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U ovom broju

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18.-22. listopada 2006.
Hotel Pical, Poreč, Hrvatska

Program i sažeci kongresa

5th Croatian Congress of Medical Biochemists with international participation
October 18-22, 2006
Hotel Pical, Poreč, Croatia

Meeting Program and Abstracts

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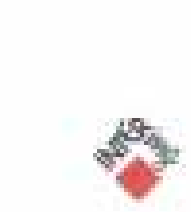				
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Biochemia Medica

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Croatian Society of Medical Biochemists - HDMB

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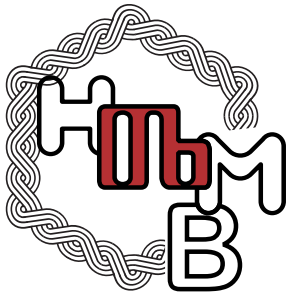
5. hrvatski kongres medicinskih biokemičara s međunarodnim sudjelovanjem

Poreč, 18.-22. listopada 2006.



5th Croatian Congress of Medical Biochemists with international participation

Poreč, Croatia, October 18-22, 2006

Pod visokim pokroviteljstvom / Under the Patronage of

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Istarska županija / Istrian Region

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ZR-2: Edukacija medicinskih biokemičara: harmonizacija s temeljnim načelima bolonjske deklaracije
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IR-3: Abbott Laboratories – Proširena primjena hematološkog brojača Cell-Dyn Saphire

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Croatia – towards a knowledge-based society

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PL-2: Pediatric laboratory medicine: why is it different?
PL-3: Pharmacogenetics, pharmacogenomics and pharmacoproteomics of cardiovascular drugs
PL-4: Medical biochemistry and laboratory medicine: future prospects

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S-4: Automation and new technologies
S-5: Tumor markers and molecular diagnosis of malignant tumors
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S-8: Markers of vascular diseases
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S-10: Toxicology and pharmacogenetics
S-11: Autoimmune and alergic diseases
S-12: Diabetes mellitus
SA: Abbott symposium – cardiovascular diseases

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P-3:Coagulation
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P-7: Diabetes mellitus
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P-9: Inflammation
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ZR-2: Education of medical biochemists: compliance to fundamental principles of Bologna declaration
ZR-3: D-dimers: definition, clinical application, standardization and harmonization of methods
ZR-4: New hematological parameters

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IR-1: Roche diagnostics – New Cobas line analytical systems
IR-2: MDLAB – ACL TOP performance in routine coagulation testing
IR-3: Abbott Laboratories – Extended application of hematology analyzer Cell-Dyn Saphire

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Raspored Schedule

	SRI/WED 18. 10.	ČET/THU 19. 10.		PET/FRI 20. 10.		SUB/SAT 21. 10.		NED/SUN 22. 10.	
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11:00–11:30									
11:30–12:15		PL1 A		PL2 A		PL3 A		PL4 A	
12:15–13:00	**Registracija** Registration								
13:00–14:00		ZR1 A	IR1 B	Izlet Excursion		ZR3 A	IR3 B	Zatvaranje kongresa Closing ceremony	
14:00–15:00		ZR2 A	IR2 C			ZR4 C			
15:00–15:30									
15:30–16:00									
16:00–17:00	**Predkongresni simpozij** Precongress symposium A	S3 B	S4 A			S9 A	S10 B		
17:00–18:00									
18:00–18:30		S Abbott A							
18:30– 19:00	Otvaranje kongresa / Opening ceremony								
19:00–20:00	**Počasno predavanje / Honorary lecture** A					Zajednička večera Gala dinner			
20:00–20:30	Koktel dobrodošlice Welcome Coctail								
20:30–21:00		Koncert Concert							
21:00–22:00									

Legenda / Legend:

- Simpozij / Symposium
- Znanstvena radionica / Scientific workshop
- Društveni program / Social program
- Plenarno predavanje / Plenary lecture
- Industrijom sponzorirana radionica / Industry sponsored workshop

A: Dvorana A / Hall A
B: Dvorana B / Hall B
C: Dvorana C / Hall C

18. listopad 2006. – srijeda

S0: Predkongresni simpozij - Laboratorijska medicina u 21. stoljeću: Povodom obilježavanja 40 godina rada prof. dr. Ane Stavljenić Rukavina, 16:00–18:00

- Centri izvrsnosti u kliničkoj kemiji i laboratorijskoj medicini
- Integracija i konsolidacija laboratorijskih specijalnosti - povezivanje laboratorija s odjelima i područjem
- Trajna edukacija: zalog za budućnost struke
- Simpozij s razlogom

O1: Otvaranje kongresa; 18:30 – 20:00

- Počasno predavanje: Hrvatska – prema društvu temeljenom na znanju

October 18, 2006 – Wednesday

S0: Precongress symposium - Laboratory medicine in the 21st century: 40th anniversary of Professor Ana Stavljenić Rukavina's activities in medical biochemistry; 16:00 – 18:00

- Centers of excellence in clinical chemistry and laboratory medicine
- Integration and consolidation of lab specialties – linking lab to the wards and territory
- Continuous education: pledge for the future of profession
- A symposium with a reason

O1: Opening ceremony; 18:30 – 20:00

- Honorary lecture: Croatia – towards a knowledge-based society

19. listopad 2006. – četvrtak

S1: Simpozij 1 - Harmonizacija u laboratorijskoj medicini; 09:00 – 11:00

- Značenje međunarodnog razumijevanja koncepata i širom svijeta prihvaćenog nazivlja u kemijskom mjeriteljstvu
- Harmonizacija medicinsko biokemijskih pretraga u Hrvatskoj
- Mjeriteljska sljedljivost i mjerna nesigurnost – mjeriteljski pogled na rezultat
- Mjerna nesigurnost u EQA

S2: Simpozij 2 - Trombociti 2006; 09:00 – 11:00

- Uloga trombocita u bolesti
- Nasljedni poremećaji funkcije trombocita
- Testovi ispitivanja funkcije trombocita
- Evaluacija trombocita protočnom citometrijom

PL1: Plenarno predavanje 1; 11:30 – 12:15

- Ateroskleroza

ZR1: Radionica 1 - Međulaboratorijske usporedbe; 13:00 – 14:00

- Utjecaj harmonizacije općih medicinsko-biokemijskih pretraga na rezultate vanjske procjene kvalitete medicinsko-biokemijskih laboratorija
- Procjena rezultata u laboratorijskoj hematologiji
- Procjena rezultata u laboratorijskoj koagulaciji
- Procjena rezultata u rutinskoj analizi mokraće
- Procjena rezultata specijalističkih biokemijskih pretraga – pH i plinovi u krvi
- Rezultati vanjske procjene kvalitete za HbA_1c

October 19, 2006 – Thursday

S1: Symposium 1 - Harmonization in laboratory medicine; 09:00 – 11:00

- Meeting the need of intercontinentally understood concepts and associated intercontinentally agreed terms for Metrology in Chemistry (MiC)
- Harmonization of medical biochemistry tests in Croatia
- Metrological traceability and measurement uncertainty – metrological view of the result
- Measurement uncertainty in EQA

S2: Symposium 2 – Platelets 2006; 09:00 – 11:00

- The role of platelets in disease
- Inherited disorders of platelet function
- Platelet function tests
- Evaluation of platelets by flow cytometry

PL1: Plenary lecture 1; 11:30 – 12:15

- Atherosclerosis

ZR1: Workshop 1 - Interlaboratory comparisons; 13:00 – 14:00

- Impact of harmonization of general medical biochemistry analyses on external quality assessment results
- Evaluation of results in laboratory hematology
- Evaluation of the NEQAS coagulation results
- Evaluation of results in qualitative urinalysis
- Evaluation of specialist biochemistry test results – pH and blood gas analysis
- Results of External Quality Assessment for HbA_1c

IR1: Industrijom sponzorirana radionica 1 - Roche Diagnostics; 13:00 – 14:00

- Analitička procjena imunokemijskog analizatora MODULAR E- 170 tvrtke Roche Diagnostics
- Roche/Hitachi Modular – Analytics E170
- Biljezi koštane pregradnje na imunokemijskom analizatoru Modular Analytics <E> tvrtke Roche Diagnostics
- Modular Analytics SWA – naše iskustvo
- Cobas analitički sistemi

ZR2: Radionica 2 - Edukacija medicinskih biokemičara: harmonizacija s temeljnim načelima bolonjske deklaracije; 14:00 – 15:00

- Nova koncepcija diplomskog Studija medicinske biokemije – magistar medicinske biokemije: stručnjak za 21. stoljeće
- Poslijediplomski studiji na Farmaceutsko-biokemijskom fakultetu
- Međunarodna suradnja u edukaciji medicinskih biokemičara
- Kako uskladiti edukaciju s potrebama medicinsko-biokemijske struke?

IR2: Industrijom sponzorirana radionica 2 - MDLAB; 14:00 – 15:00

- Rutinske koagulacijske analize na koagulacijskom analizatoru ACL TOP

S3: Simpozij 3 - Laboratorijska medicina zasnovana na dokazima; 16:00 – 18:00

- Laboratorijska medicina zasnovana na dokazima: poboljšanje ishoda
- Racionalna dijagnostika u koagulaciji
- Mjere dijagnostičke točnosti: značenje i primjenjivost u praksi
- Uvođenje EBLM u svakodnevni rad
- Pravni i etički problemi u laboratorijskoj medicini

S4: Simpozij 4 - Automatizacija i nove tehnologije; 16:00 – 18:00

- Konsolidacija i automatizacija laboratorija
- Razvoj i primjena modularnog informacijskog sustava u laboratorijskoj dijagnostici
- Biosenzori: jučer, danas i sutra
- Pretrage uz bolesnika – automatizacija i integracija
- e-Zdravstvo za sigurnost bolesnika pomoću informacijskih tehnologija

SA: Simpozij Abbott - kardiovaskularne bolesti; 18:00 – 19:00

- Kliničko značenje testova za troponin poboljšane osjetljivosti
- Abbottovi srčani biljezi u dijagnostici i motrenju
- Novi srčani biljezi akutnog koronarnog sindroma

IR1: Industry sponsored workshop 1 - Roche diagnostics; 13:00 – 14:00

- Analyitical assessment of Roche MODULAR – E 170, immunochemistry analyzer
- Roche/Hitachi Modular – Analytics E170
- Bone turnover markers on immunochemistry analyzer Modular Analytics <E> by Roche Diagnostics
- Modular Analytics SWA – our experience
- Cobas analytical systems

ZR2: Workshop 2 - Education of medical biochemists: compliance to fundamental principles; 14:00 – 15:00

- A new concept of university degree in Medical Biochemistry – Master in Medical Biochemistry: competent expert for the 21st century
- Postgraduate studies at School of Pharmacy and Biochemistry
- International collaboration in the education of medical biochemists
- How to harmonize education and requirements of the medical biochemistry profession?

IR2: Industry sponsored workshop 2 – MDLAB; 14:00 – 15:00

- ACL TOP performance in routine coagulation testing

S3: Symposium 3 - Evidence based laboratory medicine; 16:00 – 18:00

- Evidence-Based Laboratory Medicine: delivering improved outcomes
- Rational diagnostics in coagulation
- Measures of diagnostic accuracy: interpretation and transferability into praxis
- Introducing EBLM to practice
- Legal and Ethical Issues in Laboratory Medicine

S4: Symposium 4 - Automation and new technologies; 16:00 – 18:00

- Laboratory consolidation and automation
- Development and application of modular information system in laboratory diagnosis
- Biosensors: past, present and future
- Point-of-care testing – automation and integration
- e-Health for achieving patient safety through information technologies

SA: Symposium Abbott - Cardiovascular diseases; 18:00 – 19:00

- Clinical importance of troponin assays with enhanced sensitivity
- Abbott cardiac markers in diagnosis and monitoring
- New biochemical markers of acute coronary syndrome

20. listopad 2006. – petak

S5: Simpozij 5 - Tumorski biljezi i molekularna dijagnostika zloćudnih tumora; 09:00 – 11:00

- Granice nutrigenomike i prevencija raka
- Genomika i proteomika u onkologiji
- Aktivacija signalnih molekula u karcinogenezi
- Sustav aktivatora urokinaznog plazminogena: bogat izvor tumorskih biljega
- Pregled najnovijih smjernica za tumorske biljege

S6: Simpozij 6 - Pedijatrijska laboratorijska medicina; 09:00 – 11:00

- Laboratorijska dijagnostika septičnih stanja u djece
- Neonatalna hiperbilirubinemija i Gilbertov sindrom
- Laboratorijska dijagnostika alergijskih bolesti
- Proteomski i genski biljezi autoimunih bolesti u djece
- Izazovi novorođenačkog probira

PL2: Plenarno predavanje 2; 11:30 – 12:15

- Pedijatrijska laboratorijska medicina: zašto je drukčija?

October 20, 2006 – Friday

S5: Symposium 5 - Tumor markers and molecular diagnosis of malignant tumors; 09:00 – 11:00

- Frontiers in nutrigenomics and cancer prevention
- Genomics and proteomics in cancer
- Activation of signalling molecules in carcinogenesis
- The urokinase plasminogen activator system: a rich source of tumor markers
- An overview of recent guidelines on tumor markers

S6: Symposium 6 - Pediatric laboratory medicine; 09:00 – 11:00

- Laboratory diagnosis of septic states in children
- Neonatal hyperbilirubinemia and Gilbert syndrome
- Laboratory diagnosis of allergic diseases
- Proteomic and genetic markers of autoimmune diseases in children
- The challenges of neonatal screening

PL2: Plenary lecture 2; 11:30 – 12:15

- Pediatric Laboratory Medicine: Why is it different?

21. listopad 2006. – subota

S7: Simpozij 7 - Genske bolesti – novi profili pretraga; 09:00 – 11:00

- Osiguranje kvalitete – EQA
- Genetska analiza multifaktorske bolesti: osteoporoza
- Molekularni i biokemijski profili pretraga za nasljedne bolesti
- Uloga protrombotičkih rizičnih čimbenika u cerebrovaskularnim bolestima u djece
- Patobiokemija karcinoma

S8: Simpozij 8 - Biljezi bolesti krvožilnog sustava; 09:00 – 11:00

- Genetske i okolinske odrednice upalnih biljega: rezultati u Skupini Stanislas
- Infarkt miokarda i hemostaza
- NT-proBNP je vrijedan biljeg za kongestivno srčano zatajenje s dijagnostičkom i prognostičkom primjenom
- Genska podloga bolesti krvožilnog sustava
- Diferencijalna dijagnostika i prognostički biljezi moždanog udara
- Vrijednost kardiovaskularnih biljega kod morbidne pretilosti

PL3: Plenarno predavanje 3; 11:30 – 12:15

- Farmakogenetika, farmakogenomika i farmakoproteomika kardiovaskularnih lijekova

October 21, 2006 – Saturday

S7: Symposium 7 - Genetic diseases – new test profiles; 09:00 – 11:00

- Quality assurance – EQA
- Genetic analysis of multifactorial disease: osteoporosis
- Molecular and biochemical test profiles for hereditary disorders
- The role of prothrombotic risk factors in children with cerebrovascular disease
- Cancer pathobiochemistry

S8: Symposium 8 - Markers of vascular diseases; 09:00 – 11:00

- Genetic and environmental determinants of inflammatory markers: results from the Stanislas Cohorte
- Myocardial infarction and haemostasis
- NT-proBNP is a useful marker for congestive heart failure with diagnostic and prognostic applications
- Genetic background of cardiovascular diseases
- Differential diagnosis and prognostic markers of stroke
- Value of cardiovascular markers in morbid obesity

PL3: Plenary lecture 3; 11:30 – 12:15

- Pharmacogenetics, pharmacogenomics and pharmacoproteomics of cardiovascular drugs

ZR3: Radionica 3 - D-dimeri: definicija, primjena u klinici, standardizacija i harmonizacija metoda; 13:00 – 14:00

- Definicija i pregled dostupnih metodologija za kvantitativno određivanje D-dimera
- D-dimeri: različite metode, različiti rezultati - iskustvo s programom vanjske procjene kvalitete
- Kliničko značenje određivanja D-dimera u likvoru
- Dinamičko praćenje D-dimera različitim metodama za kvantitativno određivanje

IR3: Industrijom sponzorirana radionica 3 - Abbott Laboratories; 13:00 – 14:00

- Proširena primjena hematološkog brojača Cell-Dyn Saphire

ZR4: Radionica 4 - Novi hematološki parametri; 14:00 – 15:00

- Procjena novih parametara u odnosu na standarde
- Novi parametri na hematološkim analizatorima Sysmex
- Napredak u hematologiji: nedostatak željeza u svjetlu novih biokemijskih biljega i indeksa RBC (% Hypo, CHr) – Terapijski dijagram Thomas-Plot i njegova uloga u dijagnostici i terapiji

S9: Simpozij 9 - Koštani biljezi; 16:00 – 18:30

- Koštani biljezi i njihova primjena u kliničkoj dijagnostici
- Koštani biljezi u hiperparatiroidizmu
- Razvojna dinamika biljega koštane pregradnje na primjeru adolescentne anoreksije nervoze
- Gustoća kostiju i koštani biljezi u adolescentica s anoreksijom nervozom
- Osteoporoza u reumatskom artritisu
- Biološke razlike u biokemijskim koštanim biljezima

S10: Simpozij 10 - Toksikologija i farmakogenetika; 16:00 – 18:30

- Novi pristup laboratorijskoj dijagnostici u kliničkoj toksikologiji
- Štetni utjecaj nekontrolirane suplementacije: uloga laboratorijskih analiza i nutrigenetike u anti-aging medicini
- Pasivno pušenje marihuane i pozitivna doping kontrola
- Regresijski model za predviđanje doze varfarina iz farmakogenetskog statusa – klinička primjena
- Uloga farmakogenetičkih varijacija u liječenju depresije

ZR3: Workshop 3 - D-dimers: definition, clinical application, standardization and harmonization of methods; 13:00 – 14:00

- Overview and brief description of available methodologies for quantitative determination of D-dimers
- D-Dimers: different methods, different results - the experience within an external quality assessment programme
- Diagnostic value of cerebrospinal fluid D-dimer assay
- Dynamic D-dimer monitoring by different methods for quantitative determination

IR3: Industry sponsored workshop 3 - Abbott Laboratories; 13:00 – 14:00

- Extended application of hematology analyzer Cell-Dyn Saphire

ZR4: Workshop 4 - New hematological parameters; 14:00 – 15:00

- New clinical applications with WBC automated morphological analysis
- New parameters on Sysmex hematological analyzers
- Advances in hematology: iron deficiency states in the light of new biochemical markers and RBC indices (% Hypo, CHr) – Thomas-Plot Therapeutic Diagram and its relevance in diagnosis and therapy

S9: Symposium 9 - Bone markers; 16:00 – 18:30

- Bone markers and its use in clinical diagnostics
- Bone markers in hyperparathyroidism
- Developmental dynamics of bone turnover markers: an example of adolescent anorexia nervosa
- Bone density and bone markers in female adolescents with anorexia neurosa
- Osteoporosis in rheumatoid arthritis
- Biological variation of biochemical bone markers

S10: Symposium 10 - Toxicology and pharmacogenetics; 16:00 – 18:30

- New access of laboratories diagnostics in clinical toxicology
- Side effects of uncontrolled supplementation: the role of laboratory analysis and nutrigenetics in anti-aging medicine
- Passive marijuana smoking and positive doping control
- Warfarin dose prediction regression model from pharmacogenetic status – clinical application
- The role of pharmacogenetic variations in depression therapy

22. listopad 2006. – nedjelja

S11: Simpozij 11 - Autoimune i alergijske bolesti; 09:00 – 11:00

- Novi aspekti u in-vitro dijagnostici alergija
- Smjernice za testiranje na antinuklearna antitijela (ANA)
- Autoantitijela u antifosfolipidnom sindromu
- Imunodijagnostika vaskulitisa
- Imunodijagnostika autoimunih bolesti jetre

S12: Simpozij 12 - Šećerna bolest; 09:00 – 11:00

- Alotransplantacija otočića u bolesnika sa šećernom bolešću tip 1
- Uloga upalnih čimbenika u šećernoj bolesti i komplikacijama
- Hemoglobin A_1c: kamo nakon 30 godina?
- Metabolički sindrom

PL4: Plenarno predavanje 4; 11:30 – 12:15

- Medicinska biokemija i laboratorijska medicina: pogled u budućnost

October 22, 2006 – Sunday

S11: Symposium 11 - Autoimmune and alergic diseases; 09:00 – 11:00

- New aspect in in-vitro allergy diagnostics
- Guidelines for Antinuclear Antibody (ANA) Testing
- Autoantibodies in antiphospholipid syndrome
- Immunodiagnosis of vasculitis
- Immunodiagnosis of autoimmune liver diseases

S12: Symposium 12 - Diabetes mellitus; 09:00 – 11:00

- Islet allotransplantation in type 1 diabetic patients
- The role of inflammatory factors in diabetes mellitus and its complications
- Hemoglobin A_1c: 30 Years Later
- Metabolic syndrome

PL4: Plenary lecture 4; 11:30 – 12:15

- Medical biochemistry and laboratory medicine: future prospects

Ateroskleroza / Atherosclerosis
PL-1: Četvrtak / Thursday, 19. 10. 2006.
11:30 – 12:15

Prof. dr. Željko Reiner

Akademik Željko Reiner rođen je u Zagrebu 1953. godine gdje je završio osnovnu školu, Klasičnu gimnaziju i diplomirao na Medicinskom fakultetu 1976. godine. Specijalizirao je internu medicinu u KB „Sestre milosrdnice" i u Hamburgu. Za redovitog profesora izabran je prvi put 1988., a od 1997. je redoviti profesor interne medicine u trajnom zvanju. Dodijeljen mu je naziv „fellow" Europskog kardiološkog društva (FESC). Od 1995. do 2003. bio je predstojnik Klinike za unutarnje bolesti KBC Zagreb. Od 2003. pročelnik je Zavoda za metaboličke bolesti, a od 2004. i ravnatelj KBC Zagreb. Član je Akademije medicinskih znanosti Hrvatske od 1990., a predsjednik od 2004. Od 1992. je član-suradnik HAZU, a od 2006. je redoviti član HAZU. Voditelj je Odbora za aterosklerozu HAZU.Od 2000. pročelnik je Katedre za internu medicinu Medicinskog fakulteta u Zagrebu. Objavio je 314 znanstvenih i stručnih radova. Bio je gost-profesor na više sveučilišta u SAD i Europi, pozvani predavač na nizu europskih i svjetskih kongresa, član znanstvenih odbora gotovo svih međunarodnih kongresa iz područja ateroskleroze zadnjih 15-ak godina te europskih i svjetskih kardioloških kongresa, a i u nas je kao predsjedavajući organizirao više kongresa. Od 1983. do danas bio je voditelj više znanstveno-istraživačkih projekata. Član je uredništava više najuglednijih međunarodnih časopisa (Atherosclerosis, Europ J Cardiovasc Prevent Rehabil itd.) te domaćih (Liječničkog vjesnika itd.). Bio je član Upravnog odbora Europskog društva za aterosklerozu, član je Europskog odbora za prevenciju kardiovaskularnih bolesti, osnivač je i predsjednik Hrvatskog društva za aterosklerozu, osnivač Hrvatskog društva za hipertenziju, osnivač i dopredsjednik Hrvatskog društva za debljinu itd., a bio je i glavni tajnik Hrvatskoga liječničkog zbora (HLZ). Bio je predsjednik Povjerenstva za lijekove RH od 1992.-2000., te predsjednik povjerenstva za dodjelu naziva primarijus. Bio je i predsjednik Europskog odbora za borbu protiv pušenja (2000.-2002.). Od 2005. član je radne skupine za izradu Europskih smjernica za prevenciju kardiovaskularnih bolesti. Bio je član tročlanoga međunarodnog odbora za dodjelu jedne od najznačajnijih svjetskih nagrada iz medicine „Leon Bernard Prize". Od 1995. do 1998. bio je član Izvršnog odbora Svjetske zdravstvene

Academician Željko Reiner was born in Zagreb in 1953 where he completed primary school, classical grammar school, and graduated from the School of Medicine in 1976. He was a resident in internal medicine in *Sestre milosrdnice* University Hospital, Zagreb, and in Hamburg. He was appointed a full professor for the first time in 1988, and as a professor of internal medicine for life in 1997. He has become a fellow of the European Society of Cardiology (FESC). In the 1995-2003 period he was the head of the Department of Medicine, Zagreb Clinical Hospital Center. Since 2003, he has been the head of the Institute for Metabolic Diseases, and the manager of the Zagreb Clinical Hospital Center since 2004. Professor Reiner has been a member of the Academy of Medical Sciences of Croatia since 1990, and president since 2004. In the 1992-2006 period he was a collaborating member of the Croatian Academy of Sciences and Arts, whose full member he became in 2006. He is the chairman of the Committee on Atherosclerosis of the Croatian Academy of Sciences and Arts. Since 2000, he has been the head of the Department of Internal Medicine, Zagreb University School of Medicine. He has published 314 scientific and professional papers. He has been a visiting professor at several American and European universities, an invited lecturer at a number of European and worldwide congresses, a member of scientific committees of almost all international congresses on atherosclerosis during the past 15 years, as well as European and worldwide congresses on cardiology; he also chaired and organized several national congresses. Since 1983, he has been the principal investigator of a number of scientific projects. He is a member of editorial boards of several renowned international journals (Atherosclerosis, Europ J Cardiovasc Prevent Rehabil, etc.) and national journals (Liječnički vjesnik, etc.). He was a member of the Management Board of the European Society for Atherosclerosis, a member of the European Committee for Prevention of Cardiovascular Diseases, a founder and chair of the Croatian Society for Atherosclerosis, a founder of the Croatian Society for Hypertension, a founder and vice-chair of the Croatian Society for Obesity, etc., and was also a secretary of the Croatian Medical Association.

organizacije (WHO). Recenzent je znanstvenih projekata FP 6 predloženih za financiranje Europskoj Uniji. Autor je niza poglavlja u dvadesetak knjiga i udžbenika objavljenih u inozemstvu i u nas, bio je voditelj više kolegija na dodiplomskom i 7 poslijediplomskih studija. Bio je urednik 7 knjiga i priručnika ("Farmakoterapijski priručnik" s B. Vrhovcem itd.). Od 1993. do 1998. bio je zamjenik ministra zdravstva, a od 1998. do 2000. ministar zdravstva RH.

Professor Reiner chaired the Commission on Medicaments of the Republic of Croatia from year 1992 to 2000, and the commission that awarded the title of head doctor. He was the chairman of the European Commission on Battle against Smoking (2000-2002). Since 2005, he has been the member of the Working Group for preparation of European guidelines for prevention of cardiovascular diseases. He has been a member of the three-member international committee that grants one of the most prestigious awards in medicine worldwide, Leon Bernard Prize. In the 1995-1998 period, he was a member of the WHO Executive Committee. He is a reviewer of FP 6 scientific projects proposed for European Union's financial support. He is the author of a number of chapters in about twenty books and textbooks published in Croatia and abroad, he was the leader of several courses in undergraduate and seven postgraduate studies; he is also the editor of seven books and handbooks (Handbook of Pharmacotherapy, with B. Vrhovac, etc.). Academician Reiner was a deputy minister of health from 1993 to 1998, and a minister of health of the Republic of Croatia in the 1998-2000 period.

Pedijatrijska laboratorijska medicina: zašto je drukčija? / Pediatric Laboratory Medicine: Why is it different?

PL-2: Petak / Friday, 20. 10. 2006.
11:30 – 12:15

Prof. dr. Jocelyn Hicks

Prof.dr.sc. Jocelyn M. Hicks je trenutno predsjednica *JMBH Associates*, konzultacijske tvrtke za upravljanje laboratorijima. Ona je također znanstveni i marketinški savjetnik u nekoliko najznačajnijih međunarodnih dijagnostičkih tvrtki. Donedavno je prof.dr.sc. Hicks bila glavni izvršni liječnik u odjelima za genetiku te identitet Zavoda za genetiku i in-vitro oplodnju u Fairfaxu, Virginia, SAD. Prije toga je bila voditelj Laboratorijske medicine i patologije te ravnatelj Centra za kompleksne bolesti u Dječjem državnom medicinskom centru (Children's National Medical Centre, CNMC) u Washingtonu. Trenutno je prof.dr.sc. Hicks počasni ravnatelj tog Centra i počasni profesor pedijatrije i patologije na Medicinskom fakultetu Sveučilišta *George Washington*. Tijekom rada u CNMC dr. Hicks je bila zaposlena na više voditeljskih mjesta, kao predsjednik suradnika medicinskog fakulteta, član Upravnog odbora bolnice,

Prof. Dr. Jocelyn M. Hicks is currently President of JMBH Associates, a laboratory management consulting company. She is also a scientific and marketing adviser to several major international diagnostic companies.
Until recently Prof. Dr. Hicks was the Chief Operating Officer of the Genetics and Fairfax Identity Divisions of The Genetics and IVF Institute in Fairfax, Virginia. Prior to that, she was Chair of Laboratory Medicine and Pathology and Executive Director of the Center for Complex Diseases at the Children's National Medical Center (CNMC), Washington, DC. Currently Prof. Dr. Hicks is Executive Director Emeritus at CNMC and Professor Emeritus of Pediatrics and Pathology at The George Washington University School of Medicine. While at CNMC Dr. Hicks held many leadership positions, including President of the Medical Faculty Associates, member of the Hospital's Board of

te član odbora za Zakladu dječje bolnice kao bolničkog odjela za prikupljanje sredstava.

Prof.dr.sc. Hicks je diplomirala fiziologiju, magistrirala biokemiju na *University of London*, V. Britanija, te doktorirala fiziologiju i biofiziku na Medicinskom fakultetu Sveučilišta u Georgetownu, SAD. Objavila je preko 80 znanstvenih radova te mnogo knjiga, uključujući *Pretrage uz bolesnika* (Point-of-Care Testing) te *Popis rijetkih analiza* (Directory of Rare Analyses). Bila je urednica mnogih časopisa. Njeni akademski i administrativni interesi uključuju pedijatrijske referentne vrijednosti, pretrage uz bolesnika te strategijsko i poslovno planiranje.

Prof.dr.sc. Hicks je bivša predsjednica Američkoga udruženja za kliničku kemiju (American Association for Clinical Chemistry, AACC), a bila je član i upravnog odbora tog udruženja. U okviru AACC utemeljila je zakladu Van Slyke koja je posvećena obrazovanju i istraživanju te prikupljanju sredstava za sudjelovanje mladih kliničkih kemičara na stručnim sastancima u zemlji.

Prof.dr.sc. Hicks je utemeljiteljica i bivša predsjednica (u dva mandata) Međunarodnog udruženja za pedijatrijsku laboratorijsku medicinu. Predsjedavala je Odjelom za publikacije Međunarodnog saveza za kliničku kemiju i laboratorijsku medicinu (International Federation of Clinical Chemistry and Laboratory Medicine, IFCC) te je potaknula uvođenje internet-stranica tog udruženja te *IFCC Journal* zajedno s prof. Donaldom Youngom. Bila je riznicar i član Uprave IFCC-a od 2003. do 2005. godine i sadašnja je predsjednica IFCC-a.

Među mnogobrojnim počastima prof.dr.sc. Hicks mogu se izdvojiti počasno članstvo u Udruženju kliničkih biokemičara (V. Britanija), Izraelskom društvu za kliničku biokemiju, Portugalskom udruženju za kliničku patologiju i Egipatskom društvu za laboratorijsku medicinu. Dobitnica je tri nacionalne nagrade AACC i čest je pozvani predavač u SAD i inozemstvu. Godine 2006. postala je dobitnicom nagrade *Concustell* Španjolskoga društva za kliničku biokemiju i patologiju čijim će također postati počasnim članom.

Njezini osobni interesi uključuju kuhanje, igranje bridža, putovanja i vježbanje. Udana je za dr.sc. Melvina Blechera koji se bavi pravom intelektualnog vlasništva, posebice u području biotehnologije i medicine.

Directors, and Board member of the Children's Hospital Foundation, the fund-raising arm of the hospital.

Prof. Dr. Hicks obtained a BSc. (Honours) in Physiology and her MSc. in Biochemistry from the University of London (UK), and a PhD in Physiology and Biophysics from Georgetown University Medical School (USA). She has over 80 peer-reviewed publications, and many books, including Point-of-Care Testing and the Directory of Rare Analyses. She also has served as editor of many journals. Her academic and administrative interests include pediatric reference values, point-of-care testing and strategic and business planning.

Prof. Dr. Hicks is a Past President of the American Association for Clinical Chemistry (AACC) and has served on its Board of Directors. Within the AACC, she founded the Van Slyke Foundation that is devoted to education and research, as well as providing funds for young clinical chemists to attend national meetings.

Prof. Dr. Hicks is the founder and Past President (two terms) of the International Association of Pediatric Laboratory Medicine. She was Chair of the Publications Division of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and introduced the IFCC Website and the IFCC Journal together with Prof. Donald Young. She was Treasurer and a Board member of the IFCC from 2003-2005, and is the current President of the IFCC.

Prof. Dr. Hicks' many honors include honorary memberships in the Association of Clinical Biochemists (UK), the Israel Society of Clinical Biochemistry, the Portuguese Association of Clinical Pathology and the Egyptian Society of Laboratory Medicine. She has received three of the AACC's national awards, and is frequently invited to speak both nationally and internationally. She is the 2006 Awardee of the Concustell Award from the Spanish Society of Clinical Biochemistry and Pathology, and she will also be given honorary membership in that society.

Her personal interests include cooking, playing bridge, traveling and exercising. She is married to Melvin Blecher, PhD, JD, who practices Intellectual Property Law, especially in Biotechnology and Medicine areas.

Farmakogenetika, farmakogenomika i farmakoproteomika kardiovaskularnih lijekova / Pharmacogenetics, pharmacogenomics and pharmacoproteomics of cardiovascular drugs

PL-3: Subota / Saturday, 21. 10. 2006.
11:30 – 12:15

Prof. dr. Gerard Siest

Prof.dr.sc. Gérard Siest je profesor molekularne biologije i biokemijske farmakologije na Fakultetu farmaceutskih znanosti, Sveučilište Henri Poincaré, Nancy, Francuska.
Profesor Siest je glavni urednik znanstvenog časopisa *Clinical Chemistry and Laboratory Medicine* te bivši predsjednik Međunarodnog saveza za kliničku kemiju i laboratorijsku medicinu (IFCC). Organizator je godišnjih sastanaka Santorini Biologie Prospective Colloquia.
Profesor Siest je počasni doktor znanosti Sveučilišta Laval te u Krakovu te dobitnik mnogih nagrada u dvadesetak zemalja. Član je glavnog odbora Francuskog društva za aterosklerozu te Međunarodnog društva za farmakogenomiku (ISP), te kao član sudjeluje u projektu proteoma plazme Organizacije za humani proteom (HUPO). Profesor Siest je autor više od 600 radova objavljenih u znanstvenim časopisima.

Professor Gérard Siest, Pharm D, PhD, is a professor of molecular biology and biochemical pharmacology at the Faculty of Pharmaceutical Sciences, University Henri Poincaré, Nancy.
He is the editor-in-chief of the scientific journal *Clinical Chemistry and Laboratory Medicine*, and the past-president of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). He has been the organizer of the Santorini Biologie Prospective Colloquia.
Professor Siest is Doctor Honoris Causa, Laval and Krakow Universities and a recipient of awards from twenty different countries. He is the member fo the Board of the French Atherosclerosis Society, the Board of the International Society of Pharmacogenomics (ISP), member of the Human Proteomic Organization (HUPO) Plasma Proteome Project. He is also the author of more than 600 publications in peer reviewed journals.

Medicinska biokemija i laboratorijska medicina: pogled u budućnost / Medical biochemistry and laboratory medicine: future prospects

PL-4: Nedjelja / Sunday, 22. 10. 2006.
11:30 – 12:15

Prof. dr. Ana Stavljenić Rukavina

Prof. dr.sc. Ana Stavljenić-Rukavina, liječnik i magistar farmacije, specijalist medicinske biokemije, profesor Medicinskog i Farmaceutsko biokemijskog fakulteta Sveučilišta u Zagrebu u mirovini, profesor Visoke zdravstvene škole u Zagrebu, gostujući profesor Medicinskog fakulteta Sveučilišta u Splitu i Sveučilištu u Trstu.
Rođena 1939. godine u Dugoj Resi, završila studij medicinske biokemije na Farmaceutsko biokemijskom fakultetu

Prof. Ana Stavljenić-Rukavina, physician and pharmacist, specialist in medical biochemistry, retired professor at the School of Pharmacy and Biochemistry, University of Zagreb, guest professor at the School of Medicine, University of Split, and a professor at the College of Health Science in Zagreb; guest professor at the University of Trieste
Born 1939 in Duga Resa, completed the study of medical biochemistry at the Zagreb University School of Pharma-

(1963.), studij medicine na Medicinskom fakultetu Sveučilišta u Zagrebu (1968.) postdiplomsko usavršavanje u Royal Postgraduate Medical School, London; NATO Advanced Study Institute and Simon Stevin Institute, Brugge (1971., 1976., 1986.), doktorat medicinskih znanosti (1976.) i specijalistički ispit 1979. Od 1985. godine je redoviti profesor te od 1990. redoviti profesor u trajnom zvanju Sveučilišta u Zagrebu.

Tijekom 38 godina stručnog, znanstvenog i nastavnog rada u Zavodu za dijabetes, endokrinologiju i metaboličke bolesti "Vuk Vrhovac", Kliničkom zavodu za laboratorijsku dijagnostiku KBC Zagreb i Zavodu za kemiju i biokemiju Medicinskog fakulteta u Zagrebu razvila je i organizirala suvremenu laboratorijsku dijagnostiku, te dosegla vodeće mjesto u edukaciji iz laboratorijske medicine u Hrvatskoj. Radom u međunarodnim znanstvenim i profesionalnim udrugama doprinjela je harmonizaciji edukacije i razvoju laboratorijske medicine u Europi. U razdoblju od 2000.–2001. obnašala dužnost Ministra zdravstva.

Znanstveni i stručni radovi (320 od čega 140 u CC /SCI) Ane Stavljenić Rukavina nastali provedbom 23 znanstvena projekta su iz područja epidemiologije i patobiokemije dijabetesa i poremećaja metabolizma lipida, istraživanja molekularne osnove ateroskleroze i kardiovaskularnih bolesti, molekularne biokemije nasljednih i metaboličkih bolesti. Važnija otkrića su nove mutacije LDL receptor gena, CSF gena i klastera gena odgovornih za razvoj ateroskleroze. Autor je sedam knjiga i 48 priloga u knjigama drugih autora, te urednik 16 priručnika za postdiplomsku i trajnu edukaciju. Sudjelujući na brojnim međunarodnim kongresima kao pozvani i uvodni predavač, kao organizator, član znanstvenih i organizacijskih odbora stekla je značajno mjesto u međunarodnoj znanstvenoj zajednici.

Član je upravnog odbora Foruma europskih društava za kliničku kemiju (FESCC) i savjeta Europskog društva za kvalitetu u zdravstvu (ESQH).

Član je radne grupe za evaluaciju FP6 projekata i član je Savjetodavnog odbora za FP7 znanstveni program Europske komisije.

Aktivni je član znanstvenih i stručnih društava u Hrvatskoj i svijetu; bila je predsjednica Hrvatskog društva za humanu genetiku HLZ, predsjednica Hrvatskog društva medicinskih biokemičara, predsjednica Hrvatske komore medicinskih biokemičara u tri mandata, dopredsjednica Hrvatskog društva za poboljšanje kvalitete zdravstvene skrbi, član upravnog odbora Društva za aterosklerozu HLZ. Za svoj je rad primila odličje začasnog člana HLZ.

Ordenom Danice Hrvatske s likom Ruđera Boškovića za doprinos razvoju znanosti odlikovao ju je predsjednik RH 2006. godine.

Od 1994. godine je redoviti član Medicinske akademije Hrvatske u okviru koje je sudjelovala aktivno u organizaciji znanstvenih skupova Akademije te radu Glavnog odbora Akademije u mandatu 2001.–2004.

cy and Biochemistry (1963), 1968 completed the study of medicine at the School of Medicine, University of Zagreb, postgraduate studies at the Royal Postgraduate Medical School (1971, 1976, 1986), London; NATO Advanced Study Institute and Simon Stevin Institute, Brugge, PhD in biomedicine (1976) and specialist of medical biochemistry 1979. Since 1985 full professor, since 1990 full professor for life at the University of Zagreb.

During her 38 years long professional, scientific and academic experience at the *Vuk Vrhovac* Institute for Diabetes, Endocrinology and Metabolic Diseases, as well as at the Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine and Clinical Hospital Centre, and at the Department of Chemistry and Biochemistry at the School of Medicine, University of Zagreb, she has developed and organized modern laboratory diagnostics and also achieved the leading position in the laboratory medicine education in Croatia. Her work in international scientific and professional organizations has contributed to the harmonization of the education and development of the laboratory medicine in Europe. During the 2000-2001 she was the Minister of health of the Croatian Government.

Scientific and professional papers (320 of which 140 are in journals indexed in CC/SCI) of Prof. Stavljenić Rukavina are the result of the realization of the 32 scientific projects in the field of epidemiology and pathobiochemistry of diabetes, lipid metabolism, molecular basis of atherosclerosis and cardiovascular diseases, molecular biochemistry of inherited and metabolic diseases.

Her most important scientific discoveries are new mutations in LDL receptor gene, CSF gene and gene cluster responsible for the development of atherosclerosis. She is the author of seven books and coauthor in 48 books, editor of 16 continuous education postgraduate textbooks. Participating in many international congresses as invited speaker, organizer, member of the scientific and organizing committies she has gained an important position in the international scientific comunity. She is the member of the FESCC Management Board (Forum of European Societies of Clinical Chemistry) and Europe and Society for Quality in Health (ESQH). She was involved in FP6 and FP7 scientific projects evaluation of the European Community. She is the active member of national and international scientific and professional societies; she was a chairperson of the Human Genetics Society of the Croatian Medical Association, chairperson of the Croatian Society of Medical Biochemists, chairperson of the Croatian Chamber of Medical Biochemists, vice-president of the Croatian Society for Quality Improvement, member of the Management Board of the Croatian Atherosclerosis Society of the Croatian Medical Association. For her accomplishments she was awarded the honorary membership of the Croatian Medical Association. Stjepan Mesić, President of the Republic of Croatia awarded Professor Ana Stavljenić Rukavina an Order of the

Početkom 2006. godine izabrana je za redovitog člana Svjetske akademije umjetnosti i znanosti (WAAS).
Uz znanstveni, stručni i nastavni rad aktivno sudjeluje u radu humanitarnih udruga Udrugu za zaštitu zdravlja žena i vodeći programe zdravstvenog odgoja i promocije zdravlja žena i djece, član je udruge Europa Donna Hrvatska, bila je član Odbora za javno zdravstvo Instituta otvoreno društvo (OSI-NY) u New Yorku 2002.–2005. za područje središnje i jugoistočne Europe (CEE/SEE).

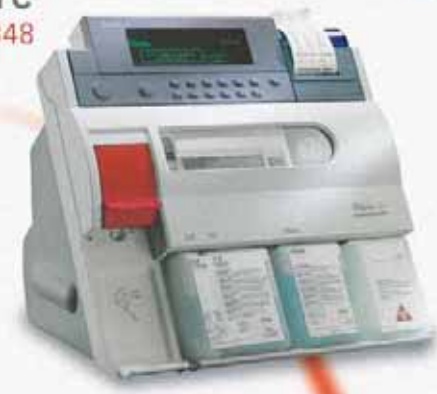
Croatian Morning Star with the image of Ruđer Bošković for her contribution to the development of science. Since 1994 she is a full member of the Academy of Medical Sciences of Croatia, where she has as a member actively participated in organization of the scientific meetings of the Academy as well as in the Management Board activities during the 2001-2004. Full member of the World Academy of Sciences and Arts (WAAS) since 2006.
Besides scientific, professional and teaching activities, she actively participates in the work of humanitarian society Association for Protection of Women's Health which organizes education courses on prevention of diseases that most often affect women. She was a member of Sub-board for Public Health, Soros Open Institute New York, for the Central and Southeast Europe region and is a member of the Europa Donna Croatia Association.

Cjelovita rješenja

dijagnostika

Critical Care

RapidLab 348



Point of Care

RapidPoint 400

Clinitek Status

Hematologija

ADVIA 120

VES-MATIC 30

Bayer

DIESSE

MEDILAB

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PL1

Ateroskleroza

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Ateroskleroza je upalni fibroproliferativni proces u kojem sudjeluju stanice stijenke krvne žile, poglavito endotelne i glatke mišićne stanice te leukociti-monociti i T-limfociti, ali i trombociti s tvarima koje proizvode, a koje potiču međusobnu aktivnost i oštećenja stanica. Zbog toga dolazi do lokalnog zadebljanja stijenke arterije koje se naziva aterom ili plak (francuski plaque - ploča, jer na stijenci žile aterosklerotične naslage često izgledaju kao masne ploče). Aterom se sastoji od meke, kašaste jezgre građene iz lipida, poglavito kolesterola i raspadnutih stanica, koju prekriva "kapa" sastavljena od izmijenjenih glatkih mišićnih stanica i veziva, poglavito kolagena, elastina i mukopolisaharida. Danas se smatra da aterogeneza započinje poremećajem funkcije endotela uzrokovanim čimbenicima rizika kao što su hiperkolesterolemija, pušenje, hipertenzija, hiperhomocisteinemija i poremećen metabolizam glukoze. U sklopu poremećaja funkcije endotela dolazi do povećane propusnosti endotela za serumske lipoproteine i ostale sastojke plazme, što je posredovano pomoću NO, PDGF, prostaciklinom, angiotenzinom-II i endotelinom. Također zbog aktivacije NF-kB dolazi do očitovanja adhezijskih molekula na endotelnim stanicama uključujući VCAM-1, ICAM-1 i selektine te do migracije leukocita i monocita/makrofaga u subendotelni prostor, što je pak posredovano oksidiranim LDL, MCP-1, PDGF i MCSF. Nakon toga dolazi do migracije glatkih mišićnih stanica iz medije krvne žile u intimu i njihovog umnožavanja (to potiču PDGF i TGF-beta), aktivacije T-stanica (to potiču TNF-alfa i IL-2), pretvaranja makrofaga pretrpanih lipidima u tzv. "pjenaste stanice" (to potiču oksidirani LDL, MCSF, TNF-alfa i IL-1) i nakupljanja trombocita, što pak potiču čimbenici kao što su tromboksan A2, tkivni čimbenik i drugi. Nakupljanje trombocita potiče i to što hiperlipidemija, a osobito hipertrigliceridemija, potiče sintezu PAI-1 u endotelnim stanicama, a on igra važnu ulogu u aterogenezi, jer smanjuje fibrinolitičku aktivnost i potiče trombogenezu. Glatke mišićne stanice stvaraju vezivnu kapu preko lipidne jezgre ateroma koja odvaja lipidnu jezgru od lumena krvne žile i krvi. Niz čimbenika, od kojih je najvažniji sastav ateroma, utječe na to hoće li aterom ostati stabilan ili će njegova kapa puknuti, pričem dolazi do stvaranja tromba na tom mjestu. Do pucanja kape ateroma poglavito dolazi zbog aktivacije makrofaga (pod utjecajem upalnih stanica, osobito T-limofocita) koji luče kovinoproteinaze (kolagenaze, elastaze) i druge

PL1

Atherosclerosis

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Atherosclerosis is an inflammatory fibroproliferative process involving vascular wall cells, especially endothelial and smooth muscle cells, leukocytes-monocytes and T lymphocytes as well as platelets with the substances they produce, which stimulate cellular interactivity and damage. This leads to local arterial wall thickening known as atheroma or plaque (Fr. *plaque*, flat area, because atherosclerotic deposits on vascular wall frequently look like adipose plates). Atheroma consists of a soft, pulpy core made of lipids, mainly cholesterol, and degraded cells, covered with a "cap" consisting of altered smooth muscle cells and connective tissue, mainly collagen, elastin and mucopolysaccharides. It is currently considered that atherogenesis begins with endothelial function impairment caused by risk factors such as hypercholesterolemia, smoking, hypertension, hyperhomocysteinemia and impaired glucose metabolism. The endothelial functional impairment includes increased endothelial permeability for serum lipoproteins and other plasma components, which is mediated by NO, PDGF, prostacyclin, angiotensin-II and endothelin. The activation of NF-κB entails expression of adhesion molecules including VCAM-1, ICAM-1 and selectins on endothelial cell surface, and migration of leukocytes and monocytes/macrophages to the subendothelial space, which is mediated by oxidized LDL, MCP-1, PDGF and MCSF. This is followed by smooth muscle cell migration from vascular media to the intima and their proliferation (stimulated by PDGF and TGF-β), T-cell activation (stimulated by TNF-α and IL-2), conversion of lipid-laden macrophages to so-called foam cells (stimulated by oxidized LDL, MCSF, TNF-α and IL-1), and platelet accumulation stimulated by the factors such as thromboxane A2, tissue factor, etc. Platelet accumulation is also stimulated by the fact that hyperlipidemia, and hypertriglyceridemia in particular, promote the synthesis of PAI-1 in endothelial cells, and PAI-1 is known to play a major role in atherogenesis by decreasing fibrinolytic activity and stimulating thrombogenesis. Smooth muscle cells form a connective cap over the lipid core of atheroma, thus separating the lipid core from the vascular lumen and the blood. A number of factors, of which the composition of atheroma is of crucial importance, determine whether the atheroma will remain stable or its cap will sustain rupture to form a thromb at the site. Rupture of the atheroma cap occurs primarily due to the activation

proteolitičke enzime koji dovode do razgradnje vezivnog tkiva kape ateroma i njenog pucanja. Pritom dolazi do prodiranja krvi iz lumena žile u aterom ili do krvarenja iz vasa vasorum, a kako su lipidi iz jezgre ateroma i pjenaste stanice izrazito trombogeni, nastaje tromb koji može povećati aterom, ali gdjekada i začepiti arteriju, a potiče se i vazospazam. Trombogenezi doprinosi i tzv. tkivni čimbenik iz stanica ateroma. S druge pak strane tromboza potiče upalni proces nastavljajući opisana zbivanja, jer potiče izraženost P selektina i liganda CD40 na površini trombocita, a te molekule potiču novačenje leukocita i upalni proces. Opisana zbivanja uzrokuju nestabilnu anginu pektoris (ako tromb ne začepi potpuno lumen koronarne arterije) i akutni infarkt miokarda (ako dođe do potpune okluzije arterije), odnosno tzv. non-Q infarkt miokarda (ako se radi o potpunoj, ali privremenoj i prolaznoj okluziji). Takvi se ateromi nazivaju nestabilnim ili vulnerabilnim. Apoptotička smrt stanica kao što su makrofazi i glatke mišićne stanice također potiče nestabilnost ateroma. Nestabilni ateromi sadrže mnogo lipida, brojne upalne stanice i imaju tanku vezivnu kapu koja sadrži malo veziva. Za razliku od njih, stabilni ateromi imaju debelu vezivnu kapu, malu lipidnu jezgru i malo upalnih stanica. Premda se smatra da samo mali broj ateroma u koronarnim arterijama koji se uoče tehnikama vizualnog prikaza spada u one nestabilne, upravo su oni odgovorni za većinu pogibeljnih koronarnih zbivanja.

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of macrophages (influenced by inflammatory cells, T lymphocytes in particular) that secrete metalloproteinases (collagenases, elastases) and other proteolytic enzymes, which in turn lead to degradation of the atheroma connective cap and its rupture. This results in blood migration from vascular lumen into the atheroma or in hemorrhage from vasa vasorum; as the lipids from the atheroma core and foam cells are extremely thrombogenic, a thromb is formed which may enlarge the atheroma, or occasionally occlude the artery, also stimulating vasospasm. The so-called tissue factor from the cells of the atheroma also contributes to thrombogenesis. On the other hand, thrombosis stimulates inflammatory process by perpetuating these events because it stimulates the expression of P selectin and CD40 ligand on platelet surface, and these molecules stimulate leukocyte recruitment and inflammatory process. All these events cause unstable angina pectoris (in case of incomplete coronary artery lumen occlusion by thrombus) or acute myocardial infarction (complete arterial occlusion) or so-called non-Q myocardial infarction (complete but temporary and transient occlusion). This type is known as unstable or vulnerable atheroma. Atheroma instability is also favored by apoptotic death of cells such as macrophages and smooth muscle cells. Unstable atheroma contains an abundance of lipids and inflammatory cells, and has a thin connective cap containing some connective tissue. In contrast, stable atheroma has a thick connective cap, small lipid nucleus, and some inflammatory cells. Although only a small proportion of coronary artery atheromas visualized by imaging techniques are considered to belong to the unstable form, they are responsible for the majority of fatal coronay events.

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PL2

Pedijatrijska laboratorijska medicina: zašto je drukčija?

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PL2

Pediatric laboratory medicine: why is it different?

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Veći dio svog radnog vijeka provela sam u dječjoj bolnici, gdje sam se neposredno uvjerila kako "djeca nisu tek male odrasle osobe", nego predstavljaju raspon od nedonoščadi do druge novorođenčadi, dojenčadi, djece u razvoju, mladeži i mladih odraslih osoba. Pred pedijatri-

Much of my career has been spent in a children's hospital, where I became acutely aware that „children are not just small adults". They range from premature babies to other neonates, infants, developing children, adolescents, and young adults. There are many challenges for the pediatric

jskim laboratorijem stoje mnogi izazovi, od potrebe za primjenom malih uzoraka, promjene referentnih intervala s dobi, potrebe za brzim pretragama do izazova što ih postavljaju adolescenti te mogućnosti izvođenja pretraga za genetičke i metaboličnе bolesti. Raspravljati će se o odgovorima na ove izazove, a oni obuhvaćaju ispravno prikupljanje novorođenačkih uzoraka, pitanja vezana uz mali volumen krvi kod novorođenčadi, dobnu raznovrsnost u pedijatriji, zbog čega adolesenti predstavljaju izazov u današnjem okruženju, testiranje uz bolesnika, te kako pristupiti testiranju za metaboličnе bolesti.

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laboratory, from the need to use small samples, changing reference intervals with age, the necessity for rapid testing, the challenges of adolescents, and the ability to test for genetic and metabolic diseases. The answers to these challenges will be discussed. They include proper collection of neonatal specimens, issues of the small blood volume of neonates, the age diversity in pediatrics, why adolesents are challenging in today's environment, Point-of-Care testing, and how to approach testing for metabolic diseases.

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PL3

Farmakogenetika, farmakogenomika i farmakoproteomika kardiovaskularnih lijekova

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Kad statin ili diuretik dajemo skupini bolesnika, neki od njih odgovaraju sniženjem kolesterola ili krvnog tlaka, a drugi pak ne. U ove različite odgovore uključeno je pet skupina gena:

- Prvu skupinu čine geni koji reguliraju farmakokinetsko ponašanje dotičnog lijeka. Najčešće su tu upleteni citokrom P450 2D6 i C9-C19. Međutim, trebamo slijediti ulogu prijenosnika, poglavito porodica ABC.
- Druga važna skupina su farmakološki ciljni geni uz receptore i enzime koji imaju glavnu ulogu u genetskoj raznolikosti koja se očituje kod statina ili antihipertenzivnih lijekova.
- Ipak, ne smijemo zaboraviti gene uključene u patologije koje mijenjaju metaboličke cikluse, fiziološke i čimbenike okoliša u regulaciji ekspresije ovih gena. Razvoj područja farmakogenomike kroz koncept biologije sustava treba isto tako obuhvatiti i proteome .
- Mnogi proteini ili peptidi izravno su odgovorni za raznolikost odgovora na terapijsku intervenciju. Polimorfizam apoE mogao bi biti dobrim modelom za objašnjavanje – kroz različite biofizikalne strukture izoforma (farmakogenomika i farmakoproteomika)

PL3

Pharmacogenetics, pharmacogenomics and pharmacoproteomics of cardiovascular drugs

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When we are giving a statin or diuretic drug to a group of patients, some are responding by a decrease in cholesterol or blood pressure but others do not. Five groups of genes are involved in these different responses :

- The first one are the genes regulating the pharmacokinetic behaviour of the drug. Cytochrome P450 2D6 and C9-C19 are the most frequently involved. But we have to follow the role of the transporters, particularly the ABC families.
- The pharmacological targets genes are the second important group with the receptors and the enzymes which play the major role in the genetic variability found with statins or antihypertensive drugs.
- Yet we should not forget the genes implicated in the pathologies modifying the metabolic cycles, the physiological and environmental factors in the regulation of the expression of these genes. The evolution of the field of pharmacogenomics through the concept of system biology should also include the proteomes.
- Many proteins or peptides are directly responsible for the variability in the response to therapeutic intervention. ApoE polymorphism could be a good

– različitih učinaka mnogih lijekova ili prehrambenih sastojaka.

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model explaining – through different biophysical structure of the isoforms (pharmacogenomics and pharmacoproteomics) – the different effects of many drugs or dietary compounds.

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PL4

Medicinska biokemija i laboratorijska medicina: pogled u budućnost

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Posljednja desetljeća obilježena su značajnim promjenama u pobolu i smrtnosti u čitavom svijetu: ekonomski razvijene zemlje su sve više pod pritiskom epidemija zaraznih bolesti poput SARS, ptičje gripe, drugih virusnih bolesti, infekcija rezistentnim sojevima bakterija ili TBC, te AIDS/HIV, a zemlje u razvoju ili nerazvijene uz postojeće zarazne bolesti pokazuju veći pobol od kroničnih nezaraznih bolesti, među kojima prevladava dijabetes. Ekonomski razvoj zemlje je nepobitno povezan sa zdravljem ljudi pa je investicija u zdravlje ujedno investicija u ekonomski razvoj isto koliko i poboljšanje pokazatelja zdravstvenog stanja stanovništva. U ciljevima milenijskog programa Svjetske zdravstvene organizacije težište se stavlja na pravo na zdravlje osobito vulnerabilnih skupina pa se od zemalja članica očekuje da takav program provedu u svojoj nadležnosti. Kako se globalne promjene u pobolijevanju i širenju bolesti mogu odraziti na laboratorijsku medicinu s organizacijskog, stručnog i znanstvenog pogleda te razvoja discipline u budućnosti? Znanstvena dostignuća u području tehnologijskog razvoja dijagnostičkih disciplina omogućuju budućnost laboratorijskoj medicini, osobito u području rješavanja javnozdravstvenih problema te u novoj disciplini javnog zdravstva nazvanoj pokret za promicanje zdravlja. Nove tehnologije potaknute minijaturizacijom strojeva, povećanjem radnog kapaciteta dijagnostičkih instrumenata i napretkom u primjeni genomike u dijagnostici značajno povećavaju ulogu dijagnostičkih disciplina u zdravstvenom sustavu. One omogućavaju dosad neviđenu brzinu postupka, sve više dijagnostičkih postupaka uz bolesnika, dok novi biomarkeri vode ka jednom od ciljeva suvremene medicine, tzv. personaliziranoj zdravstvenoj skrbi. Nove mogućnosti razvoja biomarkera za infektivne bolesti, genetički uvjetovane bolesti, molekularnu onkologiju, farmakogenomiku

PL4

Medical biochemistry and laboratory medicine: future prospects

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The last decades have been characterized by considerable changes in the morbidity and mortality rates worldwide; industrialized countries are increasingly burdened with the epidemics of infectious diseases such as SARS, avian influenza, other viral diseases, infection with resistant bacterial strains or tuberculosis, and AIDS/HIV, whereas developing and less industrialized countries show an ever greater morbidity of chronic noncommunicable diseases, primarily diabetes, in addition to the existing infectious diseases. The economic development of a country is closely related to human health, thus investment in health means investment in economy and improvement in the indicators of the population health state. The goals posed by the millennium program of the World Health Organization are focused on the right to health with special reference to the vulnerable population groups. Member countries are expected to implement the program by their authorities. What might be the impact of global changes in the prevalence and spread of diseases on laboratory medicine from the structural, professional and scientific aspects, and on the overall discipline development in the future? Scientific achievements in the field of technological development of diagnostic disciplines ensure the future of laboratory medicine, especially in the solving public health problems as well as in the new public health discipline referred to as the movement of health promotion. New technologies incited by miniaturization of devices, extended performance of diagnostic instruments and advancement in the diagnostic use of genomics have significantly increased the role of diagnostic disciplines in the health care system. They have enabled an unprecedented rapidity of procedures and an increasing proportion of point-of-care testing, whereas novel biomarkers lead to one of the

te prediktivnu medicinu stavljaju *in vitro* dijagnostiku u središnji dio zdravstvenog sustava. Razvoj biomarkera za personaliziranu medicinu dovodi dijagnostičku industriju bliže farmaceutskoj, jer je osnovni cilj jedne i druge pratiti odgovarajućim novim biomarkerima izbor odgovarajućeg lijeka, odgovor na njegovu primjenu u postizanju dobrog ishoda za pojedinog bolesnika. Zato se ubuduće očekuje promjena odnosa između stručnjaka laboratorijske medicine, proizvođača dijagnostičkih testova i proizvođača lijekova. Novi biomarkeri će usmjeravati razvoj lijekova, klinička istraživanja, praćenje i evaluaciju ishoda te doprinijeti time afirmaciji medicine zasnovane na dokazima. Primjena laboratorijske medicine zasnovane na dokazima je ekonomski zahtjevna te u cilju snižavanja troškova u zdravstvu postoji jasna tendencija proizvođača dijagnostičkih proizvoda da harmoniziraju svoje proizvode, što omogućava nižu cijenu uz kvalitetniju primjenu u svim dijelovima svijeta. Globalizacija tržišta vodi pak ka promjeni organizacije laboratorijske dijagnostike u dva smjera: primjene iste uz bolesnika odnosno konsolidaciji velikih laboratorija s obzirom na tehnologije, a ne isključivo specijalnosti i subspecijalnosti struka. Zahtjevni novi tehnološki procesi, globalizacija znanja, brza izmjena novih spoznaja zahtijevaju promjene u izobrazbi stručnjaka-specijalista laboratorijske medicine. Cjeloživotno učenje, primjena e-edukacije (*e-learning*), dobivanje i produžavanje ovlaštenja za određene specifične dijagnostičke postupke su zahtjevi bez kojih se ne mogu kompetentno obavljati profesionalne obveze. I konačno laboratorijski stručnjaci ubuduće će sve više biti promicatelji svojih profesionalnih vještina u komunikaciji s bolesnicima ili sveukupnom javnošću, što im postavlja zadaću svladavanja novih komunikacijskih vještina.

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major goals of current medicine, i.e. personalized health care. The new developments in the field of biomarkers for infectious diseases, genetic diseases, molecular oncology, pharmacogenomics and predictive medicine put the *in vitro* diagnosis in the very center of the health care system. The development of biomarkers for personalized medicine brings diagnostic industry closer to pharmaceutical industry because both of them primarily tend to follow proper drug choice and therapeutic response leading to favorable outcome in a particular patient by use of appropriate new biomarkers. Thus, the relationships between laboratory medicine professionals, diagnostic test manufacturers and drug manufacturers are expected to undergo modifications in the future. The new biomarkers will direct the development of drugs, clinical trials, follow up and outcome evaluation, and will contribute to the recognition of evidence based medicine. The use of evidence based laboratory medicine is economically demanding, therefore there is clear trend among diagnostic product manufacturers to harmonize their products, thus allowing for cost reduction along with their high quality utilization all over the world, all this in order to reduce the cost of health care in general. Market globalization, in turn, will entail dual changes in the structure of laboratory diagnosis, i.e. use of point-of-care testing and consolidation of large laboratories in terms of technology rather than exclusively specialties and subspecialties. The novel demanding technological processes, globalization of knowledge, and fast exchange of new concepts require modifications in the education of laboratory medicine professionals. Life-long learning, use of e-learning, licensing and relicensing for particular specific diagnostic procedures are the requirements without which professional commitments cannot be competently performed. And the last but not the least, in the future, laboratory professionals will increasingly take the role of their professional skill promotors in their contact with patietns or the public in general, imposing the need of mastering some new communication skills.

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S0 – Predkongresni simpozij – Laboratorijska medicina u 21. stoljeću, S0-1

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Postoji suglasje o tome da medicinski laboratoriji trebaju biti centri izvrsnosti u dijagnostičkom procesu kroz trajnu komunikaciju s kliničarima i bolesnicima. Međutim, iako polaze s vrlo bliskih polazišta (naime, bolesnika), liječnici i laboratorijski stručnjaci se tijekom radnog dana obično uvelike razilaze zbog nedostatka komunikacije i uzajamnog uvažavanja. To se može opisati kao dinamični kaotični proces gdje se zapravo bliske početne točke eksponencijalno razilaze. S jedne strane, liječnici kao oni koji spašavaju živote ne znaju što zapravo mogu očekivati od medicinskog laboratorija i koja je korist od njihovih zahtjeva za laboratorijske pretrage. Stoga njihova očekivanja često ostaju bez odgovora pa su liječnici često skloni ne uzimati laboratorij i njegovo osoblje sasvim ozbiljno. S druge strane, upravljanje kvalitetom, kontrola kvalitete i unutarnji proces optimiranja sve više postaju za mnoge kliničke kemičare prvenstveni cilj njihovog rada. Tako se zanemaruje činjenica da se 93% laboratorijskih pogrješaka događa u prijeanalitičkom ili poslijeanalitičkom dijelu dijagnostičkog procesa. Danas samo 7% grješaka nastaje u specifičnim laboratorijskim procesima. Potrebna je bolja organizacija i učinkovitost laboratorija kako bi se smanjio broj radilišta i rabili konsolidirani suvremeni analizatori, te tako izbjeglo ili na najmanju mjeru svelo cijepanje uzoraka i raspodjela poduzoraka. Tako će laboratorijska služba moći poboljšati vrijeme potrebno od primitka uzorka do izdavanja nalaza, te bolje iskoristiti raspoloživo osoblje. Reorganizacija temeljnog slijeda radnih operacija omogućava primjenu novih tehnologija i inovacija u dijagnostičkom laboratoriju. Organizacija laboratorija može se definirati kao "činiti pravu stvar na ispravan način". Izgledi da se prava stvar učini na ispravan način su veći ako su u laboratorijima ugrađeni ne samo kontrolni sustavi za tehnički proces, nego i kontrolni sustavi za tumačenje rezultata te za donošenje odluka (postavljanje dijagnoze). Kroz učinkovitost unutarnjih operacija može se poboljšati kvaliteta skrbi za bolesnika, pojačavanjem interakcije s bolesnicima i kliničarima (npr. pribivanje kliničkim vizitama i sastancima). Laboratorijski stručnjaci će morati izgraditi mrežu od laboratorija prema kliničkim odjelima uza strogo utvrđene odgovornosti uključujući

S0 – Precongress symposium – Laboratory medicine in the 21st century, S0-1

Centers of excellence in clinical chemistry and laboratory medicine

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There is agreement that medical laboratories have to be Centers of Excellence in the diagnostic process by continuous communication with clinicians and patients. However, during the clinical working day, physicians and laboratorians, although they are starting from very close points (namely, the patient), usually diverge intensely due to the lack of communication and mutual appreciation. This can be defined as a dynamic chaos-process when two arbitrarily close starting points diverge exponentially. On the one hand, physicians being in the role of life-savers, do not know what they can really expect from the medical laboratory and what is the benefit of their lab requests. Therefore, their expectations are often not answered and the physicians tend to take the laboratory and its staff not really serious. On the other hand, for many of the clinical chemists quality management, quality control, internal process optimization become ever more the primary goal in their doing. Thereby, the fact is neglected that 93% of laboratory errors occur either in the preanalytical or postanalytical part of the diagnostic process. Nowadays, only 7% of errors occur in laboratory specific processes. Improved laboratory organization and efficiency are necessary to reduce the number of work-stations and to use consolidated modern analyzers to avoid or minimize sample splitting, distribution of subsamples. Thus, the laboratory service can improve the turnaround times and use the personnel more efficiently. Reorganization of the basic workflow allows for implementation of new technologies and innovations in the diagnostic laboratory. Laboratory organization can be defined as "doing the right thing right". The chance of doing the right thing right is greater if not only technical process control systems but also control systems for data interpretation and for decision (diagnosis) making are implemented in the laboratories. By efficiency of internal operations, the quality of patient care can be improved by extending the interaction with patients and clinicians (e.g., attending clinical rounds and clinical staff meetings). Laboratorians will be obliged to build a network from the laboratory to clinical departments with a strict responsibility including diagnostic strategies, specimen collection, transportation, measurement,

dijagnostičke strategije, prikupljanje uzoraka, prijenos uzoraka, mjerenje, sastavljanje izvješća i tumačenje rezultata pomoću računalnih *on-line* sustava. Oni moraju biti ravnopravni partneri kliničarima u višedisciplinskom modelu usklađene skrbi. Dobro zamišljena (re)organizacija laboratorija, gdje su liječnici i laboratorijski stručnjaci povezani i djeluju zajednički, povećava dijagnostičku vrijednost laboratorija kao dijagnostičkog centra izvrsnosti. Uz ovakav koncept laboratorijski stručnjaci neće biti samo uspješni upravljači ljudskim, novčanim i radnim resursima, nego će isto tako davati stručno dijagnostičko mišljenje u okviru skrbi za bolesnika, kako bi se održali u okruženju usredotočenom na kliniku i bolesnika koje traži interdisplinsko poznavanje velikih načela fiziologije i patofiziologije, kao i spremnost za prihvaćanje odgovornosti za donešene odluke, što sve proizlazi iz takvih aktivnih interaktivnih odnosa.

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S0-2

Integracija i konsolidacija laboratorijskih specijalnosti – povezivanje laboratorija s odjelima i područjem

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Laboratorijska medicina sastoji se od mnoštva različitih disciplina: Kliničke biokemije, Kliničke mikrobiologije, Kliničke molekularne laboratorijske biologije, Hematologije itd. Ove discipline imaju različitu kulturalnu osnovu, ali se u smislu praktičnog organiziranja kao pomoć medicinskim strukama mogu ujediniti u Odjel laboratorijske medicine. Puno je valjanih razloga za spajanje različitih disciplina u jedinstven odjel. Zapravo, Odjel laboratorijske medicine 1) poboljšava organizaciju službe za bolesnika (upravljanje službom za uzimanje uzoraka, priprema i dostavljanje laboratorijskih nalaza itd.; 2) olakšava kontakt s bolesnicima; 3) olakšava objedinjavanje različitih analita za istoga bolesnika na znatno bolji način u vidu "medicinskih bioloških biljega"; 4) olakšava povezivanje između LIS (laboratorijski informacijski sustavi) i HIS (bolnički informacijski sustavi), te teži ka stvaranju HER (elektronski zdravstveni zapis). Sve to pak poboljšava kvalitetu usluge za bolesnika i snižava troškove. Promjena u scenariju zdravstvenih sustava širom svijeta pogoduje objedinjavanju različitih kliničko laboratorijskih disciplina u Odjel laboratorijske medicine. Pritom u obzir treba uzeti slijedeće: 1) troškovi tehnologije te potrebna visoko specifična

S0-2

Integration and consolidation of lab specialties – linking lab to the wards and territory

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Laboratory Medicine is composed of many different disciplines: Clinical Biochemistry, Clinical Microbiology, Clinical Molecular Biology Laboratory, Hematology, etc. These disciplines have a different cultural background but, from the practical organization of medical assistance, they can be unified in a Department of Laboratory Medicine. There are many good reasons for connecting the various discipline into a unique department. In fact, a Department of Laboratory Medicine 1) improves organization of the service for the patient (management of the blood sampling service, preparation and delivery of lab reports etc.; 2) facilitates contacts with patients; 3) facilitates integration of various analytes in a single patient into a more elaborated concept of "medical biomarkers"; 4) facilitates connection between the LIS (Laboratory Information Systems) and HIS (Hospital Information Systems) and trend towards the creation of HER (Electronic Health Record). All these points result in improvement of the quality of service for patient and reduction of costs. The change of the scenario in health care systems all over the world adds something more in favor of the integration of various clinical laboratory disciplines into a Department

specijalistička znanja u novim područjima laboratorijske medicine (npr. molekularna biologija) postaju preprekom za manje laboratorije i širom svijeta dovode do promjena u organizaciji laboratorija, od stapanja manjih laboratorija do pretvaranja manjih laboratorija u centre za uzimanje krvi koji te uzorke šalju na obradu u veće laboratorije (laboratorijska služba). Cilj ove nove organizacije laboratorija je s jedne strane potreba za dostupnosti usluge što bliže mjestu stanovanja ili rada bolesnika, a s druge strane iskorištavanje prednosti što ih pružaju sofisticirane i skupe tehnologije i znanja koja su financijski nedostupna manjim laboratorijima; 2) velik napredak u nekim područjima biomedicinskih znanosti (poglavito poznavanje genetičke osnove mnogih bolesti te mogućnost zaustavljanja ili odgađanja nastupa bolesti kroz odgovarajuće promjene načina života i prehrambenih navika) otvaraju vrata novoj razdoblju prediktivne/preventivne medicine gdje će, kako se očekuje, laboratorijska medicina imati važnu ulogu. Napredak u farmakogenomici drugi je važan vid personalizirane medicinske pomoći. Kako bi laboratorijska medicina preživjela ove velike promjene i uspješno se suočila s izazovima budućnosti potrebne su znatne promjene u njezinu ustroju, uza stvaranje mreže kliničkih laboratorija organiziranih u odjele, dobro povezane s istraživačkim institutima, koja će isto tako biti sposobna iskoristiti prednosti što ih donosi napredak u informacijskoj tehnologiji i digitalna revolucija. Opisuje se iskustvo bolnice San Raffaele u ovom području.

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of Laboratory Medicine. In this respect, the following points should be taken in consideration: 1) the costs of technology as well as the need of very deep specialist competences in the new areas of laboratory medicine (e.g., molecular biology) are becoming prohibitive for small laboratories and provoke, all over the world, various changes in the organization of laboratories ranging from consolidation of small laboratories to the transformation of small laboratories into blood sampling centers referring patient samples to be processed at a larger laboratory (lab service). The aim of this new organization of laboratories is the need to keep the service as close as possible to where the patients live and/or work on the one hand, and to take advantage of the sophisticated and expensive technologies/competences not affordable in a small lab on the other hand; 2) the great progress in some areas of biomedical sciences (mainly the knowledge of the genetic background of many diseases as well as the possibility of stopping or delaying the onset of a disease through an appropriate change in lifestyle/dietary habits) open the door for a new era of predictive/preventive medicine where laboratory medicine is expected to play a major role. The progress in pharmacogenomics is another important aspect of the personalization in medical assistance. For laboratory medicine to survive these major changes and to successfully face the challenges of the future, major structural changes are needed with creation of a network of clinical laboratories, organized in departments, well linked to research institutes and able to take advantage also from the progress in information technology and digital revolution. The experience of the San Raffaele in this area will be reported.

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S0-3

Trajna izobrazba: zalog za budućnost struke

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Kad se govori o kvaliteti u kliničkoj i laboratorijskoj medicini, težište se obično stavlja na analitički proces. Međutim, dobra stručna kvaliteta započinje najboljom izobrazbom. U pokušaju opisa obrazovanja i usavršavanja u laboratorijskoj medicini u Europi i u staroj Europskoj Uniji zabilježene su velike razlike u načinu usavršavanja ovih stručnjaka. Taj pregled je pokazao kako manje od jedne trećine

S0-3

Continuing Education; A pledge of the Profession Future

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When quality is referred to in clinical and laboratory medicine, the focus is mainly on the analytical process. However good professional quality starts with the best education.
In an attempt to describe the training and education aspects of Laboratory medicine in Europe and in the old European Union, it was noticed that large differences ex-

zemalja članica i ustanova članica FESCC/IFCC poklanja dužnu pozornost obrazovanju ili ga nudi. Ishod nije donio ujednačene uvjete, jer svaka zemlja regulira zdravstvo na svoj način, ovisno o svom vlastitom povijesnom razvoju, potrebama, društvenim pogledima i financijskim mogućnostima. Iz svih tih preglednih istraživanja izveli smo neke zaključke. Potrebno je propisno fakultetsko obrazovanje prije pristupanja izobrazbi za određeno zvanje, te se čini neophodnim postojanje regulirane izobrazbe u zvanju. Programi izobrazbe zahtijevaju zlatni standard koji će služiti kao vodilja za izobrazbu za dotično zvanje; uključena bi trebala biti i izobrazba iz upravljanja, dok bi ispiti mogli pomoći u poboljšanju kvalitete tog obrazovanja. Trajna izobrazba danas predstavlja pravi izazov i glavni zadatak u našoj struci. Koji su ciljevi naše trajne izobrazbe? Prvenstvena namjera je učiniti laboratorijskog stručnjaka još stručnijim na postojećem radnom mjestu; to je istodobno proces zamišljen kao pomoć laboratorijskim stručnjacima da se prilagođavaju promjenama i u njima sudjeluju. Europa ima dugu tradiciju i povijest obrazovanja, fakulteta, visokih škola i sveučilišta, kroz stoljeća, a znanost i biokemija također se diče najdužom tradicijom. FESCC se ovim tradicijama služi za podučavanje u struci kroz ljetne škole i tečajeve. FESCC je isto tako kao cilj postavio prikupljanje i širenje smjernica i modela za poslijediplomsko obrazovanje i akreditaciju. Uz to, cilj je okupiti medicinsko i znanstveno poslijediplomsko obrazovanje laboratorijskih specijalista u suradnji s UEMS. Tješnji kontakt među nacionalnim društvima rezultirao je usporedivim programima za poslijediplomsko obrazovanje te vrlo sličnim sadržajem i praktičnim radom u struci. Tijekom posljednjih deset godina provedene su mnoge aktivnosti na području sustava kvalitete medicinskih laboratorija, uz potporu nadolazećeg međunarodnog standarda ISO pod naslovom Upravljanje kvalitetom za medicinski laboratorij. ISO 15189 je dokument od velike važnosti za kvalitetan razvoj sustava kvalitete i akreditacije medicinskih/kliničkih laboratorija. FESCC organizira trajne Poslijediplomske tečajeve iz klinička kemije, poglavito u IU Centru u Dubrovniku. FESCC je zaokupljen harmonizacijom naše struke u Europi u skladu sa SZO, osobito u području izobrazbe, usavršavanja i akreditacije, što će omogućiti fleksibilnost unutar Europe. Harmonizacija izobrazbe u Europi je težak zadatak; lakše će biti postići ujednačenost izobrazbe. Standardni nastavni plan treba postati minimalnim zahtjevom za lokalnu primjenu. Poslijediplomska izobrazba u laboratorijskoj medicini treba biti opća uz naknadno subspecijalističko usavršavanje. Naša struka je multidisciplinske naravi i mi moramo okupljati medicinske stručnjake i znanstvenike te jedni i drugi trebaju proći zajedničku izobrazbu. IFCC i FESCC trebaju zajednički raditi na standardiziranim smjernicama za izobrazbu. Budući skupovi koji će se baviti neisted in the way professionals are being trained. A survey showed that less than one third of the different Member Societies and corporate members of FESCC/IFCC paid real attention to, or offered education. The outcome did not give a uniform pattern, since every country regulates health care in its own way, according to its own historical development, needs, social vision and their own financial possibilities. From all surveys we have been drawn a number of conclusions. Proper university training is required before entering vocational training and regulated vocational training seems to be necessary. The training programmed needs a golden standard indicative guide to the vocational training, management training should be included and examinations may help in improving the quality of the education.

Continuous education is now a days a real challenge and a main task in our profession. What are the goals of our continuous education? The primary purpose is to make laboratory professional more competent in their existing employment, it is at the same time a process designed to assist laboratory professionals to adapt to and to take part in changes. Europe has a long tradition and history in educations, schools, colleges and universities for centuries and also science and biochemistry have longest traditions. FESCC (The Forum of the European Societies in Clinical Chemistry) is using these traditions to teach the profession in summer schools and courses.

The aim of FESCC is also to collect and to disseminate guidelines and models for postgraduate training and accreditation. The goal is also to get together the medical and scientific postgraduate training of laboratory specialist in co-operation with UEMS. Closer contacts between the national societies have resulted in comparable programs for postgraduate training and close similarity of contents and practice of the profession. During the last ten years many activities have taken place in the field of quality systems of medical laboratories and supported the forthcoming international ISO standard. "Quality management for the medical laboratory". ISO 15189 is a document of great importance for the development of quality of quality systems and accreditation of medical/clinical laboratories.

FESCC organize continuous Postgraduate courses in clinical chemistry especially in the IU-centre of Dubrovnik. FESCC is occupied with the harmonization of our profession in Europe in accordance with WHO, especially in education, training and accreditation enabling flexibility within Europe.

Harmonization of education in Europe is difficult; equivalence of training may be more achievable. A standard syllabus should be a minimum requirement for local use. Post graduate training in laboratory medicine should be general with sub-specialty training later on. Our profession has a multidisciplinary aspect and we need medi-

riješenim pitanjima i osigurati plodne rasprave trebali bi dovesti do novih inicijativa za budućnost.

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cals and scientist together and both medical and science graduates should have a common training
IFCC and FESCC have to work together on standardized guidelines on education. There will be further meetings to address unsolved questions and fruitful discussions should lead to new initiatives for the future.

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S0-4

Simpozij s razlogom

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Svako razdoblje razvoja medicinske biokemije i profesije u pojedinoj zemlji obilježavaju nove spoznaje, napredak znanosti i struke, ali i osobe. Jedno od drugog je često nemoguće odvojiti. Ovaj je simpozij organiziran u povodu 40 godina znanstvenog rada jednoga od zaslužnih članova Hrvatskoga društva medicinskih biokemičara, no umjesto o osobi u ovom prilogu simpoziju biti će više riječi o razvoju znanstvene podloge medicinske biokemije i njenom razvoju u Hrvatskoj tijekom tog razdoblja. Razvoj biomedicinske znanosti je neprijeporno u tom razdoblju pokazao eksponencijalni rast u nizu područja važnih za medicinsku praksu. Kronične nezarazne bolesti: dijabetes, bolesti srca i krvnih žila, cerebrovaskularne bolesti, rijetke nasljedne bolesti, okolišnim čimbenicima uzrokovane bolesti uz maligne su tijekom svih proteklih 40 godina bile izazov znanstvenicima u traganju za etiološkim čimbenicima, pronalascima dijagnostičkih metoda za njihovo rano prepoznavanje ili prepoznavanje rizičnih čimbenika koji do njih dovode, kao i ustrajnom traženju novih metoda liječenja. Ako je dijabetes već sredinom šezdesetih godina prošloga stoljeća prepoznat kao javnozdravstveni problem u Hrvatskoj, to se s pravom može pripisati iznimnom timu stručnjaka i znanstvenika na čelu s profesorom Zdenkom Škrabalom, u kojem se njeguje multidisciplinarnost i zato je razumljivo da se medicinska biokemija i stručnjak iz toga područja u takovom timu razvija do prepoznatog znanstvenika u slijedećih 15 godina. Dr. Ana Stavljenić-Rukavina je svojim znanstvenim radom u tom prestižnom timu razvijala nove metode za otkrivanje dijabetesa i komplikacija te bolesti, koje su prepoznate u međunarodnim razmjerima. Sudjelovanjem u European Group for the Study of Diabetes Epidemiology Svjetske zdravstvene organizacije i međunarodnim projektima poput Multinational Study of Vascular Complications in Diabetes, opet u multidisciplinarnom timu Zavoda za dijabetes "Vuk Vrhovac", uza znanstveni iskorak učinila je medicinsku biokemiju nezaobilaznom u razvoju dijabetologije. Metabolički

S0-4

A symposium with a reason

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Every period in the development of medical biochemistry and profession in a particular country is characterized by new concepts, advancement in the science and profession as well as by some outstanding personalities. It is frequently quite difficult to consider them in separate. The present symposium has been organized to celebrate 40 years of the professional work of one of the deserving members of the Croatian Society of Medical Biochemists, however, this contribution is focused on the development of the scientific background of medical biochemistry and its development in Croatia during the respective period of time. In this period, biomedical sciences definitely showed exponential growth in a number of areas highly relevant for medical practice. Besides malignant diseases, chronic noncommunicable diseases, i.e. diabetes, cardiovascular diseases, cerebrovascular diseases, some rare hereditary diseases and diseases caused by environmental factors have posed a challenge to scientists throughout the 40-year period, in terms of searching for etiologic factors, development of diagnostic methods for their early detection or for identification of the causative risk factors, and persistent search for novel methods of treatment. The fact that diabetes was recognized as a public health problem in Croatia as early as in mid-1960s can definitely be attributed to the extraordinary team of professionals and scientists fostering multidisciplinary work, headed by Professor Zdenko Škrabalo. Thus, it is quite understandable that medical biochemistry and the respective professional had developed within such a team into a renowned scientist in the next 15 years. Dr. Ana Stavljenić-Rukavina's scientific work in this prestigious team was focused on the development of novel methods for the detection of diabetes and its complications, which have been duly recognized at the international level. With her active participation in the work of the European Group for the Study of Diabetes Epidemiology of the World Health Organization and through international projects such as Multinational Study of Vascular Complications in

sindrom u tom razdoblju nije bio poseban klinički entitet, ali u većini radova iz toga razdoblja može se zaključiti kako je bio prepoznat, premda se nazivao komplikacijom dijabetesa (hiperlipidemija, adipozitet, kardiovaskularne komplikacije). Tijekom svih 40 godina znanstvenoga rada dr. Ana Stavljenić-Rukavina bavi se epidemiologijom i etiološkim čimbenicima tih stanja primjenjujući najsuvremenije metode u datom razdoblju, od biokemijskih do molekularno bioloških i genetičkih, a niz znanstvenih projekata ima za rezultat vrijedne radove u svjetskoj bibliografiji. Vrijedi zabilježiti otkriće nove mutacije na genu za LDL receptor (mutacija Zagreb). Punih se 25 godina znanstvenoga rada u KBC i Medicinskom fakultetu Sveučilišta u Zagrebu sustavno provode projekti kojima je kao i danas u središtu znanstvenog interesa traganje za utvrđivanjem rizika za razvoj kardiovaskularnih bolesti kao jednog od značajnih javnozdravstvenih problema u globalnom smislu. Poznato je da znanstvenici ne poznaju granice u istraživanju i kadgod je moguće tragaju u širim okvirima za spoznajama koje mogu unaprijediti njihovo uže područje interesa. Tako i Ana Stavljenić-Rukavina uz metabolizam lipida, ugljikohidrata u području dijabetologije i kardiologije ulazi u područje metaboličkih bolesti i nasljednih bolesti općenito, kako zbog istih ili sličnih metoda istraživanja, tako i zbog međusobne povezanosti poremećaja metabolizma. A etiološki i okolišni čimbenici razvoja nekih kliničkih entiteta poput endemske nefropatije su dodatni izazov znanstveniku sa širim pogledom na nerazjašnjene bolesti poput ove. Za razvoj biomedicinskih znanosti je zaslužna u velikom dijelu suvremena tehnologija. U svakom od razdoblja iz radova je vidljivo da je primijenjena tehnologija i metode u projektima Ane Stavljenić-Rukavina u suglasju s onima najsuvremenijim u svjetskim razmjerima, pa je upravo zbog toga bilo moguće da izvrsno opremljeni laboratorij u kojem su mnoge tehnike bile primijenjene prije nego u drugim znanstvenim institutima iskoristi iste za razvoj drugih područja biomedicinskih znanosti (protočna citometrija, plinsko masena spektrometrija, molekularna dijagnostika). Konačna bilanca ovih 40 godina znanstvenog rada su 22 znanstvena projekta, 320 znanstvenih radova od kojih 117 u časopisima koji se citiraju u CC ili drugim časopisima namijenjenih indeksaciji. Više stotina citata navedenih radova, brojna predavanja na svjetskim i domaćim kongresima sigurno nisu potpuni iskaz, no iznad svega stoji činjenica o doprinosu Ane Stavljenić-Rukavina prepoznatljivosti naše biomedicinske znanosti u svjetskim okvirima.

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Diabetes, again in the multidiscplinary team from the Vuk Vrhovac Institute of Diabetes, along with her scientific breakthrough, she has made medical biochemistry an imperative in the development of diabetology. At that time, metabolic syndrome had not yet been established as a specific clinical entity, however, the majority of the then studies suggest that it was recognized but was referred to as complications of diabetes (hyperlipidemia, adiposity, cardiovascular complications). Throughout her 40-year scientific work, Dr. Ana Stavljenić-Rukavina was dealing with the epidemiology and etiologic factors of these conditions, using the latest methods at a particular time, from biochemistry through molecular biology and genetics, while a number of her scientific projects have resulted in valuable contributions to the international bibliography. The discovery of new mutations on the LDL receptor gene (Zagreb mutation) should be noted. During her 25-year scientific work at Zagreb University Hospital Center, projects focused on the risk identification for the development of cardiovascular diseases as one of the major public health problems worldwide, still of utmost scientific interest to the present, were systematically conducted. Scientists are known to disregard any borders in their research and to search for the concepts that may promote their close field of interest across a broad scope of activities. So, besides the lipid and carbohydrate metabolism in the fields of diabetology and cardiology, Ana Stavljenić-Rukavina tackled the field of metabolic diseases and hereditary diseases in general, both for the identical or similar methods of research and for the interrelationship among metabolic disorders. The etiologic and environmental factors for the development of some clinical entities such as endemic nephropathy pose an additional challenge to the scientist considering some as yet obscure diseases like this one. The development of modern biomedical sciences is greatly based on the current technological advancements. All her papers from any period reveal that the technology and methods used in her projects were consistent with the latest achievements at the time in the world. It was the basis for the excellently equipped laboratory, where many techniques were applied earlier than at some other scientific institutes, to be employed for the development of other fields of biomedical sciences (flow cytometry, gas mass spectrometry, molecular diagnosis). The 40 years of the scientific work of Dr. Ana Stavljenić-Rukavina have resulted in 22 scientific projects and 320 scientific papers, 117 of them in CC journals and other indexed periodicals. Hundreds of these papers citations, numerous lectures held at international and Croatian congresses certainly cannot fully reflect her scientific merits, however, the contribution made by Ana Stavljenić-Rukavina to the visibility of the Croatian biomedical science at the international level is a self-evident fact.

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S1 – Harmonizacija u laboratorijskoj medicini, S1-1

Značenje međunarodnog razumijevanja koncepata i širom svijeta prihvaćenog nazivlja u kemijskom mjeriteljstvu

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Opis rezultata kemijskih mjerenja mora sadržavati nedvosmislene i dosljedne koncepte i pojmove kao što su mjerna veličina, metrološka sljedljivost, mjerna nesigurnost, usporedivost rezultata mjerenja, nesigurnost ciljnog mjerenja itd., kako bi se omogućila valjana usporedba rezultata mjerenja. Tome još nije tako, što su tijekom proteklog desetljeća pokazale brojne radionice širom svijeta, a kemijska literatura na to stalno ukazuje. Koncepti i pojmovi su bitni kako bi međunarodna trgovina ljudskom i životinjskom hranom bila poštena, prekogranična primjena ekoloških propisa jednaka za sve uključene strane, razmjena rezultata kliničkih mjerenja postala stvarnost, a prekogranično tumačenje mjernih rezultata bilo moguće, ispravno shvaćeno i uzajamno prihvaćeno. Slično tome, njihov prijevod s jednog jezika, engleskog, na 30-40 drugih jezika treba provesti i nedvosmisleno utvrditi. Zemlje u kojima je engleski u redovnoj uporabi nisu još u potpunosti shvatile kako imaju znatnu prednost pred zemljama gdje takvi prevedeni pojmovi koji opisuju koncepte još možda nisu dostupni, a kamoli shvaćeni i prihvaćeni. Ove su zemlje stoga u znatno nepovoljnijem položaju u svim slučajevima gdje su uključena kemijska mjerenja. Opisuju se neke nejasnoće u definicijama i pojmovima, koje ukazuju na važnost revizije Međunarodnog rječnika osnovnih i općih pojmova u mjeriteljstvu (VIM), koja je u tijeku (1997.-2006.), osobito stoga što će kemijsko mjerenje prvi put u povijesti biti uključeno u VIM:

- 'mjerna veličina', 'mjerna jedinica' i 'mjerna ljestvica'
- 'rezultat mjerenja', 'metrološka usporedivost'
- 'metrološka sljedljivost'
- (uklj. 'do SI'),
- 'mjerna nesigurnost', 'ciljna mjerna nesigurnost'

Zaključuje se kako je sadašnja revizija VIM od velike važnosti za ispravno razumijevanje unutar i između mjernih zajednica širom svijeta.

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S1 – Harmonization in laboratory medicine, S1-1

Meeting the need of intercontinentally understood concepts and associated intercontinentally agreed terms for Metrology in Chemistry (MiC)

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Unambiguous and consistent concepts and terms such as measurand, metrological traceability, measurement uncertainty, comparability of measurement results, target measurement uncertainty, etc. must govern the description of results of chemical measurements in order to enable a valid comparison of measurement results. That is not yet the case as numerous workshops over the last decade have shown worldwide and as chemical literature continuously displays. For international trade in food and feed to be fair, for border-crossing implementation of environmental regulations to be the same for all parties concerned, for interchangeability of results of clinical measurements to become a reality, for any border-crossing interpretation of measurement results in chemistry to become possible, well understood and mutually accepted, concepts and terms are essential. Similarly, their translations from one language, English, to 30-40 other languages must be realized and fixed unequivocally. Countries using English as common language have not yet fully realized that they are at a considerable advantage over countries where such translated terms describing concepts may not yet be available, let alone understood and accepted. These countries are therefore at a considerable disadvantage in all cases where chemical measurements are involved. A number of ambiguities in the definitions and terms are described, which illustrate the importance of the ongoing revision (1997-2006) of the International Vocabulary of Basic and General Terms in Metrology (VIM), especially since chemical measurement will enter the VIM for the first time in history:

- 'measurand', 'measurement unit' and 'measurement scale'
- 'measurement result' 'metrological comparability'
- 'metrological traceability'
- (incl 'to the SI'),
- 'measurement uncertainty", 'target measurement uncertainty'

It is concluded that the ongoing revision of the VIM is of primordial importance for good understanding within and between the measurement communities worldwide.

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S1-2

Harmonizacija medicinsko biokemijskih pretraga u Hrvatskoj

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Osiguranje točnosti i usporedivosti rezultata laboratorijskih pretraga preduvjet je za definiranje jedinstvenih referentnih intervala ili granica odluke i njihovu primjenu na različitim lokacijama ili u različitom vremenu. Ostvarenje usporedivosti rezultata laboratorijskih pretraga omogućilo bi sveopću primjenu kliničkih smjernica temeljenih na dokazima te bi značajno doprinjelo poboljšanju kvalitete zdravstvene skrbi. Loša usporedivost rezultata laboratorijskih pretraga nastaje zbog razlika u analitičkim metodama i njihovim aplikacijama na različite instrumente ili zbog razlika u kalibracijskim postupcima i kalibratorima. Osobito se to odnosi na analite koji su heterogeni u humanim uzorcima i koji se obično mjere nekom od imunokemijskih tehnika. Posljednjih su godina pokrenuti mnogi nacionalni i međunarodni projekti standardizacije i harmonizacije rezultata laboratorijskih pretraga. Najveći utjecaj na to imalo je donošenje Europske direktive za dijagnostička medicinska sredstva *in vitro* (IVD 98/79/EC) i međunarodnih norma ISO 17511 i ISO 18153 za njenu provedbu te slijedom toga prihvaćanje koncepta mjeriteljske sljedivosti u u laboratorijskoj medicini. Sljedljivost rezultata laboratorijskih pretraga prema međunarodno priznatim i prihvaćenim referentnim materijalima i mjernim postupcima smatra se ključnim elementom u osiguranju točnosti i usporedivosti rezultata laboratorijskih pretraga. HKMB je krajem 2003. godine pokrenula nacionalni projekt harmonizacije laboratorijskih pretraga iz područja medicinske biokemije, analitičke toksikologije i laboratorijske imunologije. U njegovoj provedbi sudjeluju predstavnici referentnih centara Ministarstva zdravstva i socijalne skrbi za određena područja laboratorijske medicine i Radne skupine za izradu preporučenih metoda HKMB. Prvim dijelom projekta bile su obuhvaćene opće medicinsko-biokemijske pretrage.

U svrhu njihove harmonizacije preporučene su analitičke metode i ciljevi analitičke kontrole kao osnovni preduvjet za primjenu jedinstvenih referentnih intervala i preporučenih vrijednosti. Dokument "Harmonizacija laboratorijskih nalaza u području opće medicinske biokemije" objavljen je na www.hkmb.hr i u priručniku "Organizacija i upravljanje u medicinskom laboratoriju", Zagreb: Medicinska naklada i HKMB, 2004. U tijeku je drugi dio projek-

S1-2

Harmonization of medical biochemistry tests in Croatia

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Assurance of accuracy and comparability of laboratory test results is a precondition for definition of common reference intervals or decision limits and their application at various sites or time. Implementation of comparability of laboratory test results would enable general application of evidence-based clinical guidelines and contribute substantially to the improved quality of healthcare. Poor comparability of laboratory test results is due to differences in analytical methods and their applications on various instruments, or to differences in calibration procedures and calibrators. This particularly refers to analytes that are heterogeneous in human samples and are usually measured by some immunochemistry technique. During the past several years, many national and international projects have been launched on standardization and harmonization of laboratory test results. This mostly occurred due to the issuance of the European Directive on *In Vitro* Diagnostic Medical Devices (IVD 98/79/EC) and international standards ISO 17511 and ISO 18153 regulating its implementation, and consequently the acceptance of the concept of measuring traceability in laboratory medicine. Traceability of laboratory test results is, according to the internationally recognized and accepted reference materials and measuring procedures, considered the key element in ensuring accuracy and comparability of laboratory test results. At the end of 2003, the Croatian Chamber of Medical Biochemists (CCMB) initiated a national project of harmonization of laboratory tests in the fields of medical biochemistry, analytical toxicology, and laboratory immunology. Representatives of reference centers of the Croatian Ministry of Health and Social Welfare participate in its implementation together with members of the CCMB Working Group for Elaboration of Recommended Methods. The first part of the project comprised general medical biochemistry tests. For the purpose of their harmonization, analytical methods and analytical control targets have been recommended as an essential prerequisite for the application of commnon reference intervals and recommended values. A document entitled Harmonization of Laboratory Results in General Medical Biochemistry has been published on www.hkmb.hr and in the manual Organization and Management in

ta kojim su obuhvaćene specijalističke i visokodiferentne pretrage.

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Medical Laboratory, published by Medicinska naklada and CCMB, Zagreb, 2004. Currently, the second part of the project is under way, encompassing specialized and highly specialized laboratory tests.

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S1-3

Mjeriteljska sljedljivost i mjerna nesigurnost – mjeriteljski pogled na rezultat

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Mjeriteljstvo je znanost o mjerenjima te obuhvaća sva područja znanosti i tehnologija, pa tako i područje zdravlja ljudi. Suvremeno društvo pod pritiskom globalizacije traži točne, pouzdane, ali prije svega usporedive rezultate. Važan alat za dokazivanje usporedivosti je osiguravanje sljedljivosti rezultata, odnosno dokazano i kvantitativno povezivanje mjernih rezultata s SI jedinicom u kojoj je rezultat iskazan ili s međunarodno utvrđenom referencom. Posljednjih deset godina postignuti su značajni uspjesi na međunarodnom planu u uspostavi sljedljivosti na području mjeriteljstva u kemiji, uključujući mjerenja u kliničkoj kemiji. Napravljena je baza podataka pouzdanih referentnih materijala višeg reda te mjernih postupaka pod okriljem Dogovora o metru i sporazuma o međusobnom priznavanju (CIPM MRA), te popis potencijalnih referentnih laboratorija. Na nižim razinama točnosti temeljni je preduvjet osiguravanja sljedljivosti nabavka referetnih materijala dokazane sljedljivosti te s iskazom mjerne nesigurnosti. Mjerna nesigurnost drugo je temeljno svojstvo rezultata, a označava raspon vrijednosti unutar kojega se nalazi mjerena veličina. Međunarodne norme ISO/IEC 17025 i ISO 15189 zahtijevaju da laboratoriji procjenjuju mjerne nesigurnosti svojih rezultata. Procjena mjerne nesigurnosti zahtijeva detaljno poznavanje mjernoga procesa, uočavanje svih izvora pogrešaka te njihovo kvantificiranje. Podaci potrebni za procjenu dobivaju se iz mjera kontrole kvalitete rezultata (npr. kvantitativnih podataka za kratkoročnu i dugoročnu varijabilnost te sustavnu pogrešku), iz validacijskih podataka, kataloških podataka o opremi (npr. vaga, volumetrijska mjerila), iz certifikata (npr. nesigurnost kalibratora), ali i iz svih drugih izvora koji nam te informacije mogu dati. Laboratorij je odgovoran osigurati prikladnost mjerne nesigurnosti koju ostvaruje u svojim mjernim procesima. Prevelika mjerna nesigurnost dovodi u pitanje mogućnost donošenja ispravne odluke

S1-3

Metrological traceability and measurement uncertainty – metrological view of the result

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Metrology is a field of knowledge concerned with measurement, which covers all fields of science and technologies including the field of human health. The contemporary society, pressured by globalization, requires accurate and reliable but above all comparable results. An important tool to achieve comparability is ensurance of result traceability. It means proved and quantitative relating of the measurement results to either the respective SI unit in which the measurement result is given, or to the internationally defined reference. In the last ten years great success has been achieved at the international level in the establishment of traceability in the field of metrology in chemistry, including measurements in clinical chemistry. A database of the reliable reference material of a higher order and a database of the reference measurement procedures have been established under the auspices of the CIPM MRA (Mutual Recognition Agreement), along with a list of potential reference laboratories. At lower levels of accuracy, the basic prerequisite for providing traceability is procurement of the reference material on proved traceability and with the measurement uncertainty statement. Measurement uncertainty is the second basic property of results. It denotes the span of values which the measurand value falls within. The ISO/IEC 17025 and ISO 15189 international standards require from laboratories to evaluate the measurement uncertainties of their results. This means thorough knowledge of the measuring process, recognition of all error sources, and their quantification. Data required for the evaluation are received from the quality control results (e.g., quantitative data for short- and long-term variability and bias), validation data, catalog data on equipment (e.g., balance, volumetric apparatus), certificates (e.g., uncertainty of calibrator), and from all other sources of this type of information. Laboratory is supposed to ensure the

koja se temelji na tim rezultatima, npr. postavljanje točne dijagnoze ili procjene učinkovitosti liječenja. Mjerna nesigurnost znatno manja od očekivane može pak ukazivati na mogući problem učinkovitosti mjernoga procesa.

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adequacy of measurement uncertainty which is carried out in their measuring processes. An excessive measurement uncertainty calls into question the possibility of making the right decision based on these results (it refers to making a precise diagnosis or estimating therapeutic efficacy). Measurement uncertainty which is considerably lower than expected might indicate a potential problem in the efficiency of the measuring process.

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S1-4

Mjerna nesigurnost u EQA

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U primjeni je nekoliko vrsta ciljnih vrijednosti, ovisno o tipu EQA: vrijednosti referentne metode (RMV), usuglašene vrijednosti za koje postoji opći konsenzus, usuglašene vrijednosti za koje postoji opći konsenzus ovisno o metodi, te konsenzus ekspertnih laboratorija.

1. RMV kao ciljna vrijednost ISO 17025 je standard za ispitne i kalibracijske laboratorije. Suprotno tome, ISO 15189 odnosi se samo na pretrage u medicinskim laboratorijima. Zahtjevi za referentne mjerne laboratorije u kliničko laboratorijskoj medicini navode se u standardu ISO 15195.

Točka 5.6 govori kako svaki izdani mjerni rezultat treba biti praćen izjavom o nesigurnosti koja je procijenjena i izražena prema GUM. Izvješće o potvrdi takvog materijala sadrži referentnu mjernu vrijednost, izjavu o sljedljivosti potvrđene vrijenosti i proširenoj nesigurnosti potvrđene vrijednosti, njezinu razinu vjerodostojnosti te primijenjeni čimbenik pokrivenog raspona.

2. Usuglašena vrijednost ekspertnih laboratorija kao ciljna vrijednost. Kako ekspertni laboratoriji ne izvještavaju o standardnim nesigurnostima i ne podliježu neovisnom vrednovanju (npr. od strane tijela za akreditaciju laboratorija), standardna nesigurnost pripisane vrijednosti procijeniti će se na isti način kao za usuglašenu vrijednost dobivenu od sudionika.
3. Usuglašena vrijednost dobivena od sudionika. Standard ISO 13528 (statističke metode za primjenu kod ispitivanja stručnosti usporedbom među laboratorijima) daje pristup za izračunavanje standardne nesigurnosti na osnovi grubog prosjeka rezultata što ih objave sudionici. Ograničenja ovoga pristupa su to što: a) stvarni konsenzus među sudionicima može izostati i b) konsenzus može biti pristran uslijed opće primjene

S1-4

Measurement uncertainty in EQA

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Depending on the EQA scheme design, several types of target values are used: reference method values (RMV), overall consensus values, method dependent consensus values, and consensus of expert laboratories.

1. RMV as target value ISO 17025 is a standard for testing and calibration laboratories. On the contrary, ISO 15189 only addresses testing in medical laboratories. The requirements for reference measurement laboratories in clinical laboratory medicine are given in the ISO 15195 standard.

Clause 5.6 states that each reported measurement result shall be accompanied by an uncertainty statement estimated and expressed according to GUM. The report of certificate of such a material will contain the reference measurement value, a statement on the traceability of the certified value and the expanded uncertainty of the certified value, its level of confidence, and coverage factor used.

2. Consensus value of expert laboratories as target value

   As expert laboratories normally do not report standard uncertainties and are not validated independently (e.g., by a laboratory accreditation body), the standard uncertainty of the assigned value shall be estimated in the same way as for the consensus value from participants.

3. Consensus value from participants

   The ISO 13528 standard (statistical methods for use in proficiency testing by inter-laboratory comparisons) gives an approach for the calculation of the standard uncertainty based on the robust average of the results reported by the participants. The limitations of this approach are that: (a) there may be no real consensus amongst participants; and (b) the consensus

krive metodologije i ta se pristranost neće odraziti na standardnu nesigurnost.

U EQA se granice prihvaćanja obično izražavaju kao SD ili kao utvrđene granice oko ciljne vrijednosti. Ako je nesigurnost mala (RMV, velika skupina sudionika), tada su praktične posljedice EQA male. Granice prihvaćanja neće se značajno promijeniti uzme li se u obzir nesigurnost ciljne vrijednosti. Međutim, u malim skupinama nesigurnost ciljne vrijednosti može biti važna i treba ju dodati granicama prihvaćanja. Ubuduće će biti važno da organizacije EQA pridaju pozornost mjernoj nesigurnosti i primijene ju kadgod se to pokaže znakovitim za njihov sustav.

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may be biased by the general use of faulty methodology and this bias will not be reflected in the standard uncertainty.

Acceptance limits in EQA are usually expressed as SD or fixed limits around a target value. If the uncertainty is small (RMV, large group of participants), then the practical consequences in EQA are small. The acceptance limits will not change significantly if uncertainty of the target value is taken into account. However, in small groups, the uncertainty of the target value may be important and must be added to the acceptance limits. In the future, it will be important that EQA organizations pay attention to measurement uncertainty and apply them when relevant in their schemes.

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S2 – Simpozij 2 – TROMBOCITI 2006, S2-1

Uloga trombocita u bolesti

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Trombociti su mali fragmenti stanice koja ne sadrži jezgru i osnovna su sastavnica primarne hemostaze. Oni imaju važnu ulogu ne samo u stvaranju normalnog hemostatskog ugruška, nego i u stvaranju patološkog tromba, osobito unutar arterija gdje je područje visokog tlaka. Trombociti cirkuliraju pasivno unutar vaskulature koja je obložena endotelnim slojem. Početni korak pri ozljedi krvne žile i prekida endotelnog sloja je snažna interakcija trombocita na mjestu ozljede vaskulature ili subendotelnog područja putem specifičnih trombocitnih receptora. Nakon adhezije trombociti postaju aktivirani, mijenjaju oblik, luče sadržaj granula i agregiraju jedan na drugi pa tako stvaraju primarni hemostatski tromb, a pružaju i katalitičnu površinu za pojačanje zgrušavanja krvi. Cirkulirajući trombociti ispunjavaju mnoge kritične funkcije u održavanju hemostaze, i to adheziju na mjestu ozljede, aktivaciju koja olakšava odgovor na ozljedu, lučenje kemijskih medijatora hemostatskog odgovora, agregaciju putem vezanja fibrinogena. Trombociti imaju središnju ulogu u primarnoj, ali i u sekundarnoj hemostazi, jer pružaju fosfolipidnu površinu (kofaktor) na kojoj se odvija nekoliko ključnih koagulacijskih reakcija. Važnost trombocita u stvaranju dovoljne količine trombina i stvaranju tromba dokazna je u više modela, kao što su ispitivanja na bolesnicima s hemofilijom. Ispitivanja u kojima su se rabila antitrombocitna sredstva pokazala su kako stvaranje trombina varira izravno s funkcijom trombocita. U

S2 – Symposium 2 – PLATELETS 2006, S2-1

The role of platelets in disease

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Platelets are small anucleated cell fragments, which are essential components of primary hemostasis. They play an important role not only in the formation of a normal hemostatic plug but also in the formation of a pathologic thrombus, particularly within arteries subjected to high shear stress. Platelets circulate passively as they traverse vascular tree lined by intact endothelial cells. As an initial step, following blood vessel injury and disruption of the endothelial layer, platelets avidly interact with altered vascular surfaces or exposed subendothelial matrix *via* specific platelet receptors. Following adhesion they become activated, change shape, secrete granule contents and aggregate to each other to form a primary hemostatic plug and to provide a catalytic surface to enhance blood coagulation. Circulating platelets fulfill many critical functions in the maintenance of hemostasis, including adhesion to the sites of vascular injury, activation that amplifies the response to injury, secretion of chemical mediators of the hemostatic response, and aggregation *via* fibrinogen binding. Platelets play a central role in primary as well as in secondary hemostasis by providing a phospholipid (cofactor) surface on which several key coagulation reactions can take place. The importance of platelets for adequate thrombin generation and clot formation has been well established in a variety of model systems, including studies in patients with hemophlia. Studies utilizing antiplatelet agents demonstrate that

bolesnika s hemofilijom modulirajući učinak trombocita na stvaranje trombina je veći što je niža razina faktora, pa tako razlike između pojedinaca u funkciji trombocita mogu imati modificirajuću ulogu u kliničkom fenotipu takovih bolesnika. Trombociti mogu biti nenormalni bilo kvantitativno (trombocitopenija ili trombocitoza) ili kvalitativno. Trombocitopenija može rezultirati povećanim krvarenjem, a može biti različitog uzroka. Najčešći uzrok je imunološki posredovan ili izazvan lijekovima, ili je pak rezultat poremećaja u koštanoj srži itd. Nasljedni polimorfizmi gena trombocitnih glikoproteina mogu promijeniti njihovu antigeničnost, reguliraju razinu njihove izraženosti i moduliraju njihove funkcijske značajke pa stoga imaju važan utjecaj na izgled trombocita. Novo područje ljudske genomike je otkrivanje polimorfizama trombocitnih glikoproteina kao rizika za arterijsku trombozu. Ima dokaza kako integrin beta 3Pl A2 alela, GPIb Met 145 (VNTR A ili B) alela, a osobito integrin alfa 1 (807T) doprinose riziku od akutnog infarkta miokarda u mladih osoba i u dijabetičnoj retinopatiji. Još uvijek je potrebno klinički utvrditi je li rizik kumulativan ili sinergističan uključujući trombocitne rizike, uz već dokazane rizične koagulacijske proteine. Trombociti su također uključeni u proces upale, ali ne pasivno kao cilj upalnih medijatora oslobođenih iz leukocita, osobito faktora koji aktiviraju trombocite. Oni također imaju aktivnu ulogu u upali oslobađanjem trombocitnog faktora 4 (PF4), beta-tromboglobulina (bTG), trombocitnog faktora rasta (PDGF) i faktora koji oslobađa histamin (HRF), a koji su snažni amplifikatori aktivnosti bazofila, mastocita i neutrofila. Trombociti su uključeni u početno stvaranje ateroma, moduliranje različitih upalnih odgovora te doprinose endotelnoj disfunkciji uza svoju klasičnu ulogu u trombozi i koagulaciji.

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thrombin generation varies directly with platelet function. In hemophilia patients, the modulating effect of platelets on thrombin generation increases at lower factor levels, and interindividual differences in platelet function may play role in modifying the clinical phenotype in such patients. Platelets may be abnormal either quantitatively (thrombocytopenia or thrombocytosis) or qualitatively. Thrombocytopenia may result in increased bleeding but has many causes. The most frequent cause is immune mediated, or drug induced, as the result of bone marrow disorders, etc. Inherited polymorphisms within platelet membrane glycoprotein genes can alter their antigenicity, regulate their expression levels, and modulate their functional properties, and therefore they have a profound impact on the antigenic makeup of the platelets. The new area of human genomics is detection of platelet glycoprotein polymorphisms in the risk of arterial thrombosis. There is already substantial evidence that the integrin beta3 PlA2 allele, the GPIb Met145 (VNTR A or B) alleles and especially the integrin alfa2 allele 1 (807T) contribute to the risk of acute myocardial infarction or stroke in younger individuals and diabetic retinopathy. There is still the need of clinical risk assessment to evaluate the cumulative or synergistic effects of these platelet risk factors together with the defined coagulation protein risk factors. Platelets are also involved in inflammation but not with a passive role, as a target for inflammatory mediators released by leukocytes, in a particular platelet activating factor. They also have an active role in inflammation by the release of their own intracellular platelet factor 4 (PF4), beta-thromboglobulin (bTG), platelet-derived growth factor (PDGF) and histamine-releasing factor (HRF), which are potent amplifiers of basophil, mast cell and neutrophil activity. Platelets are involved in the initiation of atheroma, modulate various inflammation responses, and contribute to endothelial dysfunction in addition to their classic role in thrombosis and coagulation.

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S2-2

Nasljedni poremećaji funkcije trombocita

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Nasljedni poremećaji funkcije trombocita obuhvaćaju nenormalnosti 1) trombocitnih receptora za adhezivne bjelančevine, 2) trombocitne receptore za topljive agoniste, 3) putove prijenosa signala i 4) prokoagulantne fosfolipi-

S2-2

Inherited disorders of platelet function

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Inherited disorders of platelet function include abnormalities of 1) platelet receptors for adhesive proteins, 2) platelet receptors for soluble agonists, 3) signal transduction pathways, and 4) procoagulant phospholipids. Ab-

de. Nenormalnosti trombocitnih receptora za adhezivne bjelančevine uključuju nenormalnosti kompleksa GP Ib-V-IX (Bernard-Soulierov sindrom obilježen makrotrombocitopenijom i odsutnošću aglutinacije trombocita izazvanom ristocetinom, te trombocitni tip von Willebrandove bolesti, udruženih kako bi pojačali funkcijski fenotip trombocitnog GPIba, pojačanom sklonošću za vWF, što dovodi do vezanja najvećih vWF multimera za mirujuće trombocite i njihovo uklanjanje iz cirkulacije) i Glanzmannovu trombasteniju uzrokovanu oštećenjima GPIIb/IIIa koju u aktiviranim trombocitima veže adhezivne glikoproteine koji premošćuju susjedne trombocite, osiguravajući agregaciju trombocita. Ostale, manje česte nenormalnosti uključuju nenormalnosti GP Ia/IIa ili GPVI, obje obilježene selektivnom poremetnjom odgovora trombocita na kolagen. Nenormalnosti trombocitnih receptora za topljive agoniste uključuju nenormalnosti receptora tromboksana A2 te nenormalnosti receptora P2Y12 za ADP. Ovo potonje stanje obilježeno je oštećenjem trombocitne funkcije koje sliči onomu uzrokovanom prototrombocitnim lijekom klopidogrelom. Nenormalnosti trombocitnih granula uključuju nenormalnosti delta granula (deficijencija delta zaliha), nenormalnosti alfa granula (sindrom sivih trombocita i Quebec trombocitni poremećaj) te deficijenciju zaliha alfa i delta granula. Pojam 'primarni nedostatak sekrecije' obuhvaća sve one nepotpuno definirane nenormalnosti lučenja trombocita koje nisu udružene s deficijencijama trombocitnih granula. Širenjem našega poznavanja patofiziologije trombocita ova će se heterogena skupina koja okuplja većinu bolesnika s prirođenim poremećajima trombocitne funkcije sve više smanjivati, jer će iz nje otpadati bolesnici s bolje utvrđenim biokemijskim nenormalnostima odgovornim za njihovo oštećenje trombocita. Nenormalnosti putova prijenosa signala obuhvaćaju nenormalnosti puta arahidonata/tromboksana A2, nenormalnosti stimulacijske G-protein alfa podjedinice i druga oštećenja koja još nisu tako dobro opisana. Scottov sindrom je rijetka bolest krvarenja udružena s održavanje asimetrije lipidnog dvosloja u membranama krvnih stanica, uključujući trombocite, što dovodi do smanjenog stvaranja trombina. Teške ispade krvarenja treba liječiti transfuzijama trombocita, što se vidi kod bolesnika s BSS ili Glanzmannovom trombastenijom. Rekombinantni Faktor VIIa je dobra no prilično skupa alternativa transfuzijama trombocita kod refraktornih bolesnika. U svim drugim okolnostima treba primijeniti antifibrinolitike kao što je traneksemična kiselina ili analog vazopresina, dezmopresin.

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normalities of the platelet receptors for adhesive proteins include abnormalities of the GP Ib-V-IX complex (Bernard-Soulier syndrome, characterized by macrothrombocytopenia and absence of platelet agglutination induced by ristocetin, and platelet-type von Willebrand's disease, associated to gain of function phenotype of the platelet GPIba, increased avidity for vWF, leading to the binding of the largest vWF multimers to resting platelets and their clearance from the circulation) and Glanzmann's thrombasthenia caused by defects of GPIIb/IIIa, which in activated platelets binds adhesive glycoproteins that bridge adjacent platelets, securing platelet aggregation. Other, less frequent abnormalities include abnormalities of GP Ia/IIa or GPVI, both characterized by selective impairment of platelet responses to collagen. Abnormalities of the platelet receptors for soluble agonists include abnormalities of thromboxane A2 receptor and abnormalities of the P2Y12 receptor for ADP. The last condition is characterized by a platelet function defect that resembles that caused by the antiplatelet agent clopidogrel. Abnormalities of platelet granules include abnormalities of delta-granules (delta-storage pool deficiency), abnormalities of alpha-granules (gray platelet syndrome and Quebec platelet disorder), and alpha- and delta- granules storage pool deficiency. The term 'primary secretion defect' indicates all those ill-defined abnormalities of platelet secretion not associated with platelet granule deficiencies. With the progression of our knowledge in the platelet pathophysiology, this heterogeneous group, which lumps together the majority of patients with congenital disorders of platelet function, will become progressively thinner, losing those patients with better defined biochemical abnormalities responsible for their platelet defect. Abnormalities of the signal-transduction pathways include abnormalities of the arachidonate/thromboxane A2 pathway, of the stimulatory G-protein alpha-subunit and other defects, less well characterized. The Scott syndrome is a rare bleeding disorder associated with maintenance of the asymmetry of the lipid bilayer in the membranes of blood cells, including platelets, leading to reduced thrombin generation. Platelet transfusions should be used in severe bleeding episodes, which may be seen in patients with BSS or Glanzmann thrombasthenia. Recombinant Factor VIIa is a good, albeit rather expensive alternative to platelet transfusions in refractory patients. Anti-fibrinolytic agents, such as tranexamic acid, or the vasopressin analog desmopressin should be used in all other circumstances.

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S2-3

Testovi ispitivanja funkcije trombocita

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Trombociti (Trc) sudjeluju u održavanje normalne hemostaze adhezijom na mjestu ozljede krvne žile lučenjem sadržaja iz granula, agregacijom i stvaranjem primarnoga hemostatskog ugruška. Za njeno održavanje je, uz normalan broj Trc, neophodna i njihova normalna funkcija. U prošlosti se ispitivanje funkcije Trc rabilo isključivo za mjerenje i praćenje sposobnosti Trc u adheziji i agregaciji. Vrijeme krvarenja kojim se mjeri adhezija Trc *in vivo* dugi je niz godina bio jedini test za ispitivanje funkcije Trc. Uvođenje optičke agregacije Trc 1962. godine uvelike je doprinjelo boljoj dijagnostici funkcije Trc i ta je metoda ubrzo postala "zlatni standard" kao i najčešća metoda koja se rabi za ispitivanje funkcije Trc dosad. Agregacija Trc omogućuje ispitivanje međudjelovanja između receptora i liganda neophodnih za održavanje hemostaze *in vivo* mjerenjem učinka agonista na aktivaciju Trc *in vitro* i međusobno povezivanje Trc. Unatoč tome, metoda ne može u potpunosti oponašati sve aspekte normalne funkcije Trc. Uporaba lumiagregometra je još više poboljšala ispitivanje funkcije Trc, jer uz mjerenje agregacije Trc omogućuje i istodobno praćenje lučenje sadržaja iz gustih granula. U nekoliko posljednjih godina razvijeno je nekoliko novih testnih sustava za ispitivanje funkcije Trc u punoj krvi kako bi što bolje *in vitro* imitirali procese koji se događaju nakon ozljede krvne žile *in vivo*, a poglavito radi ispitivanja aktivacije Trc u fiziološkim uvjetima brzine protoka krvi u arterijama. To su: PFA 100, Impact Cone i Platelet Analyzer, sustav VerifyNow, Plateletworks, Hemostasis Analysis System, modifikacije tromboelastografije, kao i različiti testovi protočne citometrije. Tradicionalno se ispitivanje funkcije Trc najčešće rabilo za ispitivanje uzroka krvarenja. Kako Trc imaju značajnu ulogu u hemostazi i trombozi, sve je veće zanimanje za ispitivanje funkcije Trc radi praćenja učinka suvremene antiagregacijske terapije te mogućnost utvrđivanja hiperaktivnosti Trc kao mogućeg pretkazatelja sklonosti trombozi. Iako su danas dostupne različite laboratorijske metode, ne postoji niti jedan test kojim je moguće otkriti sve poremećaje funkcije Trc. Svaki od ovih testova mjeri različite aspekte funkcije Trc pa je stoga jedini mogući pristup uporaba kombinacije testova odgovarajućih za svaki pojedini slučaj.

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S2-3

Platelet function tests

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Both normal platelet number and function are essential for maintaining normal hemostasis through adhesion to the site of injury, secretion of granule contents, aggregation and participation in the primary hemostatic plug formation. In the past, platelet function tests were used to measure and monitor the platelet ability to adhere and aggregate. For many years, the bleeding time that enables measurement of platelet adhesion *in vivo* was the only available test of platelet function. The introduction of light transmission aggregation (LTA) in 1962 has greatly improved the ability to test platelet function accurately. It has become the "gold standard" and by far the most common method of assessing platelet function. Although platelet aggregation studies evaluate some important receptor-ligand interactions required for hemostasis *in vivo* by measuring the ability of the agonist to cause *in vitro* platelet activation and platelet-platelet binding, LTA does not mimic accurately all aspects of normal platelet function. The use of a lumiaggregometer has additionally improved platelet function testing since it offers the possibility to detect both platelet aggregation and dense granule secretion at the same time. In the last few years a number of new assay systems have been developed in an attempt to mimic or stimulate the processes that occur during vessel wall damage, particularly to study shear-induced platelet activation: PFA-100, Impact Cone and Platelet Analyzer, VerifyNow system, Plateletworks, Hemostasis Analysis System, modifications of thromboelastography technology (e.g., platelet mapping system) and various flow cytometric tests. The main use of platelet function tests has been traditionally to determine the cause of abnormal bleeding. However, as platelets play a key role in both hemostasis and thrombosis, it is becoming increasingly important to monitor the efficacy of modern antiplatelet therapy and to identify platelet hyperactivity as a possible predictor of thrombotic tendency. Nowadays, although multiple laboratory procedures can be used, there is no single test that can identify all potential defects of platelet function. Each of these tests measures different aspects of platelet function and the only possible approach is to use a combination of tests that are suitable for particular circumstances.

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S2-4

Evaluacija trombocita protočnom citometrijom

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Osobite biološke značajke i mnogobrojne funkcije trombocita čine te stanice zanimljivima, ali i vrlo složenima za istraživanje. Stoga analiza trombocita zahtijeva niz specifičnih testova koji trebaju biti standardizirani za svaku od sastavnica ili funkcija trombocita. Tijekom zadnja dva desetljeća protočna je citometrija postala jedno od najvažnijih analitičkih oruđa u istraživanjima trombocita. To je jedina tehnika koja omogućuje određivanje i kvantifikaciju različitih strukturnih i funkcijskih značajka trombocita, uključujući strukturne i specifične aktivacijske biljege, kao i interakciju trombocita s drugim tipovima stanicama. Protočna je citometrija stoga omogućila bolje razumijevanje patologije trombocita u različitim patološkim stanjima i bolestima. Postoje brojne studije o primjeni protočne citometrije trombocita u medicinskom laboratoriju, bilo kao uspostavljene ili kao potencijalne dijagnostičke metode. Najviše spominjani testovi u tom smislu uključuju slijedeće: a) dijagnostiku specifičnih poremećaja trombocita (kao što je urođeni manjak površinskih biljega i bolest nakupljanja); b) kvantifikaciju IgG vezanog za trombocite u cilju razlikovanja autoimune trombocitopenije od drugih stanja i za otkrivanje aloimunizacije; c) mjerenje aktivacije trombocita, uključujući određivanje aktiviranih trombocita u cirkulaciji, kao i određivanje hiper- i hiporeaktivnosti trombocita; d) praćenje učinka anti-trombocitnih spojeva; e) određivanje retikuliranih trombocita s ciljem praćenja trombopoeze; i f) primjenu u bankama krvi. Treba, međutim, istaknuti da unatoč svoje svestranosti, protočna citometrija trombocita (a osobito funkcijska procjena trombocita) još uvijek nije našla široku kliničku primjenu zbog poteškoća u optimizaciji i standardizaciji testova. U ovom trenutku pravi izazov predstavlja pretvorba čisto istraživačkih protočnocitometrijskih postupaka u praktične postupke koji bi u konačnici postali standardni laboratorijski testovi s klinički korisnom informacijom. Odnedavno dostupni komercijalni kitovi za protočnocitometrijsku analizu trombocita s oznakom IVD (kao npr. test za praćenje specifičnih antagonista ADP-receptora i test za dijagnostiku kvantitativnih poremećaja trombocitnih glikoproteina) dobar su primjer tih nastojanja.

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S2-4

Evaluation of platelets by flow cytometry

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Distinctive biological features and manifold functions make the platelets very interesting cells but difficult to study. Therefore, the analysis of platelets requires a series of specific tests standardized for each of the platelet component and/or function. During the last two decades, flow cytometry has become one of the major analytical tools in platelet research. It is the only technique capable of detection and quantitative measurement of many different aspects of platelet structure and function, including structural and specific activation markers as well as interactions with other cells. Flow cytometry has also yielded a better understanding of platelet pathology in various pathologic conditions and diseases. There have been many studies dealing with the use of platelet flow cytometry, either as established or as contemplated (potential) applications in medical laboratory. The most cited assays include the following: (a) diagnosis of specific platelet disorders (such as inherited surface marker deficiencies and storage pool disease); (b) quantification of platelet-associated IgG in order to differentiate immune thrombocytopenia from other conditions, and for detection of alloimmunization; (c) measurement of platelet activation, including activated platelets in circulation and platelet hyper- or hyporeactivity; (d) monitoring of anti-platelet agents; (e) detection of reticulated platelets for thrombopoiesis monitoring; and (f) blood bank applications. In spite of its versatility, platelet flow cytometry (especially evaluation of platelet function) has not found widespread clinical application due to difficulties in optimization and standardization of the assays. The current challenge is to translate purely research flow cytometry assays into more practical procedures that will ultimately become standard laboratory tests with clinically useful information. Recent commercially available flow cytometry kits with IVD mark (such as the test for monitoring of specific platelet ADP receptor antagonists and the test for diagnosis of quantitative platelet glycoprotein abnormalities) are good examples of these endeavors.

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S3 – Simpozij 3 – LABORATORIJSKA MEDICINA ZASNOVANA NA DOKAZIMA, S3-1

Laboratorijska medicina zasnovana na dokazima: poboljšanje ishoda

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Poput medicine zasnovane na dokazima (EBM), laboratorijska medicina zasnovana na dokazima (EBLM) može se promatrati kao formalniji pristup laboratorijskim vidovima kliničke prakse. Rečeno je kako je EBM "znanstveno sredstvo za poboljšanje kvalitete, čak i ako njegova primjena zahtijeva kombinaciju znanstvenih činjenica s vrijednosnim prosudbama i troškovima različitih vrsta liječenja". Naglašava se kako postoje zamke u smislu razlika u vrijednosnim prosudbama kad se strogo tumači provedeno kliničko istraživanjo. Neki ukazuju na izazove što ih postavlja odmak od istraživanja prema provedbi. Također se ukazuje na to da će praksa zasnovana na dokazima potisnuti kliničku ekspertizu i primorati praktičare na pristup skrbi prema određenom protokolu. Govori se da bi se tako mogla izgubiti vještina potrebna za primjenu dostupnih dokaza u vidu pretrage ili zahvata u kontekstu okolnosti kod svakog pojedinog bolesnika. Nasuprot ovoj kritici stoji činjenica da se najbolja praksa može postići samo kroz razumijevanje snage i slabosti dokaza. Tako je prepoznat utjecaj EBM na primarnu skrb kroz razvoj smjernica i standarda kvalitete, kao i na odlučivanje o raspodjeli sredstava za intervencije, ali se isto tako pokazuje kako to ne mora uvijek biti važno, poglavito u vidu sukoba s kliničarovom obvezom pružanja skrbi i u odnosu na bolesnikovu individualnost. Stoga treba postići ravnotežu između donošenja odluka na osnovi znanstvenih podataka i vrednovanja s jedne strane, te naglašene nesigurnosti i razlike u vrijednostima. Smatra se kako je utjecaj na kirurgiju bio pozitivan, ali se ukazuje na pretjerano oslanjanje na randomizirane kliničke pokuse i na ograničenja u generaliziranju dobivenih nalaza. Također se ukazuje na implikacije što ih primjena obaviještenih odluka na osnovi EBM ima u svezi s bolničkom upravom, a kao primjer se navode programi liječenja bolesti i kliničkog slijeda postupaka kakav se rabi u njemačkom zdravstvenom sustavu. Dok su neke od ovih napomena jednako primjenjive i na laboratorijsku medicinu, može se svakako kazati da su pitanja u EBLM ipak drukčija. Međutim, osnovni ciljevi poboljšane kvalitete podataka (dijagnostička točnost) i poboljšane uporabe podataka (poboljšani ishodi) ostaju isti. Jedna od glavnih prednosti prihvaćanja koncepata i kulture EBLM je to što pomiče usredotočenost laboratorijskog stručnjaka od znanosti i tehnologije pretrage prema uporabi rezultata

S3 – Symposium 3 – EVIDENCE BASED LABORATORY MEDICINE, S3-1

Evidence-Based Laboratory Medicine: delivering improved outcomes

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Evidence-Based Laboratory Medicine (EBLM), like evidence-based medicine (EBM) can be seen as a more formal approach to the laboratory aspects of clinical practice. It has been said that EBM is "a scientific tool for quality improvement, even though its application requires the combination of scientific facts with value judgements and the costing of different treatments". It has pointed out that there are pitfalls in the variation of value judgements when interpreting rigorously conducted clinical research. Several people have pointed out the challenges of moving from research to implementation. It has also been suggested that evidence based practice will stifle clinical expertise and force practitioners into a protocol driven approach to care. It has been suggested that may take away the skill required to place the evidence available on the use of a test or procedure into the context of the individual patient's circumstance. The opposing view to this criticism is the fact that only through understanding of the strengths and weaknesses of the evidence will best practice be achieved. Thus whilst it has been recognised that EBM has had an impact in primary care through the development of guidelines and quality standards, as well as influencing the decision making on allocation of resources for interventions, it has also been suggested that it may not always be relevant, particularly with respect to conflict with the clinician's duty of care and respect for patient's individuality. Thus there is a balance to be achieved between science informed and value based decision making and stressing the merits of uncertainty and the value difference. There is a belief that the impact on surgery has been positive but suggest that there is an over-reliance on the randomised controlled trial, and the limitations of generalisability of findings. It is also noted that there are implications when using EBM informed decisions in relation to hospital management, an example being the disease management programmes and clinical pathways employed in the German health care system. Whilst some of these comments are equally applicable to laboratory medicine it can also be argued that the issues in EBLM may be somewhat different. However, the basic objectives of improved quality of data (diagnostic accuracy) and improved use of data (improved outcomes) remain the same. One of the key benefits to adopting the

pretrage. To pak u osnovi mijenja odnos između kupca i pružatelja laboratorijskih medicinskih usluga.

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concepts and culture of EBLM is that it shifts the focus of the laboratory professional away from the science and technology of the test toward the utility of the test result. This in its turn fundamentally changes the relationship between the purchaser and the provider of laboratory medicine services.

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S3-2

Racionalna dijagnostika u koagulaciji

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Hemofilija – U slučaju problema s krvarenjem bazične rutinske parametre kao što su aktivirano parcijalno tromboplastinsko vrijeme (APTV), protrombinsko vrijeme (PV), fibrinogen i broj trombocita treba izvesti kao prvi korak, zajedno s temeljitom anamnezom. Aktivnost određenog pojedinačnog faktora treba mjeriti tek onda kad odgovarajući bazični parametar pokaže patološki rezultat. Ako je potrebno, valja utvrditi inhibirajuća protutijela. Sumnja li se na disfunkciju trombocita, tada funkciju trombocita treba ispitati primjenom kombinacije kolagena, epinefrina i ADP kao stimulansa. U hitnim slučajevima može se primijeniti rotacijska tromboelastografija koja omogućava analizu stvaranja ugrušaka (stvaranje fibrina) u stvarnom vremenu, kako bi se brzo dobio pregled bolesnikova stanja koagulacije.
Trombofilija – Probir na trombofiliju preporuča se u slučaju juvenilne tromboze (<45 god), ponavljajuće tromboze, obiteljske trombofilije i opetovanog gubitka fetusa. I dalje se raspravlja o tome je li takav probir koristan prije primjene oralnih kontraceptivnih sredstava. Uz rutinske parametre APTV, PV, fibrinogen i ATIII dijagnostika trombofilije treba obuhvatiti mjerenje aktivnosti FVIII, FIX, FXI i FXII, kao i određivanje razine homocisteina u krvi. Štoviše, treba motriti put proteina C pomoću odgovarajućih testova koji predstavljaju vrlo osjetljivo sredstvo za probir sustava proteina C. Rezistenciju APC, Prot C i Prot S treba mjeriti samo u pozitivnim uzorcima radi identificiranja i potvrde deficijencije. Na raspolaganju su dvije vrste testova za otkrivanje antifosfolipidnih protutijela: lupus antikoagulantni (LA) testovi, gdje se sposobnost plazme za *in vitro* inhibiciju stvaranja ugrušaka testira tzv. LA protutijelima: Dilute Russell vrijeme zmijskog otrova, vrijeme zgrušavanja kaolina, test tkivne inhibicije tromboplastina; i specifični ELISA za otkrivanje antifosfolipidnih protutije-

S3-2

Rational diagnosis in coagulation

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Hemophilia – In case of bleeding problems, basic routine parameters such as activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen and platelet count have to be performed as the first step together with profound history. A particular single factor activity should only be measured when the corresponding basic parameter shows a pathological result. If necessary, inhibiting antibodies have to be identified. If platelet dysfunction is suspected, platelet function should be tested using combinations of collagen, epinephrine and ADP as a stimulant. In emergency cases, rotational thromboelastography allowing for real-time analysis of clot formation (fibrin formation) can be used to get a quick overview of the patient's coagulation situation.
Thrombophilia – Screening for thrombophilia is recommended in case of juvenile thrombosis (<45 a yrs), recurrent thrombosis, familial thrombophilia and recurrent fetal loss. Whether such a screening is useful before the use of oral contraceptives remains under discussion. Besides the routine parameters of aPTT, PT, fibrinogen and ATIII, the diagnosis of thrombophilia should include the measurement of FVIII, FIX, FXI and FXII activities as well as determination of homocysteine levels in the blood. Moreover, the protein C pathway should be screened using appropriate test kits, which are very sensitive tools for screening the protein C system. APC-resistance, Prot C and Prot S should only be measured in positive samples for identification and confirmation of the deficiency. Two types of tests are available for the detection of antiphospholipid antibodies: lupus anticoagulant (LA) tests, in which the capacity of plasma for *in vitro* inhibition of clot formation is tested by the so-called LA antibodies: Dilute Russell viper venom time, kaolin clotting time, tissue thromboplastin inhibition test; and specific ELISA

la. Za identificiranje antifosfolipidnih protutijela prednost treba dati specifičnom ELISA pred često prilično slabo definiranim antikoagulantnim testovima. Na raspolaganju su odgovarajući testovi ELISA za probir i identifikaciju. U osoba koje boluju od autoimunih bolesti bez tromboembolijskih ispada u anamnezi probir treba provesti samo na prisutnost antifosfolipidnih protutijela. Kako ova protutijela mogu utrošiti supstrat za faktore koagulacije, mogu tako dovesti do produženja testa, poglavito APTV. Stoga u slučaju neobjašnjivog produženja APTV treba u obzir uzeti antifosfolipidna protutijela. Ukratko, valja naglasiti kako se danas bolesti hiperkoagubilnosti mogu ispravno dijagnosticirati odgovarajućim laboratorijskim pretragama u otprilike 80% osoba koje boluju od tromboembolije.

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to detect antiphospholipid antibodies. For identification of antiphospholipid antibodies specific ELISA should be preferentially used instead of the often rather poorly defined anticoagulant tests. Appropriate ELISA for screening and identification are available. Patients suffering from autoimmune diseases without thromboembolic events in their history should only be screened for the presence of antiphospholipid antibodies. Since these antibodies may consume the substrate for coagulation factors, they can lead to prolongation, especially of aPTT. Therefore, antiphospholipid antibodies should be taken into consideration in case of an unexplainable prolongation of aPTT. In summary, it should be stressed that currently, hypercoagulability disorders can be correctly diagnosed in approximately 80% of patients suffering from thromboembolism using appropriate laboratory tests.

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S3-3

Mjere dijagnostičke točnosti: značenje i primjenjivost u praksi

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Kvantitativni pokazatelji, tj. mjere dijagnostičke točnosti su dijagnostička osjetljivost i specifičnost, pozitivne i negativne prediktivne vrijednosti, omjer vjerojatnosti (engl. *likelihood ratio*), površina ispod krivulje ROC (engl. *area under the curve*, AUC), Youdenov indeks i dijagnostički omjer izgleda (engl. *diagnostic odds ratio*, DOR). S obzirom na njihovo značenje i primjenjivost mogu se podijeliti u dvije skupine: mjere diskriminacije i mjere predikcije. Mjere diskriminacije daju uvid u sposobnost nekog dijagnostičkog postupka da razluči između dva stanja (osobe s bolešću i bez nje), dok mjere predikcije rabimo kako bismo utvrdili vjerojatnost da u neke osobe možemo postaviti/isključiti dijagnozu na temelju rezultata laboratorijskog postupka. Mjere dijagnostičke točnosti često se tumače kao fiksna obilježja nekog dijagnostičkog postupka, dobivena kao rezultat istraživanja i primjenjiva u svakodnevnoj praksi. No, čak i kad je dijagnostička točnost ispitana istraživanjem provedenim u skladu s postojećim preporukama (smjernice STARD), treba znati da kvantitativni pokazatelji dijagnostičke točnosti značajno variraju u ovisnosti o relevantnom kliničkom kontekstu. Mnogi su uzroci varijabilnosti rezultata analize dijagnostičke točnosti: način

S3-3

Measures of diagnostic accuracy: interpretation and transferability into practice

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Quantitative measures of diagnostic accuracy are sensitivity, specificity, positive and negative predictive values, likelihood ratio, area under the curve (AUC), Youden index and diagnostic odds ratio (DOR). Given their interpretation and applicability, the measures of diagnostic accuracy fall into two categories: measures of discrimination and measures of prediction. Measures of discrimination assess the power of the test to discriminate between individuals with and without target condition, while measures of prediction are used to estimate the probabilities of target condition (health/disease) in individuals who have a particular test result (negative/positive). Measures of diagnostic accuracy are often interpreted and thought of as fixed attributes of a certain diagnostic procedure, obtained through the diagnostic accuracy study and applicable in routine clinical practice. However, even when such studies are designed to meet all of the methodological criteria (STARD statement), it should be stated that the measures of diagnostic accuracy may significantly vary depending on the relevant clinical context. There are many sources of this variability: exclusion/inclusion criteria, test characteristics and threshold used, the ratio

postavljanja/isključivanja dijagnoze, značajke samog dijagnostičkog postupka, brojčani odnos ispitivanih skupina (bolesnika i zdravih ispitanika) itd. Ovo predavanje daje pregled standardnih i nekih manje poznatih mjera dijagnostičke točnosti s naglaskom na njihovo tumačenje i primjenjivost u praksi.

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of individuals with/without target condition (health/disease), etc. This lecture reviews some standard and some les well known measures of diagnostic accuracy with special emphasis on their interpretation and applicability in routine practice.

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S3-4

Uvođenje EBLM u svakodnevni rad

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S3-4

Introducing EBLM to practice

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"U izobilju znanstvenih dokaza, praksa i dalje gladuje" (1), citat je koji ponajbolje opisuje stvarno stanje praktične primjene saznanja i načela laboratorijske medicine temeljene na dokazima (engl. *evidence based laboratory medicine*, EBLM). Među svim granama medicine upravo laboratorijska medicina posebno je podložna svim raspoloživim pogreškama u smislu prevelike, premale ili sasvim neprimjerene primjene. Razlog tome je s jedne strane nedovoljna obaviještenost i izobrazba kliničara uz poslovično slabu komunikaciju s laboratorijem, a s druge strane nepostojanje bilo kakvih administrativnih ograničenja opsega i učestalosti traženja laboratorijskih pretraga. Smjernice koje predstavljaju krajnji domet EBLM rijetko se spontano primjenjuju u svakodnevnom radu. Kako uvesti načela medicine temeljene na dokazima ili, kraće, kako upotrebu laboratorija učiniti racionalnom? Nažalost, jednoznačan odgovor ne postoji, ali brojne studije ukazuju na različitu razinu učinkovitosti pojedinih intervencija. Najmanji utjecaj zabilježen je za predavanja, tečajeve i jednosmjernu pismenu prepisku na relaciji laboratorij-kliničko osoblje, a bez mehanizma kasnije provjere i rasprave o učinku. Bolji rezultati opaženi su kod višekratne osobne komunikacije i redovitih audita upotrebe laboratorija. Pritom značajnu ulogu ima javno iznošenje usporedbe broja traženih laboratorijskih pretraga među pojedinim kliničarima iste struke. Zanimljiva pojava, poznatija kao Hawthorneov učinak, nerijetko se javlja kod većine projekata koji pokušavaju promijeniti ponašanje kliničara prema traženju laboratorijskih pretraga – učinak projekta vidljiv je onoliko dugo koliko postoji svijest o postojanju projekta i mogućim posljedicama. Jedan od učinkovitijih pristupa problemu u tercijarnim ustanovama svakako bi bilo osnivanje povjerenstva za uporabu dijagnostičkih resursa, u koje se moraju jednako uključiti eminentni kliničari i laboratorij-

"Practice famine amidst the evidence glut" (1); this citation quite accurately describes the current state of affairs concerning the introduction of latest laboratory medicine research evidence into practice. Among medical specialties, laboratory medicine is particularly prone to all kinds of incorrect approaches such as overuse, underuse and misuse. The reasons for this situation are multifactorial, including insufficient clinicians' education in laboratory medicine, the lack of proper communication between the ward and the laboratory as well as the lack of any administrative barriers linked to the extent and frequency of laboratory test utilization. Guidelines, the final fruit of evidence based medicine endeavors, seldom actually make a breakthrough into everyday life. So, what are the means of introducing EBLM into practice? There is no single answer to this question, but numerous studies have shown that various types of interventions tend to produce variable impact, meaning that some interventions are more effective than the others. The least effective seem to be courses, lectures and one-sided written communication which laboratory professionals send to wards, without any feedback or follow up. Better results have been recorded with regular personal communication and audits. Peer comparison among clinicians with similar case mix has also proven to be quite influential. An interesting phenomenon, known as Hawthorne's effect, has been shown in many studies – the effect of intervention lasts only as long as the physicans are aware of the project going on, or in other words, that the test ordering is being monitored. One of promising approaches within hospitals and similar institutions would certainly be to establish a diagnostic resource utilization committee, which should consist of eminent physicians and laboratory professionals. Also, good results have been recorded when

ski stručnjaci. Također, povoljni rezultati dobivaju se unošenjem promjena na uputnicama – one pretrage koje se ne nalaze na uputnici i potrebno ih je tražiti ispisivanjem imena pretrage redovito se traže daleko rjeđe. Ako postoji sustav elektronskog naručivanja pretraga, mogućnosti uvođenja načela EBLM znatno se povećavaju, npr. ispisivanjem omjera vjerojatnosti (engl. *likelihood ratio*, LR) ili ograničavanjem mogućnosti učestalog traženja pojednih pretraga. U zaključku, sve dosad provedene studije slažu se u jednom – ne postoji magična formula, ali upornim djelovanjem na razinama koje su se pokazale učinkovitima moguće je unijeti značajne promjene koje za sobom nose bolju kvalitetu zdravstvene skrbi i značajne uštede.

1. *Glasziou P, Haynes B. The paths from research to improved health outcomes. ACP Journal Club 2005;142:8-10.*

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changes were introduced in the request forms – the tests which were not offered on the form, and as such had to be hand written separately on the form, tended to be considerably less often requested. Electronic test requisition obviously offers more opportunities for introducing EBLM, such as writting LRs and post test probabilities or introducing barriers in the frequency of particular test ordering. In conclusion, all studies dealing with inappropriate laboratory use admit that there is no magic bullet, but by applying interventions that have proven to be most effective, changes can be introduced with benefical effect on the quality of care and cost containment.

1. *Glasziou P, Haynes B. The paths from research to improved health outcomes. ACP Journal Club 2005;142:8-10.*

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S3-5

Pravni i etički problemi u laboratorijskoj medicini

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Istraživači u medicini rutinski upotrebljavaju preostale uzorke tjelesnih tekućina i tkiva izvorno uzetih od bolesnika zbog medicinskih razloga. Zakonski problemi mogu nastati kada takvo istraživanje dovede do komercijalno vrijednih proizvoda i metoda, npr. vlasništvo nad uzorcima nakon što se oduzmu od bolesnika. Mogući su i drugi zakonski problemi, npr. trebaju li istraživači bolesniku odati takvu komercijalizaciju i moraju li s bolesnikom podijeliti mogući tako ostvareni profit. Biti će govora o iskustvu s takovim pitanjima u Sjedinjenim Državama. Bolesnikove "privatne" medicinske zapise uključujući rezultate kliničko laboratorijskih pretraga mogu dobiti osiguravajuća društva i poslodavci koji plaćaju stanovito zdravstveno osiguranje za svoje bolesnike, a to ide na štetu bolesnika. Police osigranja i zaposlenje mogu se uskratiti onima čiji medicinski zapisi ukazuju na mogućnost budućeg pogoršanja zdravstvenog stanja. Američki zakoni kojima bi se spriječilo ovakvo zadiranje u privatnost uvelike su neučinkoviti.

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S3-5

Legal and ethical issues in laboratory medicine

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Medical researchers routinely use left-over specimens of body fluids and tissues initially taken from patients for medical reasons. Legal issues may arise when such research leads to commercially valuable products and methods, e.g., the ownership of the specimens after they are removed from the patient. Other related legal issues may also arise, e.g., whether the researchers must divulge such commercialization to the patient and must share with the patient any profits realized. The U.S. experience with these issues will be considered. A patient's "private" medical records, including the results of clinical laboratory tests, can be obtained by health care insurance companies and employers who provide health care coverage to their employees, to the disadvantage of the patient. Insurance policies and employment may be denied to those whose medical records suggest the possibility of future ill health. National laws to prevent such invasion of privacy have largely been ineffective in the U.S.

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S4 – Simpozij 4 – AUTOMATIZACIJA I NOVE TEHNOLOGIJE, S4-1

Konsolidacija i automatizacija laboratorija

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Laboratoriji u Europi suočeni su sa sve većim ekonomskim pritiskom: sve više pretraga treba provesti sa sve manjim brojem osoblja. Kliničari traže sve kraće vrijeme za pojedine pretrage, jer trebaju ubrzati donošenje kliničkih odluka i što je moguće više skratiti vrijeme čekanja za bolesnika. Uz to, treba održati ili čak poboljšati već vrlo visoku razinu analitičke kvalitete. Svim ovim zahtjevima ne može se udovoljiti konvencionalnim sredstvima, pa se stoga razvija automatizacija prijeanalitičkih i poslijeanalitičkih procesa. Val automatizacije koji je započet u Japanu zapljusnuo je Sjedinjene Države ranih devedesetih. Nekad vrlo skupe, automatizirane sustave danas si mogu priuštiti čak i laboratoriji srednje veličine. Slijedom toga, velik broj europskih laboratorija započeo je s optimiranjem radnog procesa i uvođenjem automatiziranih sustava. U ovom prikazu daje se pregled najvažnijih pitanja u svezi s pripremom automatizacije, kao što su analiza radnog procesa, optimiranje procesa, planiranje prostornog rasporeda, konsolidacija i integracija. Objasniti će se razlika između neprekidne i automatizacije s prekidima, kao i između potpune automatizacije laboratorija i modularne automatizacije. Opisati će se neke najvažnije mogućnosti za skraćenje vremena od primitka uzorka do izdavanja nalaza, kao i za smanjenje osoblja potrebnog za pojedine radnje. Na koncu će se pojasniti neke moguće zamke na koje se može naići tijekom i nakon uvođenja automatizacije, kako bi se slušateljstvu omogućilo da izbjegne najčešće pogrješke tijekom pripreme i provedbe automatizacije.

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S4 – Symposium 4 – AUTOMATION AND NEW TECHNOLOGIES, S4-1

Laboratory consolidation and automation

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The laboratories in Europe are facing an increasing economic pressure: more tests have to be performed with less staff. Clinicians require shorter turnaround times because clinical decisions need to be expedited and patient waiting time must be minimized. Finally, a very high level of analytical quality has to be maintained or even improved. All these demands cannot be met with conventional means and therefore automation of preanalytical and postanalytical processes has been developed. A wave of automation originating from Japan has swept over to the United States in the early nineties. Once very expensive, automation systems are now affordable even for medium size laboratories. Consequently, a large number of European laboratories have started to optimize their workflow and implement automation systems. This presentation will give an overview of the most important issues concerning the preparation of automation such as workflow analysis, process optimization, floor layout planning, consolidation and integration. The difference between continuous and discontinuous automation as well as between total lab automation and modular automation will be explained. Some of the most important possibilities to decrease turnaround times and to minimize the assignment of personnel will be outlined. Finally, some possible pitfalls during and after the implementation of automation will be elucidated to allow the audience to avoid the most common mistakes during preparation and implementation of automation.

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S4-2

Razvoj i primjena modularnog informacijskog sustava u laboratorijskoj dijagnostici

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Suradnjom stručnjaka Zavoda i tvrtke Samson informatika iz Zagreba razvijen je Laboratorijski informatički sustav (LIS) koji se osniva na MS SQL bazi podataka, te informa-

S4-2

Development and application of modular information system in laboratory diagnosis

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A Laboratory Information System (LIS) has been developed by collaboration of Institute professionals and Samson informatika Co., which is based on MS SQL database

tičkom programskom paketu KLINLAB. Taj sustav je omogućio informatizaciju laboratorija, uspostavu mrežnog sustava unutar laboratorija, elektroničku vezu između administrativnog i analitičkog rada laboratorija, povezivanje laboratorijskih i kliničkih odjela za elektronički prijenos podataka te prijenos podataka u obračunsku službu bolnice. Upotrebom korisničkog imena i lozinke za svaki pristup u LIS sustav je višestruko osiguran pri kontroli upisa, rada analizatora i ispisa nalaza te pristupu i pohrani podataka.
Biološki uzorak bolesnika nakon ulaska u laboratorij dobiva naljepnicu s bar kodom, tj. zapisom koji ga povezuje s relevantnim podacima o bolesniku, kao i zahtjevom za laboratorijsku obradu. U LIS je uključen i automatizirani sustav za distribuciju uzoraka Olympus OLA 2500, koji uzorak s bar kodom distribuira na pojedina radilišta laboratorija. Na tim radilištima analize se izrađuju na analizatorima koji su također umreženi s LIS-om i nakon završetka analize rezultate spremaju u bazu podataka. Rezultati analiza koje se izrađuju ručno ili na neumreženim analizatorima ručno se upisuju u program. Završen nalaz je nakon autorizacije od strane laboratorijskog stručnjaka automatski dostupan za ispis nalaza ili za pregled na kliničkim odjelima. Veza između LIS i kliničkih odjela ostvarena je putem optičkog mrežnog sustava na razini bolnice. Na kliničkim odjelima instaliran je i dodatni informatički program nazvan Preglednik nalaza i omogućava uvid u nalaz bolesnika koji je upravo završen, kao i pregled prijašnjih nalaza istoga bolesnika. Program KLINLAB također daje uvid u poslovanje i procjenu kvalitete rada Zavoda, a zapis u bazi podataka ostaje trajan tijekom vremena predviđenog zakonom. LIS je tako pripremljen da se jednostavno može uklopiti i u bolnički informatički sustav. Uz LIS unutar bolnice, Zavod putem web stranice (www.kbsm.hr/klinkemija) i elektroničke pošte (kbsm.kzk@post.htnet.hr) ostvaruje i izravnu komunikaciju između stručnjaka Zavoda i javnosti, odgovarajući na sve pojedinačne upite.

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and KLINLAB program package. This system has enabled laboratory computerization, establishment of the network system within the laboratory, electronic connection between the administrative and analytical laboratory activities, connection of laboratory and clinical departments to the electronic data transfer, and data transfer to the Hospital accounting department. Through the usage of username and password for entering the application, LIS has been provided with multiple protection, control of data entry, analyzer performance, report printing, and data storage. On entering the laboratory, the patient's biological sample is allocated a bar code, i.e. a record that links the sample with all relevant data on the patient and test requests. LIS also includes a system of automatic sample distribution by Olympus OLA 2500, which distributes bar-coded samples to particular workplaces. At these workplaces analyzers that are included in the LIS network perform the analyses, and after the analysis has been finished, results are put to the database. Results that are obtained manually or on analyzers not included in the LIS network have to be entered manually into the program. Upon authorization by the responsible laboratory professional, the complete finding is electronically accessible for printing or for viewing by clinical departments. The connection between LIS and clinical departments is realized through the fiberoptic network system at the Hospital level. At clinical departments of the Hospital, additional software entitled Laboratory Finding Browser allows for an insight into the current patient's findings as well as in his/her previous findings. The program also offers information on the Institute business issues and performance quality assessment, the records being stored for a time period warranted by legal provisions. LIS has been so designed as to be easily incorporated in the Hospital computer system. In addition to in-house LIS, the Institute has developed direct communication between Institute professionals and the population at large, answering all individual queries by Institute's web site (www.kbsm.hr/klinkemija) and e-mail (kbsm.kzk@post.htnet.hr).

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S4-3

Biosenzori: jučer, danas i sutra

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Biosenzori su male naprave koje sadrže biološki element (enzim, antitijelo, mikroorganizame, DNA, RNA) pogodno imobiliziran na površini pretvorničkog elementa (mjerne

S4-3

Biosensors: past, present and future

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Biosensors can be defined as small compact analytical devices incoporating biologically-derived sensing element (enzyme, antibody, microorganisms, RNA, DNA)

elektrode). Zadatak biosenzora je selektivno mjerenje promjene koncentracije analita na način da je signal odziva proporcionalan koncentraciji pojedinačnog analita ili više njih. Razvoj biosenzora može se promatrati s više aspekata: s obzirom na odabir biološkog elementa, s obzirom na odabir raznih vrsta pretvorničkih elemenata, s obzirom na tehnologiju izvedbe, s obzirom na način ostvarenja mjerenja kao protočnog ili neprotočnog mjerenja injektiranjem u protok (FIA, SIA), mjerenja uz primjenu mikrofluidnih platforma (biočip) i sl. te s obzirom na način komuniciranja redoks centra enzima i mjerene elektrode. Svaki od predloženih aspekata promatranja vrlo je zanimljiv, a predstavlja metode i načine koji su učinjeni ili se čine u svrhu konstrukcije biosenzora što idealnijih svojstava. U ovom predavanju pokazati će se kratak pregled načina konstrukcije glukoznog biosenzora tijekom 50 godina te konstrukcije koje se izvode u današnje vrijeme kao i potencijalne buduće konstrukcije. Odabran je način prezentacije razvoja glukoznog senzora s obzirom na način ostvarivanja veze amperometrijske elektrode i redoks centra glukoza-oksidaze i to: a) za direktan prijenos elektrona između redoks centra i mjerne elektrode, b) za prijenos elektrona primjenom difuzijskog medijatora, c) za prijenos elektrona ostvaren električnim kontaktom enzima i medijatorom funkcionalizirane elektrode, d) za prijenos elektrona ostvaren električnim kontaktom enzima s medijatorom funkcionaliziranom sol-gel matricom i e) buduće izvedbe kontakta redoks centra enzima te mjerne elektrode uz pomoć nanočestica.

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integrated within a physicochemical transducer. The aim of a biosensor is to produce a continuous electronic signal proportional to single analyte concentration or proportional to the concentration of a related group of analytes. Development of biosensors can be considered from many different points of view including: the type of chosen biological element, different types of transducers applied, different sensor technology, measurement techniques such as batch, flow-through (flow injection analysis, FIA or sequential injection analysis, SIA), or measurements by microfluidic platforms as in case of biochip design measurements, and finally electron transfer between the enzyme redox center and transducer. Each of the mentioned aspects can be very interesting due to the methods and procedures used in order to produce the biosensor of excellent performance. In this lecture, a concise review of the glucose biosensor design during the last fifty years will be presented. Presentation of glucose biosensor development will include models which describe different options applied to obtain electrical contact between the enzyme redox center (glucose-oxidase) and amperometric transducer, and will be focused on: a) directed, non-mediated electron transfer between the enzyme and electrode, b) electron transfer provided by diffusion mediators, c) electrical connecting of dissolved enzymes at mediator-functionalized electrode, d) electrical connecting of enzymes in mediator-functionalized sol-gel matrices, and e) some perspectives of electron transfer future design based on nanotechnology.

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S4-4

Pretrage uz bolesnika – automatizacija i integracija

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Pretrage uz bolesnika razvijene su zbog potrebe da rezultati analiza ključnih za preživljavanje kritičnih bolesnika budu što prije na raspolaganju liječniku, dok ih laboratorijsko osoblje prepoznaje kao prikladne za brze rezultate u krajnjim situacijama, a ne kao zamjenu za rezultate dobivene u laboratoriju. Tehnološki napredak nastavlja se u smjeru poboljšanja robustnosti ovih uređaja u rukama osoba koje nisu primarno osposobljene za rad u laboratoriju. Na primjer, trake za određivanje glukoze temelje se na elektrokemijskim mjerenjima i znatno su manje osjetljive na varijacije u hemoglobinu i drugim optičkim interfe-

S4-4

Point-of-care testing – automation and integration

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Point-of-care testing (POCT) has developed out of the need for clinicians to have rapid tests and viewed by laboratory personnel as suitable only for rapid test results in extreme situations, not as a substitute for results generated in the laboratory. Technological advances continue to improve the robustness of these devices in hands of nonlaboratory users. For example, glucose strips are based on electrochemical measurements and are less sensitive to variation in hemoglobin levels and other optical interferents which occurred with optical measurements. The miniaturization of electrochemical

rentima od ranijih optičkih instrumenata. Minijaturizacija elektrokemijskih senzora i napredak imunokemijske tehnologije na čvrstom nosaču rezultirala je eksplozijom kvalitativnih i kvantitativnih sustava u području acido-baznih uređaja, koagulacije, opće kliničke kemije do imunoanaliza. Napredak u tehnikama dokazivanja i u proizvodnji antitijela doveo je do golemog unaprjeđenja u sustavima za pretrage uz bolesnika. Područje srčanih biljega ima najbrži porast pretraga uz bolesnika. Novi kvantitativni sustavi uključuju određivanje troponina i elektrokemijskom imunometodom, te imunofluorescentno određivanje cTnI i CKMB. S prodorom ovih uređaja na tržište bolesnici s boli u prsima dobivaju mogućnost dijagnostike koja je gotovo jednaka rezultatima u laboratoriju. Tijekom posljednjih nekoliko godina tehnologija ovih uređeja suočena je s dva pristupa. Jedan je nastojanje da se različite tehnologije integriraju u jednom instrumentu stvarajući visoko funkcionalni uređaj. Drugi pristup je integracija mnoštva različitih testova u jednu platformu.
Informatička potpora u kontroli i pohranjivanju rezultata pretraga uz bolesnika razvijena je posljednjih desetak godina. Današnji sustavi uključuju mnoge funkcije neophodne za laboratorijski rad uključujući pregled potreba instrumenta, očitavanje rezultata, indentifikaciju korisnika. Preko priključaka ovi se uređaji priključuju i na LIS. Tako se sve informacije mogu skupiti na jednom mjestu. Slijedeća razina je integriranje svih informacija i indentifikacija promjena o tijeku bolesti, što će kliničarima omogućiti donošenje odluke. Druga razina je povezivanje kliničara s testiranjem bolesnika u kući u smislu stvaranja virtualne bolnice.

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sensors and advances in the immunologic solid-phase technology have resulted in an explosion of qualitative and quantitative POCT systems ranging from acid-base systems, coagulation, general chemistry and immunoassays. Advances made in detection techniques and antibodies have brought about tremendous improvements in POCT systems. Cardiac markers appear to be one of the major growth areas for POCT. New quantitative systems include a troponion I electrochemical immunoassay, and cTnI and CKMB based on immunofluorescence technology. With penetration of these quntitative immunoassays, patients with chest pain will get an as sensitive marker as performed in the laboratory. Over several years, POCT technology has faced two distinct approaches. One effort has integrated disparate technologies into a single reader creating a highly functional system. The other approach is based on the integration of many different test types into a single platform.
Software tools needed for the control and data management have developed during the past ten years. Today's POCT data management systems integrate many tools necessary for laboratory oversight including inventory management, reader performance and identification of users. The next step will be to integrate this information and identify changes in the course of disease to aid the clinician in decision making. The next level is to connect the clinician with patient home testing in terms of virtual hospital.

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S4-5

e-Zdravstvo za sigurnost bolesnika pomoću informacijskih tehnologija

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Primjena informacijske tehnologije u zdravstvenoj skrbi brzo se povećava i novija postignuća u bežičnim i mrežnim tehnologijama već značajno utječu na današnje e-zdravstvene i medicinske usluge. E-Zdravstvo opisuje primjenu informacijskih i komunikacijskih tehnologija u čitavom nizu funkcija koje imaju učinka na zdravstveni sektor i mogu poboljšati pristup zdravstvenoj skrbi te povećati kakvoću i učinkovitost ponuđenih usluga.

S4-5

e-Health for achieving patient safety through information technologies

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The use of information technology in health care is intensifying rapidly and the recent advances in wireless and network technologies have already made a significant impact on current e-health and medical services. e-Health describes the application of information and communication technologies across the whole range of functions that affect the health sector and can improve access to healthcare and boost the quality and effective-

E-Zdravstvena rješenja obuhvaćaju proizvode, sustave i usluge koje prelaze jednostavne internetske aplikacije. Mnoge zemlje pozivaju na široko prihvaćanje mobilnih računalnih i sustava medicinskih senzora i komunikacijskih tehnologija za zdravstvenu skrb u slijedećem desetljeću. Mnogo je primjera uspješnih dostignuća e-Zdravstva uključujući usluge zdravstvene informacijske mreže, telemedicine i telebiologije, usvajanje elektroničkih medicinskih zapisa, nosive i prenosive sustave motrenja i zdravstvene portale te mnoga druga tehnološki zasnovana informacijska i komunikacijska sredstva kao pomoć u prevenciji, dijagnostici, liječenju, zdravstvenom motrenju i promjenama načina života. Uz digitaliziranje informacija što ih pružatelji usluga rabe u skrbi za bolesnike unutar organizacija, te kliničari, bolesnici i oni koji kroje zdravstvenu politiku očekuju siguran elektronički prijenos odgovarajućih informacija među organizacijama. Razvoj industrijskih standarda za mogućnost zajedničkog rada, koji omogućavaju protok kliničkih i administrativnih podataka među ključnim nositeljima interesa, pokazao se je katalizatorom dokazujući svoju važnost u poticanju IT investicija u zdravstvenoj skrbi i olakšavajući zdravstvenu reformu. Viđenje e-Zdravstva uvelike potiče potreba za što većom djelotvornošću, smanjenje medicinskih grješaka, povećanje medicinskih i znanstvenih mogućnosti, te za štednjom sredstava. Zdravstveni stručnjaci aktivno planiraju provedbu e-Zdravstva u praksi. Daljnji će razvoj ići u smjeru personaliziranih zdravstvenih sustava. Sve veća dostupnost, minijaturizacija, rad uređaja, povećane količine podataka i očekivano okupljanje bežične komunikacije i mrežne tehnologije oko mobilnih zdravstvenih sustava ubrzati će razvoj i uvođenje mobilnih zdravstvenih uređaja, sustava i usluga u idućem desetljeću. To će pak snažno utjecati na neke od postojećih laboratorijskih i zdravstvenih službi i preoblikovati će neke od mehanizama postojećih putova pružanja zdravstvene skrbi. Danas, uz bežične tehnologije, elektroničke medicinske zapise mogu procjenjivati stručnjaci s bilo kojeg mjesta spajanjem na informacijski sustav dotične ustanove. Zahvaljujući mobilnoj tehnologiji liječnicima opće prakse biti će daleko dostupniji podatci iz povijesti bolesti, laboratorijski rezultati, farmaceutski podatci, podatci iz osiguranja i oni o medicinskim sredstvima, čime se poboljšava kvaliteta skrbi i pozornost usmjerena ka bolesniku, a sve to radi sigurnosti bolesnika. Biti će riječi o novijim dostignućima u ovom novom području zdravstvene skrbi, uz podroban prikaz brzo nadolazećih promjena u zdravstvenoj politici u pitanjima financija, kliničke kvalitete, organizacije, te pravnim, tehničkim pitanjima i onima koja se odnose na očuvanje privatnosti.

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ness of the services offered. e-Health solutions include products, systems and services that go beyond simply Internet-based applications. Many countries are calling for widespread adoption of mobile computing, medical sensor systems and communication technologies for health care within the next decade. There are many examples of successful e-Health developments including health information networks, telemedicine and telebiology services, adoption of electronic medical records (EMRs), wearable and portable monitoring systems, and health portals and many other information and communication technology-based tools assisting prevention, diagnosis, treatment, health monitoring, and lifestyle management. In addition to digitising the information that providers use to care for their patients within organizations; clinicians, patients, and policymakers are looking ahead to securely sharing appropriate information electronically among organizations. The development of industry standards for interoperability, enabling the flow of clinical and administrative data among key stakeholders, has proved to be a catalyst in demonstrating its importance for encouraging health care IT investment and facilitating health care reform. The perception of e-Health appears to be largely driven by the need of increasing efficiency, reduction of medical error, increasing medical and scientific capabilities, and saving money. Health professionals are actively planning for implementation of e-Health in their practices. The next evolution will be towards personalized healthcare systems. The increased availability, miniaturization, performance, enhanced data rates and the expected convergence of wireless communication and network technologies around mobile health systems will accelerate the deployment of mobile health devices, systems and services within the next decade. These will have a powerful impact in some of the existing laboratory and health services and will reshape some of the mechanisms of the existing healthcare delivery routes. Today with wireless technologies, EMRs could be assessed by professionals from any given location by connection to the institution's information system. General practitioners' access to patient history, laboratory results, pharmaceutical data, insurance information, and medical resources would be enhanced by mobile technology, thereby improving the quality of care and greater attention to patient safety. The presentation will address the recent developments in this emerging area of healthcare areas and also include an in-depth look at rapidly emerging policy changes related financial, clinical quality, organizational, legal, technical, and privacy-related issues.

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SA – Simpozij ABBOTT – KARDIOVASKULARNE BOLESTI, SA-1

Kliničko značenje testova za troponin poboljšane osjetljivosti

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Test na srčane troponine u krvi postao je zlanim standardom u biokemijskom otkrivanju oštećenja miokarda, a može se mjeriti kao troponin T ili I. Visoka osjetljivost ovih testova omogućila je redefiniranje infarkta miokarda i osigurala nam snažno prediktivno sredstvo u bolesnika s oštećenjem miokarda. Tako je više studija jasno pokazalo povezanost razina srčanih troponina s kratkoročnim i dugoročnim ishodom u bolesnika s akutnim koronarnim sindromima, tj. infarktom miokarda ili nestabilnom anginom. U prethodnim smo studijama uspoređivali kliničku uspješnost nekoliko testova za srčane troponine i utvrdili kako se njihova stvarna klinička osjetljivost bitno razlikuje usprkos sličnostima u funkcijskom osjetljivosti. U studiji FRISC II, u velikoj skupini osoba s ne-Q-valnim infarktom miokarda ili nestabilnom anginom pretraga na srčani troponin I jednim testom identificirala je oko 10% više bolesnika sa slabim ishodom negoli drugi testovi. Naši rezultati ukazuju na kvalitativne razlike među testovima za srčane troponine. Kako bismo dodatno ispitali ovo zapažanje, izmjerili smo plazmatske razine srčanih troponina u skupini zdravih osoba (skupina SWISCH) podjednake dobi i spola kao skupina bolesnika FRISC II. Dobiveni rezultati pokazali su dobnu povezanost sa značajno povišenim razinama u starijoj skupini i ukazali na to da bi 99. percentila URL za osobe ispod 60 godina starosti trebala iznositi polovicu one utvrđene u prethodnim izvješćima. Ovi su rezultati ponovno pokazali velike razlike između pojedinih testova. Pitanje na koje rezultati studije SWISCH nisu mogli dati odgovor bilo je jesu li blago povišene razine uočene u nekih zdravih osoba povezane s bilo kakvim kliničkim posljedicama. Nastojeći naći odgovor na to, izmjerili smo razine cTnI u serumu pomoću testova AccuTnI (Beckman Coulter) i Liaison (Diasorin) u 1221 muškarca u dobi od 70 godina (studija ULSAM) koji su bili praćeni u prosjeku 10,4 godine; 835 ovih muškaraca smatrali su se zdravima u vrijeme uzorkovanja krvi. Razine cTnI mjerene pomoću testa AccuTnI jednako su pokazale snažnu povezanost sa smrću od svih uzroka i sa smrću od kardivaskularne bolesti. Taj je odnos bio manje očit kad je primijenjen test Liaison. Jedine očite razlike između testa AccuTnI i drugih testova za cTnI bile su razlike u konfiguraciji protutijela. Tako je test AccuTnI jedinstven po tome što

SA – Symposium ABBOTT – CARDIOVASCULAR DISEASES, SA-1

Clinical importance of troponin assays with enhanced sensitivity

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The assay of cardiac troponins in blood has become the gold standard in the biochemical detection of myocardial injury and may be measured either as troponin T or I. The high sensitivities of the assays have allowed for redefinition of myocardial infarction and also provided powerful predictive tools in patients with myocardial injury. Thus, several studies have clearly shown the relationship of cardiac troponin levels to both short term and long term outcome in patients with the acute coronary syndromes, i.e. myocardial infarction or unstable angina. In previous studies we compared the clinical performance of several cardiac troponin assays and found that in spite of similarities in functional sensitivities the actual clinical sensitivities differed substantially. In a large cohort of subjects with non-Q-wave myocardial infarction or unstable angina, the FRISC II study, the assay of cardiac troponin I using one assay identified about 10% more patients with poor outcome than other assays. Our results suggested qualitative differences between cardiac troponin assays. To investigate this further, we assayed plasma levels of cardiac troponins in a cohort of healthy subjects. This cohort (SWISCH) was matched according to sex and age to the FRISC II cohort patients. The results showed a relationship to age with significantly elevated levels in the elderly cohort and suggested that the 99th percentile URL for subjects below 60 years of age should be half of that defined in previous publications. The results again showed great between-assay differences. The question that could not be answered by the SWISCH results was whether the slightly elevated levels seen in some healthy subjects were related to any clinical consequences. In an attempt to answer this, we measured serum cTnI levels by the AccuTnI (Beckman Coulter) and Liaison (Diasorin) assays in 1221 70-year-old men (the ULSAM study) that had been followed for a mean of 10.4 years; 835 of these men were regarded healthy at the time of blood sampling. The cTnI levels as measured by the AccuTnI assay uniquely showed a strong relationship to all-cause death and to death from cardiovascular disease. This relationship was less obvious with the Liaison assay. The only obvious differences between the AccuTnI assay and other cTnI assays were differences in antibody configurations. Thus,

uključuje protutijelo na epitop 41-49 u molekuli cTnI, pa smo stoga pretpostavili kako je ta razlika bila vjerojatno razlog za razlike u kliničkoj uspješnosti. Zato je za nas bilo veoma važno kad je Abbott izišao s poboljšanim testom za cTnI koji uključuje ovo protutijelo. U studiji koju smo proveli u drugoj skupini osoba s ne-Q-valnim infarktom miokarda ili nestabilnom anginom (studija GUSTOIV) analizirali smo razine srčanih troponina pomoću više različitih testova u uzorcima oko 700 osoba. Rezultati su potvrdili prijašnje podatke iz skupine FRISC i pokazali kako je ovaj Abbottov test dostigao kliničku uspješnost testa AccuTnI, dajući tako daljnju potporu našem zapažanju o važnosti uključenja u test monoklonskog protutijela usmjerenog protiv epitopa 41-49. Naši podatci naglašavaju jedinstvenu kvalitetu nekih testova u identificiranju osoba s lošom prognozom, te isto tako pokazuju kako visoka funkcijska osjetljivost nekog testa ne mora uvijek značiti i njegovu visoku kliničku osjetljivost. Naši podatci također pokazuju kako se klinička iskoristivost testova na troponine širi, pa bi sukladno tome trebalo revidirati i kliničku obradu.

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the AccuTnI assay uniquely includes an antibody against the epitope 41-49 in the cTnI molecule and we therefore postulated that this difference was the likely reason for the differences in clinical performance. It was therefore of great interest to us when Abbott launched their upgraded assay of cTnI with the inclusion of this particular antibody. In a study in another cohort of subjects with non-Q-wave myocardial infarction or unstable angina, i.e. the GUSTOIV-study, we analyzed the levels of cardiac troponins with several different assays in samples of some 700 subjects. The results confirmed previous data on the FRISC cohort and also showed that the Abbott assay had acquired clinical performance of the AccuTnI assay, lending further support to our notion on the importance of the inclusion of the monoclonal antibody directed against the epitope 41-49 in the assay. Our data emphasize the unique quality of some assays in identifying subjects with unfavorable prognosis and also show that a high functional sensitivity of an assay need not always translate into a high clinical sensitivity. Our data also imply that the clinical utility of troponin assays is broadened and the clinical management should be revised accordingly.

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SA-2

Abbottovi srčani biljezi u dijagnostici i motrenju

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Test Troponin-I, te testovi BNP, CK-MB i Myoglobin dostupni su za Abbottove instrumente AxSYM i Architect. Uz to, testovi Homocystein i D-dimer dostupni su za instrument AxSYM. Izvrsna klinička osjetljivost testa TnI na instrumentu Architect može se pripisati monoklonskim protutijelima koja su upotrebljena u ovom testu (James S i sur., Clin Chem 2006.). I test Troponin-I za Architect i onaj za AxSYM rabe ista tri monoklonska protutijela koja su sva usmjerena prema stabilnom dijelu molekule troponina. Za razliku od dijagnostičkih biljega za akutni koronarni sindrom (Troponin-I, CK-MB i Myoglobin), natriuretski peptid tip B (BNP) je moćan biljeg koji pomaže u dijagnostici srčanog zatajenja. Prof. Mueller i sur. (N Engl J Med 2004.) su u prospektivnoj randomiziranoj kontroliranoj studiji potvrdili kako se može uštedjeti 26% troškova uz primjenu pretrage BNP u usporedi sa standardnom dijagnostikom bez pretrage BNP. U ovu su studiju bila uključena 452 bolesnika srednje dobi od 71 godine koji su došli na hitni prijam s akutnom

SA-2

Abbott cardiac markers in diagnosis and monitoring

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The Troponin-I assay as well as the BNP, CK-MB and Myoglobin assays are available on both the AxSYM and Architect instruments from Abbott. In addition, the Homocysteine and the D-dimer assays are available on the AxSYM instrument. The excellent clinical sensitivity of the Architect TnI assay can be attributed to the monoclonal antibodies that are used in the assay (James S *et. al.,* Clin Chem 2006). Both the Architect and AxSYM Troponin-I assays utilize the same three monoclonal antibodies, which are all directed to the stable part of the troponin molecule. In contrast to the diagnostic markers for acute coronary syndrome (troponin-I, CK-MB and myoglobin), a powerful marker to aid in the diagnosis of heart failure is the B-type natriuretic peptide (BNP). Prof. Mueller *et al*. (N Engl J Med 2004) confirmed in a prospective, randomized, controlled study that 26% of cost could be saved if BNP testing is performed compared to standard diagnosis without BNP testing. This study enrolled 452 patients, mean

dispnejom. Uz to, Mueller je pokazao da se stopa prijma u bolnicu smanjila za 10%, a medijan boravka u bolnici za 3 dana u skupini s BNP. Abbottovi testovi za BNP pružaju brzu i potpuno automatiziranu metodu za određivanje razina BNP. Prijelomna vrijednost (*cut-off*) od 100 pg/mL rabi se u svim dobnim skupinama za isključivanje srčanog zatajenja. Dok se molekula aktivnog BNP izlučuje putem receptora neutralne endopeptidaze i izlučivanja, molekula inaktivnog NT-proBNP se izlučuje putem bubrega. To pak dovodi do znatno manje ovisnosti BNP o dobi i bubregu (McCullough i sur., Rev Cardiovasc Med 2003.). Uz BNP, može se primijeniti test D-dimer na AxSYM kao pomoć u diferencijalnoj dijagnostici bolesnika s dispnejom kako bi se razlikovali srčani od plućnih uzroka. D-dimer je biljeg koagulacije koji odražava stupanj obrtaja trombina. Test D-dimer na AxSYM može se rabiti za isključivanje sumnjive venske tromboembolije, kako duboke venske tromboze tako i plućne embolije, s visokom negativnim prediktivnom vrijednošću (NPV=98,9%). Rezultat testa D-dimer dobiva se u kratkom vrremenu od 15 minuta. Ukratko, srčani testovi na uređajima Architect i AxSYM daju izvrsne rezultate u kratkom vremenu, koji su dobiveni na pouzdanim i potpuno automatiziranim instrumentima.

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age 71 years, presenting to emergency department with acute dyspnea. In addition, Mueller showed the hospital admission rate to be reduced by 10% and median length of hospital stay by 3 days in the BNP group. The Abbott BNP assays provide rapid and fully automated methods to determine BNP levels. A cut-off of 100 pg/mL to rule out heart failure is used for all age groups. Whereas the active BNP molecule is cleared *via* neutral endopeptidase and clearance receptors, the inactive NT-proBNP molecule is renally excreted. This leads to much less age and renal dependence for BNP (McCullough *et al*., Rev Cardiovasc Med 2003). In addition to BNP, the AxSYM D-dimer assay can be used to aid in differential diagnosis of dyspnea patients, distinguishing cardiac from pulmonary causes. D-dimer is a coagulation marker that reflects the degree of thrombin turnover. The AxSYM D-dimer assay can be used to rule out suspected venous thromboembolism, both deep vein thrombosis and pulmonary embolism with a high negative predictive value (NPV=98.9%). The D-dimer assay has a quick turnaround time of 15 minutes. Overall, the Architect and AxSYM cardiac assays are proven to have excellent assay performance. Quick assay results can be obtained on reliable and fully automated instruments.

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SA-3

Novi srčani biljezi akutnog koronarnog sindroma

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Akutnim koronarnim sindromom (AKS) određena su stanja od nestabilne angine, preko ne-ST akutnog infarkta do ST-akutnog infarkta miokarda s izraženom nekrozom miokarda. Najčešća etiologija uključuje pet osnovnih uzoraka: rupturu plaka s akutnom trombozom, progresivnu mehaničku opstrukciju, upalu, sekundarnu nestabilnu anginu uslijed anemije ili hipertiroidizma, dinamičku opstrukciju uslijed vazokonstrikcije. Značajnu ulogu u dijagnostici i liječenju AKS imaju biokemijski srčani biljezi. Godinama temeljena na uporabi biljega nekroze miocita, klinička primjena srčanih biljega posljednjih je desetak godina doživjela značajnu transformaciju i nastoji mjerljivim parametrima obuhvatiti sve faze AKS. Uza opće prihvaćene biljege nekroze sve veća važnost pripada biljezima upale, ishemije miokarda i biljezima srčane funkcije. Bilo sustavna ili lokalna, upala definira stabilnost ili nestabilnost ate-

SA-3

New biochemical markers of acute coronary syndrome

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Acute coronary syndrome (ACS) refers to conditions of unstable angina, non-ST myocardial infarction and ST acute myocardial infarction with pronounced necrosis. The most common pathology of ACS includes five major causes: plaque obstruction, inflammation, secondary unstable angina due to severe anemia or hyperthyroidism, and dynamic obstruction due to coronary vasoconstriction. Biochemical markers play a pivotal role in the diagnosis and management of patients with ACS. Clinical use of biochemical markers of cardiac diseases has been significantly modified over the last ten years, and today includes parameters for complete evaluation of ACS. Along with necrosis biomarkers, the biomarkers of inflammation, cardiac ischemia and ventricular overload have been firmly established in clinical studies. Local or systemic inflammation is a major contributor to plaque

rosklerotskog plaka pa se biljezi ove faze bolesti ispituju kao rani biljezi AKS, odnosno biljezi nestabilnosti plaka. Iako nedovoljno istraženi, tu se spominju C reaktivni protein, mijeloperoksidaza, transmembranski protein trombocita-CD40 ligand, monocitni hemoatraktant protein-1 (MCP-1), plazma protein A udružen s trudnoćom (PAPP-A), kolin, interleukin 6. Središnji fiziološki proces u AKS je miokardijalna ishemija pa specifični biljeg mora razlikovati akutni infarkt miokarda (AIM) od neishemijskog oštećenja miokarda. Veliku mogućnost primjene u toj fazi imaju ishemijom modificirani albumin (IMA), glikogen fosforilaza-BB (GP-BB), nevezane slobodne masne kiseline (FFAu). Među srčanim biljezima najispitivaniji su biljezi infarkta miokarda, odnosno nekroze. Nekroza je praćena otpuštanjem strukturnih proteina i staničnih makromolekula u srčani intersticijum kao posljedica oštećenja stanične membrane. Kako je pravodobno prepoznavanje AIM važno za prognozu i liječenje, određivanje biljega nekroze indicirano je u svih bolesnika sa suspektnim AKS. Najznačajniji biljezi ove skupine su mioglobin, CKMB mass, srčani troponini T i I, ali se nove nade polažu u određivanje tropomiozina i aktina. Skupinu biljega srčane funkcije čine natriuretski peptidi i to tipa B (BNP) i N terminalni dio istog prohormona (NT-proBNP).

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S5 – Simpozij 5 – TUMORSKI BILJEZI I MOLEKULARNA DIJAGNOSTIKA ZLOĆUDNIH TUMORA, S5-1

Granice nutrigenomike i prevencija raka

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Prehrambene navike mogle bi utjecati na incidenciju raka. Velik broj bioaktivnih sastavnica u hrani udružen je sa zaštitom od raka. Bioaktivne sastavnice hrane mogle bi istodobno mijenjati više od jednog procesa raka uključujući metabolizam karcinogena, hormonsku ravnotežu, proliferaciju, stanično signaliziranje, kontrolu staničnog ciklusa, diferencijaciju, apoptozu, angiogenezu i metastazu. Utvrđeno je kako genetska raznolikost i genetska osnova pojedine osobe imaju ulogu u sklonosti ka razvoju raka. Nutrigenomika je ispitivanje načina na koji bioaktivne sastavnice u prehrani djeluju uzajamno s genima i njihovim proizvodima, te kako genetska raznolikost može uvjeto-

S5 – Symposium 5 – TUMOR MARKERS AND MOLECULAR DIAGNOSIS OF MALIGNANT TUMORS, S5-1

Frontiers in nutrigenomics and cancer prevention

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Dietary habits may influence cancer incidence. A large number of bioactive components in food are associated with cancer protection. Bioactive food components may modify simultaneously more than one cancer process including carcinogen metabolism, hormonal balance, proliferation, cell signaling, cell-cycle control, differentiation, apoptosis, angiogenesis, and metastasis. It has been recognized that genetic variation and individual genetic background play a role in susceptibility to cancer. Nutrigenomics is the study of how bioactive components in the diet interact with genes and their products, and how genetic variations may cause people to respond differently

vati da ljudi različito odgovaraju na nutrijente iz prehrane. Ispitivanje interakcije između prehrane i genoma pojedine osobe, te odgovora dotične osobe na različite vrste prehrane daje važne informacije o onima u kojih odgovor nastupa te onima u kojih odgovor izostaje, a time pomaže identificirati one koji će imati najviše koristi. Tehnike koje se rabe u prehrambenoj genomici slične su onima koje se primjenjuju u suvremenom molekularno genetičkom istraživanju. Međutim, da bismo u potpunosti shvatili ulogu prehrane u karcinogenezi i identificirali one koji hoće ili neće odgovoriti na prehrambenu intervenciju, uz nutrigenomiku je neophodna istraživačka suradnja u područjima prehrambene epigenetike, prehrambene transkriptomike, proteomike i metabolomike, vodeći računa o tome da etnička pripadnost, kulturna okolina i drugi čimbenici utječu na genetsku raznolikost i odgovor na prehrambene čimbenike, te na izraženost važnih gena. Znanja dobivena iz studija udruženosti bioaktivnih sastavnica hrane i rizika za razvoj raka, na osnovi genotipa pomoći će u izradi individualiziranih prehrambenih intervencija za sprječavanje, pa čak i liječenje raka.

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to food nutrients. The study of the interaction between nutrition and an individual's genome and the response of the individual to different diets provides important clues about responders and non-responders and helps indicate who will benefit most. Techniques used in nutritional genomics are similar to those used in modern molecular genetic research. However, to fully understand the role of nutrition in carcinogenesis and to identify those who will and will not respond to dietary intervention, a collaborative research in the areas of nutritional epigenetics, nutritional transcriptomics, proteomics and metabolomics are necessary in addition to nutrigenomics, taking into account that ethnicity, cultural environments and other factors influence the genetic variability and response to nutritional factors, and the expression of important genes. The knowledge gained in studies of the associations among bioactive food components and cancer risk based on genotype will help designing individualized nutritional intervention to prevent and even treat cancer.

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S5-2

Genomika i proteomika u onkologiji

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U prošlih smo nekoliko desetljeća bili svjedoci golemog napretka u istraživanju genoma i proteoma u području molekularnog profiliranja - onkologiji. Otprilike 30.000 gena kodira za čak do deset milijuna proteina koji u (pre)malignim stanicama (de)reguliraju fiziološke procese. S obzirom na tako velik broj gena/proteina njihove promjene koje vode zloćudnoj preobrazbi su iznimno složene i teško ih je "mjeriti" tradicionalnim metodama. Mjerenje ekspresije gena cDNA mikročip tehnologijom, kao i globalno profiliranje proteina pojedinog uzorka daju složenu sliku promjena unutar stanice. Ovakve tehnologije omogućuju analizu ekspresije tisuća gena i proteina odjednom, čime istraživači raspolažu informacijama o brojnim promjenama koje su uzrok zloćudne promjene. Uz to, ovakva istraživanja daju informacije koje se mogu upotrijebiti za molekularno profiliranje tumora, što pak može rezultirati točnijom klasifikacijskom shemom kao i identifikacijom niza gena koji se mogu iskoristiti za bolju prognozu i predviđanje ishoda liječenja. Nije ni zanemari-

S5-2

Genomics and proteomics in cancer

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The past decade has seen great advances in genome and proteome research in the field of molecular profiling - oncology. Approximately 30,000 genes code for up to ten million proteins which (de)regulate physiological processes in premalignant/malignant cells. Due to such a huge number of genes/proteins, their alterations that can lead to cancer formation are dramatically complex and are difficult to measure by traditional methods. The measurement of gene expression by cDNA microarray technology, as well as global protein profiling of a particular specimen give an integrated genome-proteome picture of the changing function. Such approaches enable analysis of expression levels of thousands of genes and proteins at once, providing the researchers with information on a variety of changes that underlay malignant transformation. In addition, they give information that can be used for molecular profiling of tumors, which can result in a much more detailed classification schemes as well as in the identification of potential gene

va identifikacija molekularnih "meta" na temelju kojih se može zasnivati razvitak novog, prema svakom oboljelom "iskrojenog" liječenja. Do sada je cDNA mikročip tehnologija upotrebljena za identifikaciju gena uključenih u sklonost razvoju nasljednih oblika raka, kao što su BRCA1/2 i APC. Analizom meduloblastoma otkriveni su geni uključeni u procese adhezije stanica i njihovu mobilnost (PDGFR i Ras/*mitogen activated protein kinase* - MAPK geni). Slično tome, eskpresija proteina Wnt5a prati sposobnost metastaziranja melanoma kože. S obzirom na točniju klasifikaciju tumora i klinički ishod bolesti najbolji su rezultati postignuti u području zloćudnih tumora krvotvornog tkiva (leukemije i limfomi) i tumora dojke u osoba mlađih dobnih skupina. Usporedno sa spomenutim, nije zanemarivo ni kreiranje novih lijekova. S obzirom na to da su mnogi oblici konvencionalnog liječenja nespecifični, novi oblici liječenja usmjereni osobito na dokidanje aktivnosti proteina sve su više u središtu istraživanja. Prema tome, proteomske analize predstavljaju izravniji način analiziranja uzoraka sa svrhom kreiranja ciljanog liječenja. Sve u svemu, tehnike analize genoma i proteoma postale su moćno oruđe za proučavanje tisuća gena i proteina odjednom. Iako ćemo na rutinsku primjenu ovih tehnika još pričekati, njihovo će uključivanje u kliničke protokole biti od velikog značenja ne samo za oboljele, nego i za društvo u cjelini.

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signature sets that can be applied to both the prognosis and prediction of treatment outcome. In addition, also of great importance is identification of molecular targets that allows the development of new tailored antitumor treatments. So far, cDNA array based gene profiling has been used to identify a number of genes involved in inherited predisposition to disease such as BRCA1/2 and APC. Analysis of medulloblastomas revealed PDGFR and the Ras/MAPK genes as those responsible for cell adhesion and mobility. Similarly Wnt5a is a molecule whose expression is highly correlated with metastatic potential in melanoma. Regarding prediction and tumor classes the best achievements were obtained in leukemias, lymphomas and breast tumors from young patients. In parallel stands also changing the treatment and management of patients. As many conventional therapies are limited by the lack of specificity, novel targeted therapies mostly based on aberrant protein signaling have come into focus. Thus, proteomic analysis represents a more direct way of analyzing cancer samples for selected or targeted therapies. Alltogether, the genome-proteome techniques have become a powerful tool for the study of thousands of genes and proteins at once. Although routine application of these technologies in clinical practice will take some time, their inclusion into clinical protocols will be of great benefit not only for patients but also for the community at large.

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S5-3

Aktivacija signalnih molekula u karcinogenezi

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Tijekom posljednjeg desetljeća bazična istraživanja raka svojim su rezultatima dovela do značajnih napredaka u našem razumijevanju biologije i genetike raka. Jedna od najznačajnijih spoznaja bila je ona da programirana smrt stanice (apoptoza) i geni koji su uključeni u ovaj proces snažno utječu na maligni fenotip. Apoptoza je prirodan proces uklanjanja neželjenih stanica, poput onih s potencijalno štetnim mutacijama ili s poremećenom kontrolom tijeka staničnoga ciklusa. Poremećaji u procesu apoptoze mogu utjecati na osjetljivu ravnotežu između proliferacije stanica i njihovog umiranja, što može dovesti do razvoja različitih bolesti pa i raka. Kod mnogih tipova tumora

S5-3

Activation of signaling molecules in carcinogenesis

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In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among most important of these advances is the realization that programmed cell death (apoptosis) and the genes that control it have a profound effect on the malignant phenotype. Apoptosis is a natural process of removing unwanted cells such as those with potentially harmful mutations or alterations in the cell-cycle control. Deregulation of apoptosis can disrupt the delicate balance between cell proliferation and cell death, and can lead to diseases such as cancer. In many cancers pro-apoptotic proteins have inactivating

prisutne su inaktivirajuće mutacije u pro-apoptotičnim proteinima ili je potaknuta ekspresija anti-apoptotičnih proteina, što dovodi do nekontroliranog rasta tumora i do nemogućnosti stanice da odgovori na stresne poticaje, na štetne mutacije i na oštećenja DNA. Izbjegavanje stanice da umre programiranom smrću prepoznato je danas kao jedna od šest najvažnijih promjena u fiziologiji stanice koje dovode do malignoga rasta i značajka je većine, ako ne i svih tipova tumora. Provode se brojna istraživanja kako bi se što potpunije razjasnio mehanizam apoptoze, a sve u svrhu pronalaženja novih biokemijskih biljega koji bi se mogli rabiti u ranom dijagnosticiranju tumora te razvijanja novih terapijskih pristupa u liječenju različitih vrsta tumora, a koji bi bili manje toksični i mutageni od već postojećih.
Istražuju se različite molekule koje sudjeluju u procesu apoptoze, poglavito receptori smrti koji se nalaze na površini stanice i potiču apoptozu, Bcl-2 proteini koji imaju značajnu ulogu u mitohondrijskom putu apoptoze, kaspaze koje su glavni izvršitelji procesa apoptoze te endogeni inhibitori kaspaza. Tijekom predavanja govoriti će se o nekim od ovih potencijalno obećavajućih signalnih molekula koje bi mogle imati važnu ulogu u dijagnostici i/ili liječenju različitih vrsta tumora.

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mutations or the expression of anti-apoptotic proteins is up-regulated, leading to the unchecked growth of the tumor and the inability to respond to cellular stress, harmful mutations and DNA damage. The evasion of programmed cell death has been recognized today as one of the six essential alterations in cell physiology that dictate malignant growth and is a hallmark of most, and maybe all types of cancer. An intense research effort is uncovering the underlying mechanisms of apoptosis in order to discover new biochemical markers that could be used for early diagnosis of cancer, and to produce new therapies that are less toxic and mutagenic than current treatment regimens. Apoptosis targets that are currently being explored for cancer diagnosis and drug discovery include death receptors triggering apoptosis from the cell surface, Bcl-2 proteins as the gatekeepers of the mitochondrial pathway, caspases as the executioner enzymes, or endogenous caspase inhibitors. Some of these potentially promising signaling molecules in cancer diagnosis and treatment will be discussed.

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S5-4

Sustav aktivatora urokinaznog plazminogena: bogat izvor tumorskih biljega

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Sustav aktivatora urokinazog plazminogena (sustav uPA) sastoji se od serinske proteaze uPA, receptora (uPAR) i dva inhibitora: inhibitor aktivatora plazminogena 1 (PAI 1) i inhibitor aktivatora plazminogena 2 (PAI 2). Sustav uPA ima važnu ulogu u biološkim procesima kao što su fibrinoliza, upalni procesi, stvaranje aterosklerotičnih plakova, pregradnja matriksa, tumorska invazija, angiogeneza i metastaziranje. Vezanje uPA za njegov receptor započinje proteolitičnu kaskadu koja pretvara plazminogen u plazmin. Plazmin preko svojih vlastitih proteolitičnih funkcija razgrađuje izvanstanične sastavnice bazalne membrane i aktivira druge sastavnice kao što su metaloproteinaze. Neovisno o svojoj katalitičnoj aktivnosti, uPAR je uključen i u stanično signaliziranje, interakciju s integrinima, stanično kretanje, adheziju, invaziju i angiogenezu. Prekomjerna ekspresija uPA, uPAR ili PAI 1 je značajka malignosti i

S5-4

The urokinase plasminogen activator system: a rich source of tumor markers

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The urokinase plasminogen activator (uPA) system consists of the serine protease uPA, receptor (uPAR), and the two inhibitors, plasminogen activator inhibitor 1 (PAI 1) and plasminogen activator inhibitor 2 (PAI 2). The uPA system has an important role in biological processes including fibrinolysis, inflammation, atherosclerotic plaque formation, matrix remodeling, tumor invasion, angiogenesis, and metastasis. Binding of uPA with its receptor initiates a proteolytic cascade that results in the conversion of plasminogen to plasmin. Plasmin through its own proteolytic function degrades the extracellular basement membrane components and activates others such as metalloproteinases. Independent of the catalytic activity, uPAR is also involved in cell signaling, interactions with integrins, cell motility, adhesion and invasion, and angiogenesis. Overexpression of uPA, uPAR or PAI 1

korelira s progresijom tumora i metastaziranjem. U skladu s njihovom ulogom u diseminaciji tumora, visoke koncentracije uPA, PAI 1 i uPAR u mnogim vrstama raka koreliraju s lošim ishodom bolesti. Prognostička vrijednost uPA i PAI 1 kod bolesnica s rakom dojke koje imaju negativne limfne čvorove aksile proglašena je valjanom, rabeći i prospektivno randomizirajuće ispitivanje i udruženu analizu. Ispitivanje uPA i PAI 1 može stoga pomoći u identificiranju bolesnica s nisko rizičnim negativnim statusom limfnih čvorova aksile za koje je nepotrebna adjuvantna kemoterapija. Međutim, kod bolesnica s rakom dojke i negativnim limfnim čvorovima aksile, ali s visokim koncentracijama uPA i PAI 1 u primarnom tumoru adjuvantna kemoterapija je potrebna. Mjerenje uPA sastavnica, naročito kod bolesnica s rakom dojke, pruža mogućnost da se pomogne u individualiziranom vođenju bolesnica. Predklinička proučavanja su pokazala da ili inhibicija katalitične aktivnosti uPA ili sprječavanje vezanja uPA za njegov receptor smanjuju rast tumora, angiogenezu i metastaze. Strategije koje blokiraju uPA ili njegov receptor kako bi se poremetila njihova interakcija ili neovisno djelovanje uPAR uključuju antisensnu tehnologiju, monoklonska protutijela, citotoksične antibiotike i sintetske inhibitore uPA. Ciljana terapija je cilj budućeg liječenja tumora, a sustav uPA je dobar kandidat za takvu terapiju.

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is a feature of malignancy and is correlated with tumor progression and metastasis. Consistent with their role in cancer dissemination, high levels of uPA, PAI 1 and uPAR in multiple cancer types correlate with adverse patient outcome. The prognostic value of uPA and PAI 1 in axillary node-negative breast cancer patients was recently validated using both a prospective randomized trial and pooled analysis. Assay of uPA and PAI 1 may thus help identify low risk node negative patients for whom adjuvant chemotherapy is unnecessary. However, for lymph node negative breast cancer patients with high levels of uPA and PAI 1 in primary tumor adjuvant chemotherapy is necessary. The measurement of uPA components, especially in breast cancer, therefore has the potential to help with individualized patient management. Preclinical studies show that either inhibition of uPA catalytic activity or prevention of uPA binding to its receptor reduces tumor growth, angiogenesis and metastasis. Strategies that target uPA or its receptor with the aim to disrupt the interaction between the two or the ligand independent action of uPAR include antisense technology, monoclonal antibodies, cytotoxic antibiotics, and synthetic inhibitors of uPA. Targeted therapy is a goal of future cancer treatment and the uPA system is a likely candidate for such therapy.

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S5-5

Pregled najnovijih smjernica za tumorske biljege

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Sve je veći pritisak za pružanjem zdravstvene skrbi zasnovane na "najboljoj praksi". To poglavito vrijedi za medicinu karcinoma, gdje su dijagnostički postupci često invazivni, a liječenje skupo. Stoga je više skupina izradilo smjernice kako bi se potakla sve bolja primjena tumorskih biljega. Među njima je nedavno objavljen nacrt smjernica Američke akademije za kliničku biokemiju (NACB), koje su sad dostupne za raspravu na mreži (www.nacb.org), a usredotočene su na kliničke i laboratorijske aspekte primjene tumorskih biljega. Kako kvaliteta rezultata odražava događaje tijekom triju faza analize, primjereno je zahtjeve za svaki razmatrati zasebno. Prijeanalitički zahtjevi: implikacije zahtjeva za pretragu na tumorske biljege za bolesnika treba razmotriti prije postavljanja zahtjeva, kada

S5-5

An overview of recent guidelines on tumor markers

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There is an increasing pressure to provide health care based on "best practice". This is particularly true in cancer medicine, where diagnostic procedures are often invasive, and therapy expensive. A number of groups have therefore developed guidelines with the aim of encouraging improved use of tumour markers. Among these are recently published draft guidelines from the American National Academy of Clinical Biochemistry (NACB), which are now available for comment on the web (www.nacb.org) and which focus on both clinical and laboratory aspects of tumour marker use. As the quality of results reflects events during the three phases of analysis, it is convenient to consider the requirements of each separately. Pre-analytical requirements: the implications for

treba odabrati najvažniji tumorski biljeg. Kad se izdaje zahtjev, pažljivo treba razmotriti vrijeme uzimanja uzorka, kao i mogući utjecaj drugih vrsta liječenja i lijekova (npr. prethodno liječenje mišjim monoklonskim protutijelima) i/ili drugih medicinskih stanja (npr. PSA kod prostatitisa) na rezultate. Uzeti uzorak (serum, plazma itd.) treba uvijek zadovoljavati zahtjeve proizvođača testa. Analitički zahtjevi: testovi trebaju uvijek ispunjavati utvrđene zahtjeve za kvalitetu u smislu Unutarnje kontrole kvalitete (IQC) (zadovoljavajući objektivne kriterije za prihvatljivost rezultata za propisno utvrđene IQC uzroke) i Vanjske kontrole kvalitete (EQA) (postizanje zadovoljavajućeg rada za uzorke klinički značajne koncentracije, idealno također procjenjujući "stabilnost" testa). Isto tako, laboratoriji trebaju na najmanju mjeru svesti mogućnost pogrješke poput one uzrokovane "hvatanjem" visokih doza. Poslijeanalitički zahtjevi: usredotočenost pozornosti na to kako se rabe rezultati može ohrabriti učinkovitiju kliničku primjenu pretraga na tumorske biljege. Važni su odgovarajući referentni rasponi, zajedno s naputkom o tome što čini značajnu ili klinički važnu promjenu. U slučaju AFP i hCG poželjno je izračunavanje poluživota. Kad se mijenja metoda, laboratorij treba napomenuti je li za očekivati da to utječe na tumačenje trenda u rezultatima biljega.

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the patient of requesting a tumor marker test should be considered before making the request, at which time the most relevant tumour marker should be selected. When making the request, careful consideration must also be given to specimen timing, as well as to the possible influence on results of other treatment and medication (e.g., previous treatment with mouse monoclonal antibodies) and/or other medical conditions (e.g., prostatitis for PSA). The specimen taken (serum, plasma, etc.) should always meet the requirements of the assay manufacturer. Analytical requirements: assays should meet defined quality requirements for both Internal Quality Control (IQC) (fulfilling objective criteria for acceptability of results for appropriately constituted IQC samples) and External Quality Assessment (EQA) (achieving satisfactory performance for specimens of clinically relevant concentration, ideally also assessing assay "stability"). Laboratories should also minimise the possibilities of error such as that caused by high dose "hooking". Post-analytical requirements: focusing attention on how the results are used can encourage more effective clinical use of tumour marker tests. Appropriate reference ranges, together with advice as to what constitutes a significant or clinically relevant change, are important. For AFP and hCG, calculation of the half-life may be desirable. When method changes are made, the laboratory should highlight whether it is likely to have affected interpretation of the trend in marker results.

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S6 – Simpozij 6 – PEDIJATRIJSKA LABORATORIJSKA MEDICINA, S6-1

Laboratorijska dijagnostika septičnih stanja u djece

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Bez obzira na sav napredak u medicini, dijagnostika septičnih stanja u djece, osobito novorođenčadi, i dalje predstavlja izazov za kliničare i laboratorijske znanstvenike. Zbog iznimno malog volumena krvi dostupnog za dijagnostičke svrhe samo u malim količinama nedonoščad ispod 1000 g čini podskupinu kojoj valja posvetiti osobitu pozornost. Već dugo i dobro utvrđen proteinski biljeg za otkrivanje i motrenje sepse, CRP, čini se "presporim", jer mu koncentracije rastu tek nakon što upalno stanje traje duže od 24 h. Citokini koji se oslobađaju ranije u tijeku upalnoga procesa, npr. interleukini 6 ili 8, postupno preuzimaju ulogu dijagnostičkih parametara izbora; međutim,

S6 – Symposium 6 – PEDIATRIC LABORATORY MEDICINE, S6-1

Laboratory diagnosis of septic states in children

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Regardless of all medical progress, the diagnosis of septic states in children, especially in newborns, remains a challenge to clinicians and laboratory scientists. Due to their extremely small blood volume which is available for diagnostic purposes only in minute amounts, preterm newborns below 1000 g represent a subgroup to which special attention has to be paid. The long-time well-established marker protein for the detection and monitoring of a sepsis, CRP, appears to be too "slow" since concentrations rise only after the inflammatory state has persisted for more than 24 h. Cytokines released earlier during the course of an inflammatory process, e.g.,

njihova je uporaba ograničena zbog nepostojanja visoko točnih i preciznih mehaniziranih metoda koje bi se mogle provoditi u rutinskom laboratoriju u kratkom vremenu do dobivanja nalaza kroz svih 24 sata i uz primjenu uzoraka volumena manjeg od 50 μL sreuma ili plazme. Interleukin 8, koji se može pouzdano odrediti primjenom samo 10 μL krvi, obećava kao biljeg septičnih stanja, ali za njega nema potpuno automatizirane metode. U novije vrijeme uvedeno je više POCT aplikacija semikvantitativnih metoda za određivanje citokina. Međutim, njihovu pouzdanost tek treba u potpunosti dokazati. Upravo se ispituje korisnost proteina koji veže lipopolisaharide (LBP). Uloga prokalcitonina (PCT) je proturječna u novorođenčadi zbog brze promjene njegove koncentracije tijekom prvih sati života. Osobito je važno otkrivanje uzročnika sepse, tj. (najčešće) bakterijskog patogena. Molekularne metode mogle bi uskoro postati moguće alternative klasičnim mikrobiološkim tehnikama za otkrivanje, identificiranje, pa čak i za ispitivanje osjetljivosti mikroorganizama.

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interleukins 6 or 8, have gradually occupied the role of the diagnostic parameters of choice; however, their use is limited by the lack of highly accurate and precise mechanised methods which can be performed in a routine laboratory with short turnaround times 24 hours a day using sample volumes below 50 μL serum or plasma. Interleukin 8, which may reliably be determined using only 10 μL blood, shows promise as a marker of septic states, but no fully automated method is available. A number of POCT applications of semiquantitative methods for cytokine determinations have recently been introduced. Their reliability, however, has not yet been proven sufficiently. Lipopolysaccharide binding protein (LBP) is currently under investigation for its usefulness. The role of procalcitonin (PCT) is discussed controversely in newborns due to the rapid change of its concentrations during the first hours of life. Of special importance is the detection of the causative agent of the sepsis, i.e. the (mostly) bacterial pathogen. Molecular methods may soon emerge as potential alternatives to classic microbiology techniques for the detection, identification and even susceptibility testing of microorganisms.

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S6-2

Izazovi novorođenačkog probira

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Novorođenački probir podrazumijeva sustavno pretraživanje cjelokupne populacije novorođenčadi određenog dijela ili cijele države na one bolesti koje su dostupne liječenju, a koje se klinički ne mogu dovoljno rano prepoznati. Da bi se neka bolest uvrstila u nacionalni program novorođenačkog probira, preporuka je zadovoljiti uvjete Svjetske zdravstvene organizacije koje su još 1968. godine postavili Wilson i Junger: razumno visoka učestalost, dostupnost liječenju, nemogućnost rane kliničke dijagnoze, prikladan laboratorijski test te povoljan odnos troškova programa prema ekonomskoj koristi od ranog otkrivanja i liječenja. No, ovisno o ekonomskim mogućnostima i zdravstvenoj politici nekih zemalja, u nacionalne programe novorođenačkog probira postupno su se uvodile i bolesti koje samo djelomice zadovoljavaju prije navedene uvjete: nedostatak biotinidaze, galaktozemija, cistična fibroza, neuroblastom, Duchenneova mišićna distrofija i

S6-2

The challenges of neonatal screening

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Neonatal screening implies systematic screening of the entire neonate population in a part of or in a country as a whole for the diseases eligible to treatment which cannot be clinically recognized early enough. For a disease to be included in the national program of neonatal screening, the requirements posed by the World Health Organization and originally proposed by Wilson and Junger as early as 1968 should be met: reasonably high prevalence, treatment availability, impossible early clinical diagnosis, appropriate laboratory test, and favorable ratio of the program cost to economic benefits of early detection and treatment. However, diseases that have only partially met the above criteria, e.g., biotinidase deficiency, galactosemia, cystic fibrosis, neuroblastoma, Duchenne's muscular dystrophy, and many others have been gradually included in the national programs of neonatal screening, depending on the economic resources and health policy

brojne druge. Prije deset godina počelo je i uvođenje tandemske spektrometrije masa (MS-MS) u programe novorođenačkog probira. Ta moćna tehnologija omogućila je otkrivanje specifičnih metabolita iz suhe kapi krvi za preko 30 nasljednih metaboličnih bolesti, ali samo mali dio njih je moguće i odgovarajuće liječiti. Mogućnost te tehnologije koristi se na razne načine i u pokusnim programima novorođenačkog probira lizosomskih bolesti nakupljanja. Programi novorođenačkog probira predstavljaju važan dio preventivne medicine. U Republici Hrvatskoj zasad je takav program organiziran za nasljednu metaboličnu bolest fenilketonuriju i konatalnu hipotireozu. U posljednje vrijeme nove tehnologije stvorile su mogućnosti za proširenje tog programa, ali mišljenja stručnjaka o toj problematici u pojedinim državama još se bitno razlikuju. Na njih utječu razlike u organizaciji zdravstvenog sustava, kulturni i vjerski običaji, te utjecaj javnog mišljenja i nevladinih organizacija. Valja napomenuti kako već i sama mogućnost predsimptomatske dijagnostike otvara niz ozbiljnih etičkih dilema i potrebno je u raspravu aktivno uključiti sve koji skrbe za takve bolesnike u pojedinoj državi.

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of particular countries. Some ten years ago, tandem mass spectrometry (MS-MS) was initially introduced in the program of neonatal screening. This powerful technology has enabled identification of specific metabolites from a dry blood drop for more than 30 hereditary metabolic disorders of which only a minor part can be properly treated. The opportunities offered by this technology have also been used in a number of modes in the pilot programs of neonatal screening for lysosomal storage diseases. The programs of neonatal screening are an important segment of preventive medicine. In Croatia, such a program has been launched for the hereditary metabolic disease phenylketonuria and perinatal hypothyroidism. Novel technologies have lately opened the possibility of program expansion, however, expert opinions on the issue still greatly vary among different countries, being influenced by differences in the structure of health care system, cultural and religious conventions, public opinion, and non-governmental structures. It should be noted that the very possibility of presymptomatic diagnosis opens an array of serious ethical issues, thus the respective debate should actively include all those providing care for these patients in a particular country.

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S6-3

Neonatalna hiperbilirubinemija i Gilbertov sindrom

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Hiperbilirubinemija je stanje povišene koncentracije bilirubina u krvi. Ovdje govorimo o patološkoj nekonjugiranoj neonatalnoj hiperbilirubinemiji kad koncentracija bilirubina u serumu prelazi 220 µmol/L. Toksični bilirubin, koji je lipofilan i može slobodno prolaziti kroz krvno-moždanu barijeru, ugrožava život novorođenčeta. Nekonjugirana hiperbilirubinemija može biti rezultat prirođenog poremećaja funkcije enzima glukuronizacije. Ako je aktivnost uridin difosfat glukuronil transferaze smanjena na 20%-50%, govorimo o Gilbertovu sindromu (GS). GS je uzrokovan polimorfizmom gena UGT1A1 u promotorskoj regiji. Izraženost gena je smanjena zbog insercije dodatnog TA para u element A(TA)6TAA, tako da nastaje A(TA)7-8TAA. Fototerapija je najuspješniji oblik liječenja neonatalne hiperbilirubinemije, ali može biti praćena nuspojavama. Kako bismo optimirali trajanje ozračivanja, pratili smo sniženje serumske koncentracije bilirubina za vrijeme ozra-

S6-3

Neonatal hyperbilirubinemia and Gilbert syndrome

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Hyperbilirubinemia is a state of increased bilirubin concentration in blood. We talk about pathological non-conjugated neonatal hyperbilirubinemia when the bilirubin concentration in serum exceeds 220 µmol/L. Toxic bilirubin, which is lipophilic and can freely pass through the blood-brain barrier, threatens the life of newborn infants. Non-conjugated hyperbilirubinemia can be the result of congenital impaired function of the glucuronization enzyme. If the activity of the uridine diphosphate (UDP) glucuronyl transferase is decreased to 20%-50%, we talk about Gilbert syndrome (GS). GS is caused by the gene UGT1A1 polymorphism at the promotor region. Expression of the gene is decreased due to an insertion of additional TA pair into the A(TA)6TAA element, so that A(TA)7-8TAA is formed. Phototherapy is the most successful treatment of neonatal hyperbilirubinemia, but side effects can occur. In order to optimize the duration

čivanja pomoću dviju laboratorijskih metoda. Cilj nam je isto tako bio procijeniti učestalost GS u slovenskoj populaciji. Koncentracija bilirubina određivala se je primjenom Jendrassik-Grofove (JG) metode (ukupni bilirubin) i modificirane kapilarne elektroforeze (CE) (nekonjugirani bilirubin) u 27 novorođenčadi podijeljene u tri skupine prema trajanju ozračivanja (4, 8 i 12 sati). Nakon izolacije DNA plazme, polimorfizam gena UGT1A1 određen je u 236 zdravih odraslih osoba u segmentu DNA umnoženom pomoću PCR primjenom SSCP analize. Osmosatna fototerapija dovela je do statistički značajnog sniženja vrijednosti bilirubina prema objema metodama: uz JG metodu 17,3% (p<0,01) i uz CE 17,8% (p=0,048). Nakon 12 sati ozračivanja sniženje koncentracije bilirubina bilo je također statistički značajno: uz JG 20,0% (p=0,029) i uz CE 26,2% (p=0,012). Incidencija TA polimorfizma u slovenskom pučanstvu sukladna je prosječnoj učestalosti u bjelačkoj populaciji. Mi smo utvrdili 13,9% homozigota s genotipom 7/7, 47,8% heterozigota s genotipom 6/7 i 37,7% osoba s genotipom 6/6. Studije dokazivanja polimorfizma u novorođenčadi s hiperbilirubinemijom još su u tijeku. Na osnovi rezultata dobivenih dvjema laboratorijskim metodama preporučamo 8-satnu fototerapiju za postizanje statistički značajnog sniženja koncentracije bilirubina. Kako je enzim UDP glukuronil transferaza uključen u glukuronizaciji određenih lijekova, dokazivanje GS je vrlo važno u slučaju novorođenčadi s hiperbilirubinemijom.

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of irradiation we studied the serum bilirubin concentration decrease during irradiation using two laboratory methods. Our objective was also to assess the prevalence of GS in the Slovenian population. In 27 newborn infants divided into three groups on the basis of the duration of irradiation (4, 8 and 12 hours), we determined the concentration of bilirubin using the Jendrassik-Grof (JG) method (total bilirubin) and modified capillary electrophoresis (CE) (non-conjugated bilirubin). After plasma DNA isolation, the UGT1A1 gene polymorphism was determined in 236 healthy adults in the DNA segment multiplied by PCR using the Single Strand Conformation Polymorphism analysis. The 8-hour phototherapy resulted in a statistically significant decrease in bilirubin values as assessed by either method: a decrease by 17.3% (p<0.01) by JG method and by 17.8% (p=0.048) by CE. After 12 hours of irradiation the decrease in the bilirubin concentration was also statistically significant: 20.0% (p=0.029) by JG method and 26.2% (p=0.012) by CE. The incidence of TA polymorphism in the Slovenian population was found to be consistent with the average prevalence in the Caucasian population in general. We detected 13.9% of homozygotes with 7/7 genotype, 47.8% of heterozygotes with 6/7 genotype, and 37.7% of individuals with 6/6 genotype. The studies of the polymorphism detection in newborns with hyperbilirubinemia are still in progress. Considering the results obtained by use of two laboratory methods, we recommend 8-hour phototherapy to achieve a statistically significant decrease in the bilirubin concentration. Because the UDP glucuronyl transferase enzyme takes part in the glucuronization of certain medications, detection of GS is of utmost importance in newborns with hyperbilirubinemia.

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S6-4

Laboratorijska dijagnostika alergijskih bolesti

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S6-4

Laboratory diagnosis of allergic diseases

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Među kroničnim dječjim bolestima alergijske su bolesti najčešće. Uglavnom se očituju simptomima na gornjim (rinitis) i donjim (astma) dišnim putovima, te na koži (atopijski dermatitis, urtikarija, angioedem) i probavnom sustavu (ezofagitis, gastroenterokolitis). Mogu biti različitih stupnjeva težine: od blagih atopijskih (ekcem, rinitis, as-

Allergic diseases are the most common chronic diseases in childhood, which can manifest on upper (rhinitis) and lower (asthma) airways, skin (atopic dermatitis, urticaria, angioedema) and gastrointestinal system (esophagitis, gastroenterocolitis). The severity of allergic diseases can vary from mild atopic (eczema, rhinitis, asthma) or malab-

tma) i malapsorpcijskih simptoma (dijareja, blago usporen rast djeteta) do akutne, za život opasne anafilaksije. Alergijski hod označava postupan prelazak simptoma alergijskih bolesti s jednog organskog sustava na drugi: u najranijoj dobi na probavnom sustavu, potom na koži; od 3. godine na donjim dišnim putovima; u školskoj dobi odnosno pubertetu na gornjim dišnim putovima. Zahtjevi što se postavljaju medicinskim biokemičarima odnose se na odabir pretraga koje bi trebale:

1. odrediti vrstu alergijske reakcije dokazivanjem humoralnih i staničnih posrednika alergijske reakcije. Primjenjuje se određivanje:
   - koncentracije ukupnog IgE
   - broja eozinofilnih i bazofilnih granulocita (test degranulacije), funkcija limfocita
   - koncentracija ECP
   - membranskih biljega bazofilne aktivacije, CD63 i CD45
   - oslobođenoga histamina iz bazofilnih granulocita.
2. otkriti pokretače alergijske reakcije određivanjem:
   - koncentracije specifičnih IgE (katkad i specifičnih IgG) na uzročne alergene
   - koncentracije antiglijadinskih (AGA-IgA,G), endomizijalnih (EMA-IgA) antitijela, te IgA-antitijela na tkivnu transglutaminazu (IgA-tTG) u djece sa celijakijom.
3. procijeniti klinički tijek reakcije (rana, kasna, produljena) određivanjem:
   - postojećih medijatora: triptaza (rana reakcija), ECP (kasna reakcija)
   - sintetiziranih posrednika alergijske reakcije: leukotrijena nakon izlaganja bazofilnih granulocita uzročnim alergenima (CAST).
4. omogućiti praćenje uspješnosti specifične imunoterapije određivanjem:
   - koncentracije specifičnih IgG
   - koncentracije blokirajućih podrazreda IgG4.
5. odrediti dijagnostičku djelotvornost određivanja pojedinih humoralnih ili staničnih posrednika alergijske reakcije određivanjem:
   - dijagnostičke osjetljivosti
   - dijagnostičke specifičnosti
   - pozitivne i negativne prediktivne vrijednosti .

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sorption (diarrhea) to acute, life-threatening anaphylaxis. Allergic march describes the chronologic progression from one clinical manifestation of allergy to the next one. Early life allergy under 3 years of age usually involves the gastrointestinal system, from 3 years on the skin, and thereafter up to puberty it involves lower airways (asthma). As asthma begins to stabilize, allergic rhinitis (upper airways) becomes a common manifestation of allergic disease in adolescents. Clinical chemist must be capable to implement such analyses that can:

1. define the type of allergic reaction (IgE-mediated, non-IgE mediated) – by determination of:
   - concentration of total IgE,
   - number of eosinophil granulocyte, basophil granulocyte (degranulation test), lymphocyte function tests,
   - concentration of ECP,
   - markers of basophilc activation, CD63 and CD45,
   - histamine release from activated basophil granulocytes.
2. find out the triggers of allergic reaction – by determination of:
   - concentration of allergen-specific IgE (sometimes allergen-specific IgG),
   - concentration of antigliadin antibodies (AGA-IgA,G), anti-endomysial antibodies (EMA-IgA), and human tissue transglutaminase IgA (IgA-tTG) in children with celiac disease.
3. assess the clinical coarse of allergic reaction (early, late, extended) – by determination of:
   - primary mediators: tryptase (early reaction), ECP (late reaction),
   - secondary mediators: leukotrienes after exposure of basophil granulocyte to provocative allergens (CAST).
4. monitoring of specific immunotherapy – by determination of:
   - concentration of specific IgG,
   - concentration of IgG4 as blocking antibodies.
5. estimate diagnostic efficiency of particular humoral and cellular mediators of allergic reaction – by determination of:
   - diagnostic sensitivity,
   - diagnostic specificity,
   - positive and negative predictive value.

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S6-5

Proteomski i genski biljezi autoimunih bolesti u djece

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Iako je etiopatogenetski koncept autoimunosti poznat već pola stoljeća, čak i uz enormnu ekspanziju biomedicinske tehnologije zadnjih dvadesetak godina, autoimune bolesti su još uvijek i kliničarima i biokemičarima svojevrsna "zona sumraka", često predstavljajući zamršeni dijagnostički i terapijski izazov. Klasični dijagnostički pristup autoimunosti, još davno utemeljen na imunološkim i serološkim tehnikama (raznovrsnim metodama detektiranja cirkulirajućih autoantitijela i prikaza njihove imunopatološke interakcije sa oboljelim stanicama i tkivima), zahtijeva korištenje složenih dijagnostičkih algoritama čija kumulativna senzitivnost, specifičnost i prediktivnost često ne dosižu klinički zadovoljavajuću razinu pouzdanosti.

Brzo i precizno uspostavljanje ispravne dijagnoze autoimune bolesti u pedijatrijskih bolesnika je zaseban imperativ, jer pravovremena i ispravna dijagnoza ne znači samo brzo započinjanje adekvatnog terapijskog postupka, nego i priliku za sekundarnu i tercijarnu prevenciju komplikacija i kroničnih posljedica sistemnih ili organskih autoimunih bolesti u djece.

Napredak kliničkih spoznaja o etiopatogenezi autoimunih bolesti, potpomognut uvođenjem suvremenih tehnika analize pojedinačnih humanih gena, ljudskog genoma u cijelosti, kao i razumijevanjem neposredne povezanosti proteinskih produkata ljudskih gena (proteoma) sa kliničkom ekspresijom autoimunih bolesti, omogućio je razvijanje novih analitičkih postupaka koji u sebi objedinjavaju dostignuća i biokemijske i genetičke tehnologije, a sa ciljem preciznog definiranja genskih i proteinskih biljega involviranih u patogenezu autoimunosti. Štoviše, definiranje točnih genetskih, genomskih i proteomskih biljega autoimunih bolesti u djece nije samo od neposredne dijagnostičke vrijednosti u prepoznavanju već razvijene slike bolesti, nego ima i visoku prediktivnu vrijednost u autoimunih bolesti, jer one načelno imaju dugotrajnu asimptomatsku, preklиničku fazu.

Perspektivno, takvi biljezi bi mogli služiti i kao sredstvo biokemijsko-genetičkog probira potencijalnih pedijatrijskih bolesnika unutar zdrave populacije. Naposljetku, laboratorijsko prepoznavanje genetičkih uzroka autoimunih bolesti u svjetlu snažnog razvoja novih tehnologija sekvencijske analize humanog genoma i genetskog dizajniranja rekombinantnih proteina, otvara neslućene mogućnosti u sve izvjesnijoj budućoj primjeni specifične genske terapije autoimunih bolesti u djece.

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S6-5

Proteomic and genetic markers of autoimmune diseases in children

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Although the etiopathogenic postulates of autoimmunity have been established almost a five decades ago, autoimmune diseases still present a kind of "twilight zone" for clinicians and biochemists, being a complex diagnostic and therapeutic challenge in spite of tremendous expansion of biomedical technologies during the recent years. Classical diagnostic approach to autoimmunity, based on immunological and serological techniques (including numerous methods of circulating antibodies detection and their interactions with affected cells and tissues), engages the complicated diagnostic algorithms, often with a questionable degree of cumulative sensitivity, specificity and positive predictive value.

It is a clinical necessity to establish a fast and accurate diagnoses in pediatric patients suffering from the autoimmune diseases, because the early and correct diagnoses enables not only the adequate therapeutic measures, but also the opportunity for secondary and tertiary prevention of complications and chronic consequences of the systemic or organ-specific autoimmune diseases in children.

The still expanding clinical knowledge about the etiopathogenesis of the autoimmune diseases, facilitated by introducing of the novel techniques for analysis of individual genes and the whole human genome, together with the understanding of etiological links between the peptide products of human genes (proteoms) and clinical expression of the autoimmune diseases, have lead to development of new analitic procedures based on biochemical and genetic accomplishments, aiming to precisely define the genetic, genomic and proteinomic markers involved in autoimmune pathogenesis. Moreover, the accurate genetic, genomic and proteinomic autoimmune markers definition could not only be of the immediate diagnostic value, but could also be highly predictive for the autoimmune diseases which usually have a long asymptomatic preclinical period.

In the future, these markers will probably be the useful tool for the biochemical and genetic screening of autoimmune diseases among the healthy pediatic population. Finally, propelled by enormous development of new technologies of human genome sequential analysis and genetic design of recombinant proteins, recognition of the genetic causes of autoimminuty will open a huge variety of possibilities for designing of specific gene therapy for autoimmune diseases in children.

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S7 – Simpozij 7 – GENSKE BOLESTI – NOVI PROFILI PRETRAGA, S7-1

Osiguranje kvalitete – EQA

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Molekularni testovi našli su put do kliničkog laboratorija. Sve više dijagnostičkih laboratorija rabi sve veći broj genetičkih pretraga. Za razliku od posljednjih godina, danas je to dopunjeno sve većim izborom komercijalnih setova i dijagnostičkih testova. Nadalje, u skoroj budućnosti biti će dostupni višeparametrijski sustavi testova, koji će laboratorijskim znanstvenicima omogućiti dobivanje desetaka i stotina genotipova iz samo jednog uzorka. Uz genotipiziranje, rutinski je dostupna kvantitativna analiza genske ekspresije, kao i epigenetičko profiliranje. Bez obzira na pitanje etičkih, zakonskih i socijalnih implikacija široko rasprostranjenog genetičkog testiranja, osiguranje kvalitete mora biti središnje pitanje u nadolazećim godinama, jer se je pokazalo kako se nedostatci u genetičkom testiranju mogu utvrditi u prijeanalitičkoj, analitičkoj i poslijeanalitičkoj fazi ispitivanja. Valja ustanoviti i unutarnje protokole i vanjske programe procjene kvalitete (EQAP) za genetički laboratorij. U posljednjih 10 godina donešeno je više EQAP u području humane genetike i kliničke kemije za provjeru dijagnostičkih testova u laboratorijima sudionicima. Prikazati će se izvori za takve EQAP. Nedavno je ustanovljen metodološki program EQAP (EQUAL) unutar programa EU FP6, koji se bavi procjenom kvalitete u genotipiziranju, kvantitativnoj PCR i sekvencioniranju DNA. Čini se kako metodološki, tj. tehnički problemi nisu rijetki u području genetičkog testiranja usprkos široko dostupnim sastavnicama laboratorijskih setova, te mogu dovesti do pogrješnih rezultata. EQUAL je otišao korak dalje nudeći radionice za izobrazbu i semestralnu poduku za one koji postižu slabije rezultate u okviru programa EQUAL. Nakon izobrazbe sudionici su bili daleko uspješniji na slijedećem EQAP, dokazujući kako procjena analitičke i dijagnostičke sposobnosti treba biti važno mjesto u kontekstu genetičkog testiranja (i vjerojatno u svim područjima laboratorijskih znanosti). Uz raspravu će se prikazati primjeri za gore navedeno.

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S7 – Symposium 7 – GENETIC DISEASES – NEW TEST PROFILES, S7-1

Quality assurance – EQA

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Molecular testing has found its way into clinical laboratory. An increasing number of genetic tests are being used by a rapidly growing number of diagnostic laboratories. In contrast to recent years, this is now complemented by a growing number of commercial kits and diagnostic tests. Furthermore, the very near future will bring multiparametric test systems allowing the laboratory scientist to obtain dozens and hundreds of genotypes from a single specimen. Next to genotyping, quantitative gene expression analysis and epigenetic profiling are also routinely available. Regardless of the question of ethical, legal and social implications of wide-spread genetic testing, quality assurance has to be a central issue in the coming years, since it has been shown that failures in genetic testing can be identified in preanalytical, analytical and postanalytical investigational steps. Both internal schemes and external quality assessment programs (EQAPs) need to be defined for the genetic lab. Over the last 10 years, a number of EQAPs have been established in the area of human genetics and clinical chemistry challenging diagnostic tests in the participating labs. Sources for such EQAPs will be given. Very recently, a methodological EQAP program (EQUAL) has been established within the EU FP6 program dealing with quality assessment in genotyping, quantitative PCR and DNA sequencing. It appears that methodological, i.e. technical problems are not uncommon in the genetic testing area despite the widely available laboratory kit components and can lead to failures in the results. EQUAL has gone one step beyond offering training workshops and sessions to under-achievers in the EQUAL program. Following training, the participants scored significantly higher in the next EQAP demonstrating that the assessment of analytical and diagnostic proficiency should be an important issue in the context of genetic testing (and probably in all areas of laboratory sciences). Examples will be given and discussed.

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S7-2

Molekularni i biokemijski profili pretraga za nasljedne bolesti

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Danas postoji više od 3000 poznatih nasljednih bolesti koje uzrokuju mutacije u DNA kodu. Srećom sve su te bolesti u općoj populaciji rijetke, no znakovite za određene etničke skupine i mogu se očitovati u djetinjstvu, ali i odrasloj dobi. U većini slučajeva liječenje nije moguće ili je ograničenog dometa, a očekivani životni vijek i kvaliteta života su ozbiljno ugroženi. Navedene se bolesti uobičajeno prenose autosomno-recesivnim, dominantnim ili X-vezanim nasljeđivanjem, a u njihovim ispitivanjima rabe se genetika, genomika i proteomika. Ispitivanje nositelja i prijenatalna dijagnostika za ove bolesti uglavnom nisu mogući konvencionalnim metodama ili su obilježeni ograničenom točnošću i osjetljivošću. Nasuprot tome, molekularno ispitivanje nukleinskih kiselina (DNA, RNA) primjenom analize ulomaka, sekvenciranjem i *chip* tehnologijom, uz obvezno sudjelovanje u međunarodnim kontrolama (EMQN), visoko je osjetljivo i pouzdano u prijenatalnoj i poslijenatalnoj dijagnostici za slijedeće nasljedne poremećaje: cistična fibroza, spinalna mišićna atrofija, mišićna distrofija tipa Duchenne, miotonična distrofija I i II, nasljedne motorne i senzorne neuropatije (CMT, HNPP), sindrom fragilnog X, Friedreichova i spinocerebelarne ataksije, Huntingtonova koreja, hemokromatoza, deficit alfa-1-antitripsina, hiperkolesterolemija, muška neplodnost, prirođena agenezija *ductus deferensa* i genodermatoze. Modernu laboratorijsku dijagnostiku nasljednih bolesti čine profili koji uključuju biokemijske i molekularne pretrage; tako, na primjer, kod X-vezane mentalne smetnje profil laboratorijskih pretraga čine T4, TSH, amino i organske kiseline u mokraći, citogenetika i molekularna genetika fragilnog X-kromosoma; kod cistične fibroze određuju se kloridi u znoju i mutacije u genu CFTR; kod mišićne distrofije profil pretraga uključuje CK i analizu gena i proteina; kod spontanih pobačaja to su LH, T4, TSH, FSH, SHBG, estradiol, prolaktin, kardiolipinska protutijela, lupus antikoagulant i analiza kromosoma; kod muške neplodnosti FSH, LH, prolaktin, testosteron, SHBG i FAI, status željeza, citogenetička analiza kromosoma i molekularna genetika koja uključuje mikrodeleciju kromosoma Y (regije AZFa, b i c), te probir na mutacije gena CFTR za cističnu fibrozu; kod hemokromatoze kao najčešće nasljedne bolesti profil pretraga obuhvaća željezo, TIBC, feritin i DNA pretrage

S7-2

Molecular and biochemical test profiles for hereditary disorders

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Currently there are over 3000 known hereditary genetic diseases caused by mutations in the DNA code. Fortunately, they all are rare in the general population, yet common in specific ethnic groups where they may manifest in childhood but also during adult age. In most cases, treatment is either unavailable or of limited benefit, and life expectancy and quality of life are severely compromised. These conditions are typically transmitted by autosomal recessive, dominant or X-linked inheritance, and their investigation involves genetics, genomics and proteomics. Carrier testing and prenatal diagnosis for these conditions by conventional methods are either unavailable or of limited accuracy and sensitivity. In contrast, molecular analysis of nucleic acids (DNA, RNA) that includes fragment analysis, sequencing and chip technology, and obligatory international quality control (EMQN), is extremely sensitive and reliable in prenatal and postnatal diagnosis of the following hereditary disorders: cystic fibrosis, spinal muscular atrophy, Duchenne muscular dystrophy, myotonic dystrophies I and II, hereditary motor and sensory neuropathies (CMT, HNPP), fragile X syndrome, Friedreich's and spinocerebellar ataxias, Huntington's chorea, hemochromatosis, alpha-1-antitrypsin deficiency, hypercholesterolemia, male infertility, congenital agenesis of ductus deferens, and genodermatoses. Modern laboratory diagnosis consists of profiles that include biochemical and molecular tests. Thus, e.g., laboratory test profile for X-linked mental handicap involves T4, TSH, urine for amino and organic acids, cytogenetics and molecular genetics of fragile X chromosome; cystic fibrosis profile includes determination of sweat chlorides and CFTR gene mutations; muscular dystrophy profile includes CK and analysis of dystrophin gene and protein; recurrent miscarriage profile (female) includes LH, T4, TSH, FSH, SHBG, estradiol, testosterone, prolactin, cardiolipin antibodies, chromosome analysis and lupus anticoagulant; male infertility profile includes FSH, LH, prolactin, testosterone, SHBG and FAI, iron status, cytogenetic chromosome analysis, and molecular genetics that involves Y chromosome microdeletion (AZFa, b and c regions), and screening for CFTR gene mutations for cystic fibrosis; test profile for hemochromatosis as the

mutacija C282Y, H63D; kod duboke venske tromboze pretrage KKS, kardiolipinska protutijela, faktor II (G20210A), faktor V Leiden (G1691A). Profili biomarkera za nasljedne bolesti danas su ključni dio laboratorijske dijagnostike, jer pružaju informacije na staničnoj i biokemijskoj razini pri identifikaciji nositelja, dijagnostici oboljelog fetusa te u dijagnostici ovih bolesti u odrasloj dobi.

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most frequent hereditary disease comprises iron, total iron binding capacity, ferritin, and DNA tests for C282Y and H63D mutations; deep vein thrombosis screening includes FBC, cardiolipin antibodies, factor II prothrombin mutation (G20210A) and factor V Leiden mutation (G1691A). Biomarker profiles for hereditary diseases are currently the essential part of laboratory diagnosis as they provide information at both cellular and biochemical level during carrier identification, diagnosis of an affected fetus, and diagnosis of the above disorders at adult age.

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S7-3

Uloga protrombotičnih rizičnih čimbenika u cerebrovaskularnim bolestima u djece

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Cerebrovaskularne bolesti (CVB) u dječjoj dobi su relativno rijetke s učestalošću od 2,3/100.000 na godinu, ali se ubrajaju u deset vodećih uzroka smrti. Za nastanak cerebrovaskularnih događaja poznati su rizični čimbenici bez genetičke podloge, a unatoč iscrpnim ispitivanjima etiološki čimbenik ili udruženi uzročnici ostaju nepoznati u 20–50% slučajeva. Dobro ustanovljeni genetički poremećaji povezani s moždanim udarom su anemija srpastih stanica, Fabrijeva bolest, MELAS, *moya-moya*, homocistinurija i nasljedni poremećaji metabolizma lipida. Postoje također podaci koji upućuju na važnost protrombotičnih poremećaja zbog promjena na razini gena koji reguliraju čimbenike zgrušavanja i fibrinolize. Iako su različiti genetički poremećaji proteina koji reguliraju sustav zgrušavanja i fibrinolize (FV Leiden, PT 20210A, nedostatak antitrombina, proteina C i proteina S) ustanovljeni kao rizični čimbenici za nastanak venske tromboze u odraslih, povezanost tih čimbenika s razvojem arterijske tromboze nije dokazana. Kako se rezultati ispitivanja u odraslih ne mogu preslikati na dječju dob zbog fiziološke razlike u hemostazi, učestalosti tromboze i uključenosti pojedinih rizičnih čimbenika, u posljednje vrijeme sve je veće zanimanje za utvrđivanje moguće genetičke podloge za nastanak CVB u djece. Rezultati dosadašnjih istraživanja ukazali su na povezanost nekoliko genetičkih rizičnih čimbenika tromboze s moždanim udarom: izolirani nedostatak proteina C, FV Leiden, PT20210A, MTHFR C677T i povišene vrijednosti lipoproteina(a). Za ostale trombofilne čimbenike poput PAI-1 4G/4G, FV HR2 haplotipa, nedostatka proteina

S7-3

The role of prothrombotic risk factors in children with cerebrovascular disease

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Although relatively rare in childhood, cerebrovascular diseases (CVD) are among the ten leading causes of death in children, with an incidence of 2.3/100,000 *per* year. Non-genetic risk factors responsible for the occurrence of cerebrovascular events are widely known, whereas the etiologic factor or combined causative agents remain obscure in 20%–50% of cases despite exhaustive investigations. The well established genetic disorders associated with stroke are: sickle cell anemia, Fabry disease, MELAS, moya-moya disease, homocystinuria and familial lipid abnormalities. There also are data indicating the importance of prothrombotic abnormalities due to defects in the coagulation and fibrinolytic system. Although various genetic disorders of the proteins regulating coagulation system and fibrinolysis (FV Leiden, PT 20210 A, antithrombin, protein C and protein S deficiency) have been established as risk factors for the occurrence of venous thrombosis in adults, the correlation between these factors and the development of arterial thrombosis is not conclusive. As results of studies in adults cannot be valid for the child's age due to physiologic difference in hemostasis, thrombosis frequency and involvement of individual risk factors, a growing interest has recently been observed in determination of the possible genetic basis of CVD in children. The results show that several genetic coagulation defects can be associated with stroke including isolated protein C deficiency, PT20210A, FV Leiden, methyltetrahydrofolate reductase (MTHFR C677T polymorphism) and elevated lipoprotein(a). Other factors

S i antitrombina nije dokazano da su povezani s povećanim rizikom moždanog udara u djece. Udruženost rizičnih čimbenika povezanih s nastankom moždanog udara najčešće uključuje FV Leiden i nedostatak proteina C, FV Leiden i povišenu vrijednost lipoproteina(a) ili MTHFR C677T. Također je pokazano da je prisutnost genetičkih rizičnih čimbenika povezana s ponovljenim moždanim udarom kod 3,3% do 19% djece. Učestalost genetičkih čimbenika razlikuje se također prema dobi, geografskom području i etiologiji moždanog udara. U zaključku, genetički čimbenici igraju značajnu ulogu u genezi moždanog udara u djece, a genetička ispitivanja kod djece mogu dati važne informacije za terapiju, prevenciju i ishod moždanog udara u djece.

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including plasminogen activator inhibitor 4G/4G, Factor V HR2 haplotype, protein S and antithrombin deficiency alone have not demonstrated an increased risk of pediatric stroke. Frequent combinations of genetic risk factors associated with stroke include FV Leiden and protein C deficiency, FV Leiden and elevated lipoprotein(a) or MTHFR C677T polymorphism. It has also been shown that genetic risk factors are associated with stroke recurrence in 3.3%-19% of children. The frequency of genetic factors differs according to age, geographic region, and stroke etiology. In conclusion, genetic factors play a greater role in the genesis of stroke in children than in adults, and genetic studies in children could provide important information regarding the treatment, prevention and outcome in children and adults with stroke.

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S7-4

Patobiokemija karcinoma

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Kolorektalni karcinom (CRC) je jedan od vodećih uzroka smrti od malignih bolesti u zapadnim civilizacijama. Tijekom posljednjih desetljeća incidencija CRC je u porastu. CRC je isto tako među najbolje istraženim karcinomima, bitan za naše razumijevanje razvoja neoplazije. Osobito je pokazano kroz uredan slijed genetskih defekata kako će se neoplazija razvijati i napredovati u potpun karcinom i metastaze (višestupanjska karcinogeneza, prema B. Vogelsteinu). Međutim, manje se zna o ranim fazama tumorskog razvoja koje prethode genetskim defektima kritičnih gena "vratara" ili "njegovatelja" poput wnt-signaliziranja/APC odnosno hMLH1/hMSH2. Danas postaje jasno da bi ovi dugo vremena smatrani prvim uzrocima mogli imati preteče u smislu negenetičkih defekata predstavljanih epigenetičkim pojavnostima poput genske metilacije ili drugih mehanizama koji utječu na razvojnu ili tkivno-specifičnu gensku izraženost zbog zasad nepoznatog razloga. Nas su zanimale najranija poznata tumorska oštećenja, te smo opisali izraženost staničnih adhezijskim molekula CEACAM tijekom diferencijacije stanica sluznice kolona. Primjerice, utvrdili smo kako je izraženost CEACAM1 regulirana naniže ili se gubi u velike većine vrlo ranih tumorskih stadija. Gubitak CEACAM1 zapaža se s istom učestalošću i u hiperplastičnim i u neoplastičnim tumorskim entitetima, te nastupa prije mutacije APC. Isto tako, gubitak CEACAM1 se zapaža u ranom i uznapredo-

S7-4

Cancer pathobiochemistry

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Colorectal cancer (CRC) is one of the leading causes of death from malignancy in western civilizations. During the last decades, the incidence of CRC has increased. CRC is also one of the best studied cancers and has been seminal to our understanding of the development of neoplasia. Specifically, it has been shown that through an orderly sequence of genetic defects neoplasia will develop and progress to full-fledged cancer and metastasis (multistep carcinogenesis according to B. Vogelstein). However, not much is known about the early steps of tumor development preceding the genetic defects of critical gatekeeper or caretaker genes like wnt-signaling/APC or hMLH1/hMSH2, respectively. It is becoming clear now that these long-thought first causes may have precedents in terms of non-genetic defects represented by epigenetic phenomena like gene methylation or other mechanisms influencing developmental or tissue-specific gene expression for some as yet unknown reason. We have been interested in the earliest tumor lesions known and have characterized expression of the CEACAM cellular adhesion molecules during the differentiation of colonic mucosa cells. For example, we find that CEACAM1 expression is down-regulated or lost in the great majority of very early tumor stages. CEACAM1 loss is observed at equal frequencies in both hyperplastic and neoplastic tumor entities, and occurs prior to APC mutations. Also,

valom adenomu, kao i kod CRC, no čini se da nije uzrokovan genetičkim mutacijama u samom genu. Zanimljivo je da je gubitak CEACAM1 pokazuje jednaku učestalost u tumorima CIN (wnt-signalizirajući defekti) i MSI (defekti u popravku krivo sparenih baza). Sveukupno, rezultati dobiveni za molekule u obitelji CEACAM ukazuju na to da bi molekularni defekti važni za razvoj tumorskih oštećenja mogli prethoditi fiksnim genetičkim mutacijama koje su se smatrale primarnim prvim uzrokom u višestupanjskoj karcinogenezi. Prikazati će se eksperimentalni rezultati koji upućuju na biološke funkcije kojima molekule CEACAM iskazuju svoja svojstva tumorske supresije.

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CEACAM1 loss is observed in early and advanced adenomas as well as in CRC, but appears not to be caused by genetic mutations in the gene itself. Interestingly, CEACAM1 loss is equally frequent in CIN (wnt-signaling defects) and MSI (DNA mismatch repair defects) tumors. Taken together, the results obtained for molecules in the CEACAM family suggest that molecular defects important for the development of tumor lesions can precede fixed genetic mutations that have been held as primary first causes in the multistep carcinogenesis. Experimental results that suggest biological functions by which CEACAMs exert their tumor-suppressing properties will be presented.

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S8 – Simpozij 8 – BILJEZI BOLESTI KRVOŽILNOG SUSTAVA, S8-1

Genetske i okolinske odrednice upalnih biljega: rezultati u Skupini Stanislas

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Upala je biološki proces koji leži u osnovi više kroničnih bolesti, uključujući rak i srčanožilne bolesti. Ispitivali smo varijabilnost intermedijarnog fenotipa upale uzrokovanu genetskim, biološkim i okolinskim (pojedinačnim i obiteljskim) čimbenicima. Ispitivanja su provedena u poduzorcima ispitne skupine Stanislas Family Cohort. Kvantifikacije su provedene primjenom proteinskog biochipa (evidence®) ili klasičnog ELISA testa. Genotipiziranje je provedeno mnogostrukim oruđem. Određivali smo biološke i okolinske čimbenike upletene u varijabilnost plazmatskih koncentracija IL-8 i MCP-1 (2 kemokina) te EGF i VEGF (2 faktora rasta) među pojedincima u navodno zdravih odralih osoba i djece. Uz to, opisane su referentne vrijednosti ovih četiriju količina. Također smo pokazali kako opća nasljednost EGF iznosi 26,9%, te da ju u potpunosti objašnjava obiteljska okolina. Polimorfizam 61A>G u EGF nije bio udružen s plazmatskom koncentracijom ovoga peptida. Kod VEGF smo utvrdili da genetika objašnjava 60,6% varijacije u koncentraciji, kao i sveukupnu opću nasljednost. Međutim, tri polimorfizma VEGF koje smo ispitivali, 460C>T, 405G>C i 936C>T, nisu imali učinka na koncentraciju VEGF u plazmi. Uz to, pokazali smo kako je polimorfizam 252A>G u genu LTA udružen s porastom koncentracije MCP-1 u plazmi. Konačno smo utvrdili odnose između novih upalnih biljega i klasičnog upalnog biljega, hs-CRP. Našli smo da broj leukocita i trombocita,

S8 – Symposium 8 - MARKERS OF VASCULAR DISEASES, S8-1

Genetic and environmental determinants of inflammatory markers: results from the Stanislas Cohort

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Inflammation is a biological process underlying several chronic diseases, including cancer and cardiovascular disorders. Therefore, we investigated the variability of intermediate phenotype of inflammation due to genetic, biological and environmental (individual and familial) factors. Studies were conducted on subsamples of the Stanislas Family Cohort. Quantifications were done either by using a protein biochip (evidence ®) or classic ELISA test. Genotyping was performed with a multiplex tool. We determined biological and environmental factors involved in the interindividual variability of plasma IL-8 and MCP-1 (2 chemokines) and EGF and VEGF (2 growth factors) concentrations in supposedly healthy adults and children. In addition, reference values of these four quantities were described. We also showed that general heritability of EGF was 26.9%, and that it was explained in total by familial environment. The 61A>G polymorphism of EGF was not associated with plasma concentration of this peptide. Concerning VEGF, we found that genetics explained 60.6% of the concentration variance and overall general heritability. However, the three VEGF polymorphisms we tested, i.e. 460C>T, 405G>C and 936C>T, had no effect on plasma VEGF concentration. In addition, we showed that the 252A>G polymorphism of the LTA gene was associated with an increase in plasma MCP-1 concentration. Finally, we determined relations

te koncentracije orozomucida, haptoglobina, IL-6 i ICAM-1 koreliraju s koncentracijom hs-CRP, dok koncentracije IL-8, IL-18, MCP-1, EGF, VEGF, IGF-1, IGFBP-3, TNF-α, TNF-RII te selektina E, L i P nisu korelirale s koncentracijom hs-CRP u fiziološkim uvjetima. Svi ovi rezultati pomoći će nam u tumačenju bioloških rezultata dobivenih u bolesnika te u razumijevanju regulacije ovih količina u fiziološkim uvjetima. Štoviše, naši rezultati potvrđuju složenost regulacije upalnoga procesa i mreže citokina.

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between the emerging inflammatory markers and a classic inflammatory marker, hs-CRP. We found that leukocyte and platelet counts, and orosomucoid, haptoglobin, IL-6 and ICAM-1 concentrations correlated with the concentration of hs-CRP, whereas IL-8, IL-18, MCP-1, EGF, VEGF, IGF-1, IGFBP-3, TNF-alpha, TNF-RII and selectins E, L and P concentrations did not correlate with hs-CRP concentration in physiological conditions. All these results will help us in the interpretation of biological results of patients and in the understanding of the regulation of these quantities in physiological conditions. Moreover, our results confirm the complexity of the regulation of the inflammatory process and the cytokine network.

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S8-2

Infarkt miokarda i hemostaza

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Infarkt miokarda (IM) predstavlja veliko opterećenje za zdravstvo kako u razvijenim tako i u novonastalim državama. Utvrđen je širok raspon rizičnih čimbenika, kao što su dob, spol, pretilost, pušenje, hipertenzija i razine kolesterola. Daljnja istraživanja bavila su se prognostičkom vrijednošću promijenjenih koncentracija hemostatskih sastavnica, kao što su fibrinogen i čimbenik aktiviranja trombocita (PAF). Protrombotski stadiji poput manjka proteina C i S, rezistencije aktiviranog proteina C ili antifosfolipidna protutijela odgovorni su za IM mlađe dobi. Napredak u razumijevanju genoma otkrio je višestruke polimorfizme gena kandidata povezane s upalom i metabolizmom lipida, kao i s hemostazom i trombozom. Patofiziološki se ateroskleroza opisuje kao proces koji je u tijeku, potaknut promijenjenim metaboličkim stanjima kao što su visoke razine LDL-kolesterola. Struktura arterijskih žila drastično se mijenja kroz godine i desetljeća. Ovi procesi su dobro dokumentirani i razjašnjeni. Ruptura aterosklerotskih plakova otkriva kolagenska vlakna, uzrokujući aktiviranje trombocita i time stvaranje tromba. Blokada žile nastupa kroz nekoliko sekunda. Sve veća usredotočenost na aktiviranje i inhibiciju trombocita obogatiti će već utvrđene primarne i sekundarne terapijske strategije kao što su inhibitori COX i HMG-CoA. Statini jasno smanjuju aterosklerotski rizik snižavanjem razina kolesterola u serumu i možda moduliranjem koagulacijskog sustava. Aspirin nepovratno deaktivira arahidonski metabolizam i suzbija sintezu tromboksana A2, što dovodi do smanjene

S8-2

Myocardial infarction and hemostasis

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Myocardial infarction (MI) poses a major health burden in industrialized and emerging countries. A broad range of risk factors have been established such as age, sex, obesity, cigarette smoking, hypertension, and cholesterol levels. Further insight considered changed concentrations of hemostatic components such as fibrinogen and platelet-activating factor (PAF) to be of prognostic value. Prothrombotic stages like protein C and S deficiency, activated protein C resistance or antiphospholipid antibodies are blamed for early age MI. Progression in understanding genome has unveiled multiple candidate gene polymorphisms to be related to inflammation and lipid metabolism as well as to hemostasis and thrombosis. Pathophysiological understanding describes atherosclerosis as an ongoing process triggered by altered metabolic conditions such as high LDL-cholesterol levels. Over years to decades, the structure of arterial vessels is severely changed. These processes are well documented and understood. Rupture of atherosclerotic plaques uncovers collagen fibers, causing platelet activation and therefore thrombus formation. This blockage of the vessel takes place within seconds. The increased focus on platelet activation and inhibition will enrich the established primary and secondary therapeutic strategies such as COX- and HMG-CoA inhibitors. Statins clearly reduce atherosclerotic risk by lowering serum cholesterol levels and possibly modulating the coagulation system. Aspirin inactivates irreversibly the arachidonic metabolism and suppresses

funkcije trombocita. Nove generacije protutrombocitnih sredstava već su dostupne za kliničku primjenu: inhibitori fosfodiesteraze, tienopiridini i antagonisti receptora glikoproteina IIa-IIIb. Ispituju se nove terapijske strategije poput antagonista trombinskih receptora, koji imaju moćan antitrombotski učinak u arterijskom trombu bogatom trombocitima, suzbijajući sposobnost trombina da proizvodi fibrin. Tkivni faktor (TF), ključni pokretač koagulacijske kaskade, oslobađa se iz endotelnih stanica, krvožilnih glatkomišićnih stanica i monocita. Suzbijanje djelovanja TF čini se privlačnim ciljem u liječenju akutnog koronarnog sindroma. Potrebna su daljnja istraživanja radi rješavanja novonastalih problema uzrokovanih otpornošću na aspirin i klopidogrel, te kako bi se osigurale bolje dijagnostičke i terapijske strategije u liječenju IM.

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thromboxane A2 synthesis leading to a decreased platelet function. New classes of antiplatelet agents have already become available for clinical use: phosphodiesterase inhibitors, thienopyridines and glycoprotein IIa-IIIb receptor antagonists. New therapeutic strategies are under investigation such as thrombin receptor antagonists, which exert potent antithrombotic effect in platelet-rich arterial thrombus by inhibiting the ability of thrombin to generate fibrin. Tissue factor (TF), a key initiator of the coagulation cascade, is released from endothelial cells, vascular smooth muscle cells and monocytes. Inhibition of TF action seems to be an attractive target for the treatment of acute coronary syndrome.
Further research is necessary to handle the emerging clinical problems caused by aspirin and clopidogrel resistance, and to provide better diagnostic and therapeutic strategies to treat MI.

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S8-3

NT-proBNP je vrijedan biljeg za kongestivno srčano zatajenje s dijagnostičkom i prognostičkom primjenom

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Lijeva ventrikulska disfunkcija (LVD) može nastati kao dio koronarne srčane bolesti, arterijske hipertenzije, bolesti zalistaka i primarne bolesti miokarda. Kongestivno srčano zatajenje (CHF) je klinički sindrom uzrokovan poremećajem srčane crpne funkcije. Za dijagnosticiranje LVD primjenjuju se klinički testovi i tehnike slikovnog prikaza, međutim, ta je dijagnoza djelomice subjektivna, dugotrajna i skupa. Natriuretski peptid tipa B (BNP) je jedan od četiriju natriuretskih hormona koji reguliraju krvni tlak, ravnotežu elektrolita i tekućinski volumen u odgovoru na tlačno preopterećenje. Biološki neaktivan prohormon, proBNP, uglavnom se luči iz lijeve srčane klijetke te se u tom procesu cijepa u fiziološki aktivni BNP i N-terminalni fragment NT-proBNP. Nekoliko je studija objavilo kako se BNP i NT-proBNP mogu rabiti za dijagnosticiranje kliničkih problema udruženih s LVD. Mi smo procijenili kliničku upotrebu NT-proBNP pomoću testa VITROS® NT-proBNP kao srčanog biljega uspoređujući koncentraciju NT-proBNP iz 484 uzorka dobivenih od osoba s dijagnozom CHF ili bez nje. Test VITROS® NT-proBNP uz VITROS® ECi/ECiQ Immunodiagnostic System i uporabu tehnologije Intellicheck® je brz, potpuno automatizirani test za NT-proB-

S8-3

NT-proBNP is a useful marker for congestive heart failure with diagnostic and prognostic applications

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Left ventricular dysfunction (LVD) can occur as part of coronary heart disease, arterial hypertension, valvular disease and primary myocardial disease. Congestive heart failure (CHF) is a clinical syndrome caused by impairment of the cardiac pumping function. Clinical tests and imaging procedures are used to diagnose LVD, however, the diagnosis is partially subjective, time consuming and costly. B-type natriuretic peptide (BNP) is one of four natriuretic hormones that regulate blood pressure, electrolyte balance and fluid volume in response to pressure overload. The biologically inactive prohormone, proBNP, is secreted mainly by the left ventricle of the heart and, in this process, is cleaved into physiologically active BNP and the N-terminal fragment NT-proBNP. Several studies have reported that BNP and NT-proBNP can be used for the diagnosis of clinical problems associated with LVD. We assessed clinical utility of NT-proBNP using VITROS® NT-proBNP assay as a cardiac marker by comparing the NT-proBNP concentration of 484 samples from individuals diagnosed with and without CHF. VITROS NT-proBNP combined with VITROS® ECi/ECiQ Immunodiagnostic System using Intellicheck® Technology is a rapid, fully auto-

NP u ljudskom serumu i plazmi. Rezultate za NT-proBNP analizirali smo prema odgovarajućim dogovorenim pragovima kako bismo procijenili učinkovitost ovoga biljega. Naši rezultati su pokazali kako postoji povezanost između težine kliničkih znakova i simptoma CHF i koncentracija NT-proBNP. Medijan koncentracija NT-proBNP u bolesnika svrstanih prema klasifikaciji NYHA kao I.-IV. iznosio je 741, 917, 1870, odnosno 1665 pg/mL. Usporedba kliničke osjetljivosti i specifičnosti provedena je pomoću ROC analize. Izračunato područje ispod krivulje iznosilo je 0,950. Ovako visoka vrijednost potvrđuje da ovaj test ima dobru sposobnost razlikovanja između bolesnih i referentnih skupina. Naši rezultati isto tako pokazuju kako test ima visoku negativni prediktivnu vrijednost, te se očekuje da rezultati za NT-proBNP u bolesnika s CHF prelaze dogovorene pragove u 97,4–100% vremena. U zaključku, potvrđujemo da je NT-proBNP koristan srčani biljeg i može se učinkovito rabiti kao pomoć u dijagnostici osoba sa sumnjom na CHF.

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mated assay for NT-proBNP in human serum and plasma. NT-proBNP results were analyzed against appropriate decision thresholds to assess the effectiveness of the marker. Our results showed that there was a relationship between the severity of the clinical signs and symptoms of CHF and NT-proBNP concentrations. The median NT-proBNP concentrations in patients classified as New York Heart Association classification I-IV were 741, 917, 1870 and 1665 pg/mL, respectively. The comparison of clinical sensitivity and specificity was performed using a Receiver Operator Curve. The area under the curve was calculated to be 0.950. This high value confirmed the assay to have a good capability of differentiating between the diseased and reference cohorts. Our results also showed the assay to have a high negative predictive value and NT-proBNP results in patients with CHF are expected to exceed decision thresholds 97.4%-100% of the time. In conclusion, we confirm that NT-proBNP is a useful cardiac marker and can be effectively used as an aid in the diagnosis of individuals suspected of having CHF.

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S8-4

Genska podloga bolesti krvožilnog sustava

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Ateroskleroza je multifaktorska bolest uzrokovana međudjelovanjem nasljednih i čimbenika okoliša. Vodeći je uzrok smrtnosti i smanjene radne sposobnosti u razvijenom svijetu. Neka od njenih temeljnih svojstava i dalje su slabo poznata. Iako mnogi rizični čimbenici utječu na njen razvoj, ateroskleroza je proces koji se češće događa na određenim mjestima u cirkulaciji i uzrokuje zasebne kliničke manifestacije. Ateroskleroza koronarnih arterija često uzrokuje infarkt miokarda i anginu pektoris. Kad zahvati arterije koje opskrbljuju središnji živčani sustav, uzrokuje moždanu kap i prolaznu moždanu ishemiju. U perifernoj cirkulaciji uzrokuje intermitentnu klaudikaciju i gangrenu. Na molekularnoj razini ateroskleroza je proces u kojem sudjeluju mnogi važni (pato)fiziološki mehanizmi, poput metabolizma lipoproteina, koagulacije i upale. Mutacije gena uključenih u bilo koji od tih mehanizama mogu rezultirati viškom ili nedostatkom ključnih proteina i tako narušiti homeostatsku ravnotežu. Intermedijerni fenotipovi poput hipertenzije, dijabetesa, pušenja i debljine također djeluju i na taj način mijenjaju ukupni rizik za pojavu i napredovanje bolesti. Budući da je koronarna bolest

S8-4

Genetic background of cardiovascular disease

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Atherosclerosis is a multifactorial disease caused by interaction of hereditary and environmental factors. It is the leading cause of death and work disability in industrialized countries. Some of its fundamental characteristics remain unknown. Although its development is influenced by numerous risk factors, atherosclerosis is a process which takes place more frequently at specific locations within the circulation thus causing separate clinical manifestations. Atherosclerosis of coronary arteries frequently causes myocardial infarction and angina pectoris. Once the arteries supplying the central nervous system are affected, it results in stroke and transitory brain ischemia. In peripheral circulation it results in intermittent claudication and gangrene. At the molecular level, atherosclerosis is a process which involves many important (patho)physiological mechanisms such as lipoprotein metabolism, blood clotting and inflammation. Mutations in the genes involved in any of these mechanisms may result in excess or lack of key proteins and thereby lead to imbalanced homeostasis. Intermediate phenotypes such as high blood pressure, diabetes, smoking and obe-

srca poligenski uvjetovana, svaka pojedina mutacija ili polimorfizam pridonijeti će tek umjereno ili u maloj mjeri ukupnom riziku. Na konačan rizik utjecati će i interakcije među genima, kao i interakcije između gena i okoline.

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sity also contribute and alter the overall risk of onset and progression of the disease. Since coronary heart disease is a polygenic disease, each mutation or polymorphism adds to the total risk only moderately. The final risk is influenced by gene interactions as well as by gene-environment interactions.

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S8-5

Diferencijalna dijagnostika i prognostički biljezi moždanog udara

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Moždani udar (MU) je vrlo heterogena bolest. Velike su razlike među bolesnicima s obzirom na težinu i podtip MU. Tehnike slikovnog prikaza su temelj pravilnog prepoznavanja i liječenja, jer do 25% slučajeva s kliničkom slikom MU čine stanja koja oponašaju MU. Stoga se intenzivno istražuju novi biokemijski pokazatelji koji bi bili nadopuna tehnikama slikovnog prikaza i pomogli u razlučivanju radi li se o MU ili ne, radi li se o ishemiji ili hemoragiji, ishemiji ili tranzitornoj ishemijskoj ataki, koliki je stupanj penumbre i površina oštećenja, ako se potvrdi ishemija i razmatra terapija trombolizom, mogu li se predvidjeti komplikacije, intracerebralna hemoragija ili maligni edem mozga. Do danas su proučavani brojni biokemijski pokazatelji, oni podrijetlom iz neurona ili glija stanica, posrednici upalne reakcije, no nisu zaživjeli u kliničkoj praksi, jer nisu pokazali dovoljnu dijagnostičku točnost. Imali su relativno nisku osjetljivost i specifičnost, i relativno bi se kasno otkrili u sistemskoj cirkulaciji (nakon 6-12 sati). Metaloproteinaze matriksa (MMP) i njihovi inhibitori (TIMP) imaju važnu, ali različitu ulogu u pojedinim fazama i podtipovima MU. U akutnom MU djeluju štetno, dok u već razvijenom MU imaju učinka u procesima oporavka. Kako je u više radova potvrđena dijagnostička učinkovitost MMP-9 u akutnom MU, ispitivali smo promjene u koncentraciji odabranih MMP (-2, -9) i TIMP (-1, -2) u cirkulaciji bolesnika s različitim podtipovima MU podijeljenih prema klasifikaciji Oxfordshire Community Stroke Project (OCSP). Obuhvaćeno je 126 bolesnika s akutnim ishemijskim MU i 124 kontrolna ispitanika. U bolesnika je ustanovljena niža koncentracija MMP-2, omjera MMP-2/TIMP-2 ($p<0,001$) i viša koncentracija TIMP-2 ($p<0,001$) nego u kontrolnih ispitanika. Koncentracija MMP-9 i omjer MMP-9/TIMP-1 su bili viši u bolesnika s potpunim cirkulacijskim infarktom prednje cirkulacije (TACI) nego u bolesnika s drugim podtipovima MU prema

S8-5

Differential diagnosis and prognostic markers of stroke

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Human stroke is a very heterogeneous disease. Stroke varies widely in severity and may present different subtypes. Imaging is the mainstay of identifying stroke type because it is not possible to distinguish ischemia from hemorrhage reliably on clinical grounds alone. Stroke mimics comprise even 25% of cases presenting as a stroke. Consequently, establishing a rapid and accurate diagnosis has recently emerged as a pivotal issue. The new biochemical markers may in the future be used in combination with neuroimaging techniques to help the diagnosis and other crucial issues raised during the acute phase of stroke: these include early distinction between ischemic and hemorrhagic stroke, transient ischemic attack and established stroke, the degree of ischemic penumbra, the extent of definitive brain tissue damage, the measurable effects of thrombolytic therapies, and the prognosis and risk of complications, intracerebral hemorrhage and malignant edema. Previously studied plasma markers, mainly neuronal and glial markers of stroke, inflammation mediators, have not found application in routine clinical practice. They display a relatively low sensitivity and specificity, and their serum/plasma concentrations tend to increase rather late in the course of brain injury (beyond 6 to 12 hours). Matrix metalloproteinases (MMPs) and their natural inhibitors (TIMPs) play a key but different role in different stroke phases and stroke types. In the acute phase of stroke MMPs have a detrimental effect, whereas in established stroke MMPs participate in the healing process. Several recent publications have suggested MMP-9 as a reliable marker in the differential diagnosis of stroke. Our aim was to determine modulation of serum levels of selected MMPs (-1, -2) and TIMPs (-1, -2) in stroke types subdivided according to Oxfordshire Community Stroke Project (OCSP) classification. The study

klasifikaciji OCSP (p=0,0019 odnosno p=0,0065) i kontrolnih ispitanika (p<0,0001 odnosno p=0,0024). Negativna je korelacija uočena između koncentracije MMP-2 s MMP-9 i omjerom MMP-9/TIMP-1 u svim podtipovima MU osim u TACI. Analiza ROC je pokazala podjednaku dijagnostičku točnost koncentracije MMP-9 i Barthelova indeksa u diferencijalnoj dijagnozi TACI. Visoki je omjer MMP-9/TIMP-1 (omjer izgleda 3,263) povezan s TACI. Na temelju naših rezultata zaključujemo da omjer MMP-9/TIMP-1 može pružiti dodatnu informaciju s ciljem boljeg praćenja bolesnika i to kao pokazatelj proširenosti infarkta.

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included 126 patients with acute stroke within the first 24 hours of symptom onset, and 124 healthy volunteers. The stroke group had lower MMP-2 concentration and MMP-2/TIMP-2 ratio (p<0.001) but higher TIMP-2 (p<0.001) than controls. The level of MMP-9 and MMP-9/TIMP-1 ratio were higher in patients with total anterior circulation infarct (TACI) than in patients with other stroke subtypes according to OCSP classification (p=0.0019 and p=0.0065, respectively) or controls (p<0.0001and p=0.0024, respectively). A negative correlation of MMP-2 levels with MMP-9 and MMP-9/TIMP-1 ratio was recorded in all stroke subtypes except for TACI. The Receiver Operating Characteristic analysis showed a similar discriminating power for MMP-9 levels and Barthel index in the differential diagnosis of TACI. High MMP-9/TIMP-1 ratio (odds ratio 3.263) was associated with TACI. Our results demonstrate that the MMP-9/TIMP-1 ratio may provide information to help in assessing stroke patients in the future as a baseline biomarker of the infarct extent.

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S8-6

Vrijednost kardiovaskularnih biljega kod morbidne pretilosti

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S porastom indeksa tjelesne mase (BMI) proporcionalno raste volumen krvi i ejekcijska frakcija, što dovodi do hipertenzije i hipertrofije stijenke lijeve klijetke (LVH). U asimptomatskih pretilih osoba je dijagnosticiranje ovih patologija složeno, jer ehokardiografija ne spada među rutinske pretrage u ovih osoba. Rano dijagnosticiranje LVH osigurava brzo liječenje u pretilih osoba i time znatno poboljšava stopu smrtnosti. Sve je više dokaza za to da pretilost može aktivirati reninsko-angiotenzinski i neurohumoralni sustav kroz bubrežnu disfunkciju praćenu povišenim lipidima i netolerancijom glukoze. To je pak udruženo s višim razinama triglicerida, povišenim LDL-C, niskim HDL-C i aterogenim LDL, pa predstavlja stvaran i potencijalan rizik za kardiovaskularnu bolest (CVD). Inzulinska rezistencija je povezana s nizom metaboličnih nenormalnosti, uključujući dijabetes, dislipoproteinemiju, hipertenziju i aterosklerozu, protrombotična stanja s vrlo visokim rizikom za koronarnu bolest. Kao primjer, uveli smo razine BNP podrijetlom iz lijeve ventrikulske mase.

S8-6

Value of cardiovascular markers in morbid obesity

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With the increasing body mass index (BMI), blood volume and ejection fraction rise in the same manner, leading to hypertension and hypertrophy of the left ventricular wall (LVH). Diagnosing pathologies in the asymptomatic obese is complicated, as echocardiography is not part of the routine checkup in these individuals. Especially the early onset diagnosis of LVH ensures an immediate treatment of the obese and therefore markedly improves the mortality rate. There is increasing evidence that obesity may activate renin-angiotensin and neurohumoral systems through renal dysfunction, accompanied by increased lipids, and glucose intolerance. It is associated with higher levels of triglycerides, elevated LDL-C, low HDL-C and atherogenic LDL, posing actual and potential risk for cardiovascular disease (CVD). Insulin resistance is related to a constellation of metabolic abnormalities, including diabetes, dyslipoproteinemia, hypertension, and atherosclerosis, prothrombotic states which are at a very high risk of coronary disease. As an example, I introduce

Razine BNP koreliraju s ehokardiografski potvrđenom LVH i klasifikacijom NYHA u pretilih bolesnika. Mi rabimo BNP kao parametar probira u osoba s morbidnom pretilošću. Ispitali smo 61 bolesnika s morbidnom pretilošću (52 žene/9 muškaraca srednje dobi od 41,15±10,13 godina) s BMI od 45,27±6,1 kg/m^2. Skupina od 57 osoba normalne tjelesne težine podjednake dobi i spola služila je kao kontrolna skupina (BMI 22,58±3,14 kg/m^2). Nakon 8-satnog razdoblja gladovanja odredili smo BNP (normalna vrijednost <250 fmol/L), te glukozu i profil lipida. BNP smo mjerili pomoću BNP-ELISA (Biomedica, Beč).

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BNP levels deriving from the left ventricular mass. BNP levels correlate with echocardiography verified LVH and NYHA-classification in the obese patient. We use BNP as a screening parameter in the morbidly obese. We examined 61 patients suffering from morbid obesity (52 female/ 9 male; aged 41.15±10.13 years) with a BMI of 45.27±6.1 kg/m^2. A group of 57 normal-weight age- and sex-matched individuals served as controls (BMI 22.58±3.14 kg/m^2). After an 8-hour fasting period, BNP (normalvalue <250 fmol/L), the glucose and lipid profile were determined. BNP was measured with BNP-ELISA (Biomedica, Vienna).

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S9 – Simpozij 9 – KOŠTANI BILJEZI, S9-1

Koštani biljezi i njihova primjena u kliničkoj dijagnostici

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Broj kliničkih slučajeva osteoporoze brzo se povećava širom svijeta. SZO predviđa da će se incidencija osteoporotskih fraktura (proksimalni femur) povisiti više nego dvostruko kroz slijedećih 50 godina (u zemljama EU, 414.000 Fx u 2000. godini, uz očekivani porast na 972.000 u 2050. godini). Svaki prijelom umnožava rizik od naknadnih prijeloma, poglavito kralježnice. Odraz koštanog premodeliranja, što je najbolji predskazatelj osteoporoze i rizika od frakture može procijeniti pomoću:

- mjerenja mineralne gustoće kostiju (BMD) pomoću DXA
- histomorfometrije kostiju
- posebnih metoda slikovnog prikaza i naknadnih biopsija
- mjerenja koštanih biljega u krvi i/ili mokraći

Danas se od biljega koštane resorpcije najviše rabe CTx i NTx, dok se od biljega koštane sinteze najviše upotrebljavaju aktivnost ALP, te koncentracije osteokalcina i PINP. Nova znanstvena dostignuća u patobiokemiji i patofiziologiji donose primjenu novih biljega, uglavnom iz obitelji interleukina (OPG, RANK, RANKL itd.). Glavne prednosti primjene koštanih biljega su slijedeće:

- širok spektar biljega
- laka dostupnost
- brz odgovor na terapiju
- cijena (biljezi su relativno jeftini)

S9 – Symposium 9 – BONE MARKERS, S9-1

Bone markers and its use in clinical diagnosis

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The number of clinical cases of osteoporosis is growing rapidly worldwide. WHO has estimated the incidence of osteoporotic fractures (proximal femur) to increase more than twice during the next 50 years (EU countries 414,000 Fx in 2000, expected to rise to 972,000 in 2050). Every fracture multiplies the risk of subsequent fractures, of the spine in particular. The reflection of bone remodeling, which is the best predictor of osteoporosis and fracture risk, can be estimated by:

- measurement of bone mineral density (BMD) by use of DXA
- bone histomorphometry
- special imaging methods and subsequent biopsies
- measurement of bone markers in blood and/or urine

CTx and NTx are bone resorption markers most widely used today. Concerning markers of bone synthesis, the activity of ALP and concentrations of osteocalcin or PINP are most frequently used. New scientific developments in the pathobiochemistry and pathophysiology propose the use of new markers, mostly from the interleukin family (OPG, RANK, RANKL, etc.). The main advantages of the use of bone markers re:

- broad spectrum of markers
- easy access
- quick response to therapy
- price (markers are relatively inexpensive)

Glavni nedostatci uporabe koštanih biljega su slijedeći (u analitičkom dijelu):
- nema sljedljivosti
- nema standardizacije
- visoka razina nesigurnosti mjernih postupaka
- visoka varijabilnost

(u biološkom dijelu):
- nema specifičnosti
- visoka biološka varijabilnost

Glavni ciljevi (i pitanja) kod primjene koštanih biljega su slijedeći:
- razvoj novih biljega koji će koštani metabolizam odražavati bolje od klasičnih
- bolje razumijevanje podloge koštane resorpcije i sinteze
- specifičan odraz učinaka lijekova pomoću biljega koji najbolje odgovaraju određenoj vrsti liječenja
- bolja prediktivna vrijednost

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The main disadvantages of the use of bone markers are (in the analytical part):
- no traceability
- lack of standardization
- high uncertainty of measurement procedures
- high variability

(in the biological part):
- no specificity
- high biological variability

The main targets (and questions) in the use of bone markers are:
- development of new markers, reflecting bone metabolism better that classic ones
- better understanding of the backgrounds of bone resorption and synthesis
- specific reflection of drug effects by use of markers according to type of therapy
- better predictive value

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S9-2

Koštani biljezi u hiperparatiroidizmu

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Jedna od komplikacija hiperparatiroidizma je metabolična koštana bolest. Primarni se hiperparatiroidizam liječi kirurškim zahvatom, nakon čega odmah slijedi normalizacija koštanog metabolizma koja se može pratiti padom koštanih biljega u krvi i u mokraći. Složenije je naravi metabolična koštana bolest bubrežna osteodistrofija koja se javlja u 70% do 100% kroničnih bubrežnih bolesnika kada se glomerularna filtracija snizi ispod 60 mL/min. Bubrežna koštana bolest javlja se u jednom od tri oblika koji se lako mogu transformirati jedan u drugi. Najčešći je sekundarni hiperparatiroidizam s ubrzanom koštanom pregradnjom, potom miješani oblik te, poglavito u starijih bolesnika, adinamična koštana bolest s osteomalacijom (usporeni koštani metabolizam). Od bubrežne osteodistrofije pate gotovo svi bolesnici na hemodijalizi, što im izrazito povećava pobol i smrtnost. Kalcitriol i kalcijeve soli suprimiraju parathormon, ali ubrzavaju vaskularnu kalcifikaciju, kardiovaskularni pobol, adinamičnu koštanu bolest i sklonost frakturama. Metoda histomorfometrije zlatni je standard u dijagnostici koštane lezije, no invazivna je, skupa, ograničena samo na kost zdjelice i nije rutinska. Stoga je primjena biokemijskih biljega važna u dijagnostici i kontroli liječenja bubrežne osteodistrofije. Fragmenti kolagena – biljezi koštane pregradnje filtriraju se insuficijentnim

S9-2

Bone markers in hyperparathyroidism

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Metabolic bone diseases develop as a complication of primary and secondary hyperparathyroidism. Patients with primary hyperparathyroidism are surgically treated, which results in normalization of bone metabolism, as indicated by bone markers. Bone disease observed in 75%-100% of patients with chronic renal failure is more complex, as the glomerular filtration rate falls below 60 mL/min. Renal bone disease can be subdivided into three groups which are not fully separate entities and may easily transform from one to another. Hyperparathyroid (high-turnover) bone disease is most frequently followed by mixed osteodystrophy and low-turnover osteodystrophy (osteomalacia and adynamic bone disease). The resulting abnormalities in bone and mineral metabolism play a significant role in the morbidity and mortality of chronic renal failure patients. Nearly all patients requiring chronic maintenance dialysis therapy develop abnormal bone histology. Calcitriol and calcium salt are used to suppress PTH and improve osteomalacia but these agents predispose to the development of vascular calcification, cardiovascular morbidity, low-turnover bone disease and fracture. Urinary markers of collagen breakdown are filtered by the glomeruli and chronic renal failure patients cannot effectively clear it, which results in

glomerulima pa su njihove serumske razine povišene. Istodobno je pojačana i koštana izgradnja. Sadržaj biljega razgradnje u krvi i mokraći važan je podatak kojim se procjenjuje aktivnost osteoklasta te usmjerava kliničare u izboru i kontroli učinkovitosti terapije. Biljezi izgradnje dobro koreliraju s promjenama mineralne gustoće kostiju i prednjače vremenski pred denzitometrijom. Klinička ispitivanja ističu osteokalcin i N-terminalni propeptid prokolagena P1NP za procjenu aktivnosti osteoblasta i predviđanje gubitka koštane mase. Povišen serumski osteokalcin odražava smanjeni bubrežni klirens ili metaboličnu koštanu bolest, ili pak oboje. Klirens intaktnog P1NP posredovan je endotelnim stanicama jetre pa je njegov serumski sadržaj neovisan o bubrežnoj disfunkciji i zato najobjektivniji pokazatelj koštane dinamike u uremičnih bolesnika. U zaključku, biokemijski koštani biljezi vrijedan su klinički pokazatelj koštane pregradnje i dinamike cjelokupne koštane mase, što otvara stvarne mogućnosti da bi danas nenadomjestiva biopsija kostiju mogla naći zamjenu u dijagnostici razlikovanja ubrzane od usporene koštane pregradnje.

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elevated serum levels. Besides, a developed bone disease affects bone formation and breakdown. Bone resorption markers are used in an attempt to assess the extent of osteoclastic bone resorption as well as guidance in selecting therapy and therapeutic response monitoring. Bone formation markers correlate with changes in the bone mineral density *per* year. Especially, N-terminal propeptide of type I procollagen P1NP and osteocalcin provide better discrimination and indices of osteoblast function in hemodialysis patients and thus are clinically useful for predicting bone loss. P1NP marker may provide a more reliable assay for bone formation, particularly in uremic patients, because specific receptor-mediated clearance of intact PINP occurs by hepatic endothelial cells and is unaffected by renal dysfunction. An elevated level of osteocalcin reflects either reduced renal clearance or metabolic bone disease, or an interaction of both conditions. Bone markers are important clinical tools in patient management and would be useful, as they may replace the invasive methods (bone biopsy and histomorphometry remains the gold standard) that are required to distinguish between the high and low bone turnover.

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S9-3

Razvojna dinamika biljega koštane pregradnje na primjeru adolescentne anoreksije nervoze

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S9-3

Developmental dynamics of bone turnover markers: an example of adolescent anorexia nervosa

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Sticanje optimalnog mineralnog sadržaja kostiju tijekom dječje i adolescentne dobi ovisno je o međusobnom djelovanju prehrambenih i hormonskih činitelja, te općenito načina života. Za razliku od stabilnih koštanih dimenzija u odraslih, razvojne promjene odnose se na veličinu i geometriju kostiju, a mineralni sadržaj ovisan je o kronološkoj dobi, ali prije svega o koštanoj zrelosti i stadiju pubertetskog razvoja. U vrijeme prije puberteta biljezi osteogeneze i razgradnje kostiju održavaju se na jednoj konstantnoj razini s koncentracijama koje su više nego u odraslih, ali niže nego u dojenčadi, te nisu spolno ovisne. Tijekom puberteta odvijaju se dvije važne pojave: ubrzani longitudinalni rast i pojačana mineralizacija kostiju. U djevojčica najveće ubrzanje rasta odvija se u ranom pubertetu (P1-P3), a mineralno nakupljanje je najveće u kasnim fazama (P3-P4), kada se postiže glavnina koštanog

The acquisition of optimal mineral bone content during childhood and adolescence depend on the interaction of dietary and hormonal factors and lifestyle in general. In contrast to stable bone dimensions in adults, developmental changes refer to bone size and geometry, while bone mineral content depends on chronologic age and primarily on bone maturity and stage of pubertal development. During the prepubertal period, the markers of osteogenesis and bone degradation are maintained at a constant level with concentrations exceeding those in adults but lower than those measured in infants, and are not sex dependent. Two important phenomena occur during puberty: accelerated longitudinal growth and enhanced bone mineralization. In female children, the highest rate of growth acceleration occurs in early puberty (P1-P3), while highest mineral accumulation is recorded

miraza. Mineralizacija se usporenim tempom nastavlja nakon spolne i koštane zrelosti, te negdje krajem drugog desetljeća postiže oko 90% vršne koštane mase. Tijekom pubertetskog modeliranja i remodeliranja kostiju biljezi anabolizma i katabolizma su povišeni, a u vrijeme postizanja vršne koštane mase oba procesa su u ravnoteži. Najviše kocentracije osteokalcina (OC) u djevojčica postižu se s 12 godina života, a zatim se već s 15-16 godina dosižu vrijednosti odraslih. Slična dinamika opisana je i za resorpcijske biljege. Anoreksija nervoza (AN) tipično se javlja u vrijeme kada se očekuju sticanja glavnine koštanog miraza, pa je deficit koštane mase ocijenjen s DEXA veći u adolescentnoj nego adultnoj AN. U akutnoj fazi bolesti prevladava teška pothranjenost uz konstelaciju adaptivnih metaboličnih i hormonskih promjena, prije svih hipogonadotropni hipogonadizam. U predpubertetskoj pojavi pubertet je odgođen, bolest u vrijeme puberteta usporiti će pubertetski razvoj, uključujući zasotatak u rastu i smanjenu mineralizaciju kostiju. Pojava nakon menarhe rezultirati će sekundarnom amenorejom. AN je tipičan primjer neravnoteže koštane pregradnje, obilježene smanjenim stvaranjem i pojačanom resorpcijom kosti, što se biokemijski odražava sniženim koncentracijama biljega osteogeneze poput OC, PINP (N-propeptid prokolagena 1) uz povećanje koncentracije resorpcijskih biljega poput CTX (C-telopeptid kolagena 1) ili NTX (N-telopeptid kolagena 1). Unatoč napretku koje je donijelo određivanje biljega koštane pregradnje, bilo u fiziološkim procesima modeliranja i remodeliranja ili u kostima zahvaćenim bolešću, interpretacija rezultata neće uvijek biti laka, osobito u nedostatku razvojnih, referentnih podataka u vršnjaka.

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in later phases (P3-P4) when most of the bone dowry is acquired. Mineralization is continued at a slower pace after sexual and bone maturity to reach some 90% of the peak bone mass towards the end of the second decade of life. During the pubertal bone modeling and remodeling, the anabolism and catabolism markers are increased to reach balance at the time of attaining peak bone mass. In female children, the highest osteocalcin concentration (OC) is achieved at age 12 to reach adult values as early as age 15-16. Resorption markers follow a similar dynamics. Anorexia nervosa (AN) typically occurs at the time of the expected acquisition of the majority of bone dowry, thus the bone mass deficit evaluated by DEXA is greater in adolescent AN than in adult AN. The acute stage of the disease is predominated by severe malnutrition associated with an array of adaptive metabolic and hormonal changes, primarily hypogonadotropic hypogonadism. In case of prepubertal manifestation, the onset of puberty is delayed, while the disease concurrence with puberty will slow down pubertal development, including growth retardation and reduced bone mineralization. The disease manifestation after menarche results in secondary amenorrhea. AN is a typical example of bone turnover imbalance, characterized by a decreased bone formation and enhanced bone resorption, which is biochemically reflected by decreased concentrations of the markers of osteogenesis, e.g., OC, PINP (procollagen 1 N-propeptide), and increased concentrations of bone resorption markers such as CTX (collagen 1 C-telopeptide) or NTX (collagen 1 N-telopeptide). In spite of the advancement brought along by determination of the markers of bone turnover, either in the physiological processes of modeling and remodeling, or in bone involved by a disease, interpretation of the results may not always be easy, especially when reference developmental data on peer groups are lacking.

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S9-4

Gustoća kostiju i koštani biljezi u adolescentica s anoreksijom nervozom

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S9-4

Bone density and bone markers in female adolescents with anorexia nervosa

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Anoreksija nervoza je kompleksni poremećaj kod kojega dolazi do smanjenja unosa kalcija, gubitka težine, te posljedično brojnih metaboličnih i endokrinih poremećaja uključujući primarnu ili sekundarnu amenoreju, te gu-

Anorexia nervosa is a complex disorder characterized by reduced calcium uptake, weight loss, and consequentially numerous metabolic and endocrine impairments including primary or secondary amenorrhea and loss of bone

bitak koštane mase. Osteopenija i osteoporoza su česte komplikacije koje mogu dovesti do klinički značajno povišenog rizika za nastanak fraktura kasnije u životu. Cilj studije bio je utvrditi koštanu gustoću (BMD), te serumske koncentracije inzulinu sličnog faktora rasta (IGF-I), biljega koštane izgradnje (osteokalcin, OC) i razgradnje (C-terminalni telopeptid tipa 1 kolagena, CTX) u adolescentica s anoreksijom nervozom s obzirom na duljinu trajanja bolesti. U analizu je bilo uključeno 28 bolesnica u aktivnoj fazi bolesti srednje dobi od 15,1±2,4 godina (χ±SD) sa sekundarnom amenorejom (χ±SD 9,6±9,2 mjeseci) i indeksom tjelesne mase (BMI u kg/m^2) od 15,8±2,1 (χ±SD). Vrijednosti BMD L-kralježnice uspoređene su s normalnim vrijednostima za istu dob, spol i rasnu pripadnost (Z-skor; Hologic QDR400) i unutar tjedan dana mjerene serumske vrijednosti IGF-I, OC i CTX. S obzirom na trajanje bolesti bolesnice su podijeljene u skupinu A (≤ 12 mjeseci) i skupinu B (>12 mjeseci). U skupini B zabilježen je veći broj bolesnica sa sniženim vrijednostima Z-skora (63% *vs.* 10%), te je utvrđena značajna negativna korelacija vrijednosti Z-skora s duljinom trajanja bolesti (r=-0,58; p=0,001). Trajanje bolesti pozitivno je koreliralo s duljinom amenoreje (r=0,89; p=0,000) i dobi bolesnica (r=0,64; p=0,000). Srednja dob bolesnica u skupini A bila je 14,2±1,9 godina, a u skupini B 17,2±2,2 godine (p=0,002). Skupine se nisu razlikovale prema vrijednosti koštanih biljega, IGF-I i BMI, a nije utvrđena ni korelacija između duljine trajanja bolesti i navedenih parametara. Zabilježena je značajna negativna korelacija između vrijednosti IGF-I i biljega razgradnje (CTX) (r=-0,49; p=0,008). Zaključeno je kako je duljina trajanja bolesti značajno utjecala na vrijednosti koštane gustoće u adolescentica s anoreksijom nervozom, što je rezultiralo nižim vrijednostima Z-skora u bolesnica s duljim trajanjem poremećaja. Nije utvrđena razlika u vrijednostima biljega koštane pregradnje, što je moguće posljedica značajne razlike u dobi među skupinama. Bolesnice s kraćim trajanjem bolesti bile su značajno mlađe, kada su i fiziološke vrijednosti koštanih biljega povišene, što otežava tumačenje nalaza koštanih biljega kod poremećaja koštanog metabolizma. Niže vrijednosti IGF-I korelirale su s povišenim vrijednostima biljega razgradnje, što ukazuje na ulogu IGF-I u etiopatogenezi osteoporoze.

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mass. Osteopenia and osteoporosis are frequent complications that may lead to clinically significant increase in the risk of fractures later in life. The aim of the study was determine bone mineral density (BMD) and serum concentrations of insulin-like growth factor (IGF-I), bone synthesis marker (osteocalcin, OC) and bone resorption marker (collagen type 1 C-terminal telopeptide, CTX) in female adolescents with anorexia nervosa according to the duration of the disease. The study included 28 patients in the active stage of the disease, mean age 15.1±2.4 years (χ±SD) with secondary amenorrhea (χ±SD, 9.6±9.2 months) and body mass index (BMI in kg/m^2) of 15.8±2.1 (χ±SD). BMD values of L-spine were compared with normal values for the respective age, sex and ethnicity (Z-score: Hologic QDR400), and serum levels of IGF-I, OC and CTX were measured within a week. According to disease duration, patients were divided into group A (≤ 12 months) and group B (<12 months). Results showed a greater proportion of patients with decreased Z-score values in group B than in group A (63% *vs.* 10%) and a significant negative correlation of Z-score value with the disease duration (r=-0.58; p=0.001). Duration of the disease showed positive correlation with the duration of amenorrhea (r=0.89; p=0.000) and patient age (r=0.64; p=0.000). The mean patient age was 14.2±1.9 and 17.2±2.2 years in group A and B, respectively (p=0.002). There was no between group difference according to bone marker values, IGF-I and BMI, and no correlation between the disease duration and these parameters. A significant negative correlation was recorded between IGF-I and bone resorption marker (CTX) (r=-0.49; p=0.008). In conclusion, duration of the disease had a significant effect on the values of bone density in adolescents with anorexia nervosa, which resulted in decreased Z-score values in patients with prolonged duration of the disorder. There was no difference in the values of bone resorption marker, which may have been due to the significant between group age difference. Patients with a shorter duration of the disorder were significantly younger, when the physiological values of bone markers are known to be higher, thus posing difficulty on interpreting the results on bone markers in bone metabolism impairment. Lower values of IGF-I correlated with increased values of bone resorption markers, suggesting a role of IGF-I in the etiopathogenesis of osteoporosis.

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S9-5

Osteoporoza u reumatoidnom artritisu

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Reumatoidni artritis (RA) je upalna bolest koja je obilježena sinovitisom, razgradnjom hrskavičnog tkiva i subhondralnom erozijom kosti. Osteoporoza koja se javlja u RA posljedica je više rizičnih čimbenika kao što su upala, imobilizacija, uporaba kortikosteroida itd. Progresija bolesti u vidu oštećenja zgloba je vrlo teško predvidiva i varira od bolesnika do bolesnika. Nekoliko studija je pokazalo da klinički znakovi bolesti kao i radiološka mjerenja često nisu dobri prediktivni čimbenici erozije zgloba. Ti nalazi upućuju na potrebu pronalaska biokemijskog parametra koji će pouzdano odražavati dinamiku tkivnog metabolizma u RA, koji će dobro korelirati s radiografijom te biti dobar prognostički pokazatelj napredovanja bolesti. Cilj je bio utvrditi koji biljeg koštanog metabolizma najbolje korelira s osteopenijom u RA. U radu smo obradili 26 bolesnika s dijagnozom RA i 20 zdravih kontrolnih osoba. Određivali smo slijedeće biljege koštanog metabolizma: serumski osteokalcin pomoću ELISA (Quidel), amino terminalni propeptid kolagena tip 1 (PINP) pomoću ECLIA (Roche) kao biljezi koštane izgradnje, te beta C-terminalni telopeptid (CTx) pomoću ECLIA (Roche) kao biljeg koštane resorpcijei. Određivani su i C-reaktivni protein (CRP; Olympus) kao biljeg upale te ciklički citrulinirani peptid (CCP) protutijela pomoću ELISA (Euroimmun) kao serološki biljeg RA. Nije nađena statistički značajna razlika između skupine bolesnika (P) i kontrolne skupine (C) za osteokalcin (4,958±1,30 ng/mL) (P), (4,672±1,64 ng/mL) (C); i PINP (49,600 ng/mL (29,525-62,197) (P), 41,235 (29,710-73,980) (C). Statistički značajna razlika nađena je za beta-CTx (0,445±0,26 ng/mL) (P), (0,302±0,19 ng/mL) (C). Također nije nađena korelacija između beta -CTx i CRP (p>0,050) te beta -CTx i CCP (p>0,050). Iako dobiveni rezultati ne pokazuju značajnu razliku između dviju ispitivanih skupina osim za beta -CTx, potrebna su daljnja ispitivanja uključujući i biljege metabolizma hrskavičnog tkiva (COMP, PIINP) i sinovitisa (YLK-40, PIIINP) kako bi se otkrio dovoljno osjetljiv i specifičan biokemijski pokazatelj razaranja zgloba.

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S9-5

Osteoporosis in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune, inflammatory disease characterized by synovitis, cartilage degradation and subchondral bone erosion. Osteoporosis in RA is characterized by a complexity of risk factors such as inflammation, immobilization, and use of corticosteroids. The progression of joint damage is highly unpredictable and variable from patient to patient. Predictive factors based on disease activity or radiographic damage have some limitations. These consideration suggests the need of accurate, precise assays reflecting the dynamics of tissue metabolism in RA, which can be used for prognosis. The aim was to investigate the markers of bone metabolism in RA. Twenty-five patients with the diagnosis of RA and twenty age- and sex-matched healthy controls were included in the study. Serum osteocalcin was determined by ELISA test (Quidel), PINP (amino-terminal propeptide of type 1 collagen) by ECLIA (Roche) as markers of bone formation, and beta -CTx (C-terminal telopeptide) by ECLIA (Roche) as a marker of bone resorption. Also, C-reactive protein (CRP; Olympus) and CCP antibody (cyclic citrullinated peptide antibody) were determined by ELISA (Euroimmun). There was no statistically significant between group difference for osteocalcin (4.958±1.30 ng/mL) (P), (4.672±1.64 ng/mL) (C) and PINP (49.600 ng/mL (29.525-62.197) (P), 41.235 ng/mL (29.710-73.980) (C). We found a statistically significant difference only for beta-CTx (0.445±0.26 ng/mL) (P), (0.302±0.19 ng/mL) (C). There was no correlatin between beta-CTx and CRP (p>0.050), or between beta-CTx and CCP (p>0.050). Our data suggest that only beta -CTx could be a valuable marker of bone turnover; however, joint is a complex organ and RA alters the metabolism of different tissues including bone, cartilage and synovial membrane. To evaluate the mechanisms involved in joint destruction, the markers of cartilage degradation (COMP, PIINP) and synovitis (YLK-40, PIIINP) should also be determined. Also, the question is whether beta -CTx is superior as a predicitive marker to the markers already in use.

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S9-6

Biološke razlike u biokemijskim koštanim biljezima

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Biološke varijacije sastoje se od intra-individualne i inter-individualne varijacije. Ove sastavnice bioloških varijacija rabe se za postavljanje specifikacija analitičke kvalitete glede otklona i nepreciznosti, za ocjenu serijskih promjena u pojedinim analitima, te za procjenu kliničke korisnosti referentnih raspona utvrđenih za dotičnu populaciju. Relativno velika varijacija iz dana u dan u izlučivanju nekih koštanih biljega mokraćom naglašava potrebu za primjenom višestrukih uzoraka kako bi se utvrdilo njihovo izlučivanje u neke osobe. Ostale biološke promjene poput menstrualnog ciklusa pokazuju porast tijekom srednjeg i kasnog folikularnog razdoblja, te pad tijekom srednjeg i kasnog lutealnog razdoblja. Ova varijacija ukazuje na to da ciklične promjene ovarijskih spolnih steroida u serumu mogu promijeniti biljege koštane resorpcije za vrijeme menstrualnog ciklusa. U trudnoći su vrijednosti u mokraći značajno porasle u trećem trimestru i ostale visoke tijekom babinja u usporedbi sa ženama bez trudnoće ili u ranoj trudnoći, pokazujući kako su to korisni biljezi za procjenu koštane resorpcije u vrijeme oko babinja. Međutim, biološka promjenjivost biljega koštane resorpcije u mokraći mjerena u zdravih žena prema ženama s osteoporozom u postmenopauzi u drugom mlazu mokraće prikupljane u tjednim razmacima kroz 5 tjedana pokazala je analitičku promjenjivost, kritične vrijednosti razlika i indeks individualnosti; stoga rutinska primjena biokemijskih koštanih biljega u mokraći u pojedine osobe ima ograničenu vrijednost ako se ove varijable ne uzmu u obzir. Prednost se daje ranim jutarnjim uzorcima; rezultati u mokraći trebaju se izražavati kao omjer kreatinina; referentni rasponi trebaju se grupirati prema spolu; treba izraziti analitičku nepreciznost (CV ≤9%), izvedenu iz biološke varijacije; također, razlika između serijskih rezultata pojedine osobe mora biti <50% da bi bila statistički značajna; u značajnom broju bolesnika će procjena rizika za osteoporotsku frakturu zahtijevati analizu višestrukih uzoraka mokraće. Sve ove varijable i pojedinačne čimbenike treba uzeti u obzir kad se postavljaju referentni rasponi, prikupljanje uzoraka i prijeanalitički postupci.

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S9-6

Biological variation of biochemical bone markers

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Biological variation consists of intra-individual and inter-individual variation. These components of biological variation are used to set analytical quality specifications for bias and imprecision, to evaluate serial changes in individual analytes, and to assess the clinical utility of population-based reference intervals. The relatively large day-to-day variation in urinary excretion of some bone markers emphasizes the need to use multiple samples to characterize this excretion of an individual. Other biological changes such as menstrual cycle show a rise during the mid- and late follicular period, and a fall during the mid- and late luteal periods. This variation suggests that cyclic changes in serum ovarian sex steroids might modulate bone resorption markers during the menstrual cycle. In pregnancy, urinary values significantly increased in the 3rd trimester of pregnancy and remained high during the puerperium as compared with nonpregnant or early pregnant women, demonstrating that these are useful markers to assess bone resorption during peripuerperal periods. Nevertheless, the biological variability of urinary bone resorption markers measured in healthy *vs.* postmenopausal osteoporotic women in second flow urine collected at weekly intervals for 5 weeks showed analytical variability, critical difference values and index of individuality; therefore, the routine use of urinary biochemical bone markers in an individual patient is of limited use if these variables are not taken into consideration. Early morning specimens are preferred; results in urine should be expressed as creatinine ratio; reference intervals should be stratified according to sex; necessary analytical imprecision (CV ≤9%), derived from biological variation; also, the difference between serial results from an individual must be >50% to be statistically significant; and assessment of risk for osteoporotic fracture would, in a significant number of patients, require analysis of multiple urine specimens. All these variables and individual factors should be considered when establishing reference intervals, sample collection and preanalytical procedures.

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S10 – Simpozij 10 – TOKSIKOLOGIJA I FARMAKOGENETIKA, S10-1

Novi pristup laboratorijskoj dijagnostici u kliničkoj toksikologiji

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Odavno se govori kako neka kemijska tvar u maloj količini može biti dodatak hrani, u umjerenoj količini lijek, dok u velikoj količini može biti otrov. Prema podacima mnogih autora, i kod nas su lijekovi na prvom mjestu kao najčešći uzroci akutnih otrovanja odrasle populacije, zatim slijede alkoholi, opijati, gljive i ostalo. Dijagnoza otrovanja temelji se na anamnezi, fizikalnom pregledu, kliničkom tijeku bolesti i selektivnim laboratorijskim pretragama. Izbor laboratorijskih pretraga ovisi o specifičnostima svakog pojedinog slučaja otrovanja, kao i o težini kliničke slike. Ne postoje nigdje na svijetu pretrage koje mogu otkriti i dokazati sve otrove. Kliničko toksikološka analitika sastoji se od metoda direktnog kvalitativnog dokaza i direktnog kvantitativnog određivanja otrova, kao i od indirektnih metoda dokaza djelovanja otrova. Glavni zadatak kvalitativne analitike je da prepozna ili isključi prisutnost jednog ili više otrova. Važnost kvalitativnog dokaza otrova za dijagnostiku u kliničkoj toksikologiji proizlazi iz iskustva da dijagnoza postavljena u vrijeme uzimanja uzorka u usporedbi s rezultatima kliničko toksikoloških ispitivanja može biti točna u 22% slučajeva, u 36% djelomice točna, te u 42% pogrješna. Negativan rezultat ukazuje na to da analit nije prisutan u klinički značajnoj koncentraciji. Svaki pozitivan nalaz dobiven testovima probiranja treba potvrditi postupkom veće specifičnosti. Testovi probiranja imaju mogućnost određivanja jedne supstance ili skupine supstancija, a uključuju: jednostavne kolorimetrijske testove, razliku osmolalnosti, imunokemijske testove, testove probiranja koji otkrivaju skupinu lijekova a uključuju tehnike razdvajanja (TLC, GC, HPLC). Kvantitativno određivanje otrova omogućava u pravilu "zaključke unatrag" o stupnju težine otrovanja. Ono često daje naznake za indikaciju terapije uklanjanja otrova, te u mnogim slučajevima dopušta djelotvornu kontrolu tijeka uklanjanja otrova. Važno je naglasiti da je za procjenu toksikoloških rezultata potrebna uska suradnja između kliničara i medicinskog biokemičara, a toksikološki rezultati tumače se uz kliničku sliku te poznavanje farmakokinetike i toksokinetike pojedinih otrova.

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S10 – Symposium 10 – TOXICOLOGY AND PHARMACOGENETICS, S10-1

New approach to laboratory diagnosis in clinical toxicology

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It is a well known fact that the same chemical compound used in small quantities can be considered a food additive, in moderate quantities may be a medicine, but used in large quantities can be very toxic. The main causes of acute poisoning in the population are drugs, followed by alcohol, opiates, mushrooms, etc. Diagnosis is based on history, physical examination, clinical evaluation and selective laboratory analysis. Which laboratory tests should be done vary from case to case. There is no laboratory test that could detect and confirm all toxins. Clinical toxicological analysis includes methods of direct qualitative and direct quantitative toxin measures, but also indirect methods that measure toxin activity. The main task of qualitative analysis is to determine or exclude the presence of one or more toxins. The importance of qualitative toxin detection lies in the fact that, if the diagnosis is set at the time of sample collection and compared to the results of clinical toxicological analysis, it is accurate in 22%, partially accurate in 36% and false in 42% of cases. Negative result means that the toxin is not present in a clinically relevant concentration. Each positive result should be confirmed using a test of higher specificity. Screening tests can be used to detect only one or several substances; these include simple colorimetric tests, immunochemistry based tests, GC, HPLC, TLC. Tight collaboration between clinicians and medical biochemists is needed for proper evaluation of toxicological results, and results should be interpreted using the knowledge on the specific toxin pharmacokinetics and toxokinetics.

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S10-2

Štetni utjecaj nekontrolirane suplementacije: uloga laboratorijskih analiza i nutrigenetike u *anti-aging* medicini

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Produženje života i svakodnevna dostignuća u području medicinske znanosti doveli su do razvoja nove grane medicine: *anti-aging* medicine. Po definiciji *anti-aging* medicina je primjena dijagnostičkih i terapijskih metoda u cilju ranog otkrivanja, prevencije i liječenja s procesom starenja povezanih poremećaja i bolesti, što dovodi do unaprjeđenja kvalitete života i produžetka ljudskog vijeka. Obilježava ju individualizirani pristup svakom bolesniku, a uloga laboratorijskih analiza u utvrđivanju optimalne funkcije organizma je bitna. Kako je hrana osnovni izvor energije, pravilan odabir i količina namirnica nezaobilazni su čimbenik za postizanje kvalitete života. Zbog brzog tempa života i svakodnevnog stresa suvremeni čovjek često poseže za tabletama vitamina, minerala i ostalim suplementima, što može dovesti do ozbiljnog narušavanja fiziologije organizma. Uz metode koje nudi nutrigenetika (analiza DNA), na raspolaganju su i analize iz opsega rada medicinsko-biokemijskog laboratorija. Prvi je cilj dijagnosticirati "štetnu hranu": analiza DNA, test na individualne alergene i test intolerancije na hranu. Prije negoli započnemo suplementaciju na raspolaganju su na nam brojne specijalističke laboratorijske analize: antioksidansi oksidativnog stresa, analiza minerala i toksičnih elemenata u kosi, masne kiseline u eritrocitima i dr. Cilj je upozoriti na opasnost od nekontrolirane uporabe dodataka prehrani, kao i prikaz laboratorijskih analiza kojima utvrđujemo razinu vitamina, minerala, masnih kiselina i drugih oblika suplemenata. Istraživanje se provodilo na dvojaki način: na temelju dostupnih podataka iz svakodnevnog rada laboratorija i dostupne literature. Vitamin C uz niz pozitivnih kliničkih značajka izaziva probavne smetnje i sniženje razine bakra u organizmu. Velike količine kalcija dovode do gubitka apetita, mučnine, povraćanja, glavobolje, nepravilnog srčanog ritma i svrbeža. Nuspojave pretjeranog unosa željeza su porast razine slobodnih radikala u organizmu, povećan rizik od razvoja karcinoma, SLE i Huntingtonove bolesti, te kod bolesnika s reumatoidnim artritisom pogoršanje simptoma osnovne bolesti. Visoka razina vitamina A u tijelu može rezultirati pojavom porođajnih anomalija, smanjivanjem gustoće kosti i promjenom lipidograma (porast). U zaključku, nekontrolirana uporaba neprimjerene (kvantitativno i kvalitativno) suplementacije je riskantna za zdravlje bolesnika, jer su utvrđene broj-

S10-2

Side effects of uncontrolled supplementation: the role of laboratory analysis and nutrigenetics in anti-aging medicine

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Prolonged life expectancy and daily achievements in the field of medical sciences have led to the development of a novel branch of medicine, anti-aging medicine. By definition, anti-aging medicine implies the use of diagnostic and therapeutic methods for early detection, prevention and treatment of impairments and disorders associated with the process of aging, thus improving the quality of life and extending life expectancy. Anti-aging medicine is characterized by individual approach to each patient, with laboratory analyses playing the crucial role in assessing the optimal body function. As food is the main source of energy, an appropriate choice and amount of foodstuffs is an unavoidable factor to achieve a favorable quality of life. Due to the fast lane life and daily stress exposure, modern man frequently reaches for tablets of vitamins, minerals and other supplements, which may severely impair the body physiology. In addition to the methods offered by nutrigenetics (DNA analysis), analyses from the scope of medical biochemistry laboratory are also available. The primary goal is to diagnose "harmful food": DNA analysis, testing for individual allergens, and testing for food intolerance. Prior to initiating supplementation, numerous specialist laboratory analyses can be performed: oxidative stress antioxidants, analysis of hair minerals and toxic elements, erythrocyte fatty acids, etc. The objective is to point to the risk associated with uncontrolled use of food additives and to present the array of laboratory tests to determine the levels of vitamins, minerals, fatty acids and other forms of supplementation. The study had a dual design: on the basis of data deriving from daily laboratory routine and from the literature. In addition to a number of favorable clinical characteristics, vitamin C may cause gastrointestinal discomforts and reduce the level of copper in the body. High amounts of calcium lead to inappetence, nausea, vomiting, headache, cardiac rhythm impairment, and pruritus. Side effects of excessive iron intake include an increased body level of free radicals, increase in the risk for the development of carcinoma, SLE and Huntington's disease, and exacerbation of the underlying disease symptoms in patients with rheumatoid arthritis. A high level of vitamin A in the body may result in birth defects, reduced bone density and lipidogram alteration (increase). In conclusion, uncontrolled

ne štetne posljedice. Prethodne laboratorijske analize te metode dostupne u okviru nutrigenetike jamče sigurnu i učinkovitu suplemenetaciju.

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use of (quantitatively and qualitatively) inappropriate supplementation poses a risk for patient health, since a number of associated adverse effects have been demonstrated. Previous laboratory analyses and methods available in the frame of nutrigenetics ensure safe and efficient supplementation.

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S10-3

Pasivno pušenje marihuane i pozitivna doping kontrola

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Uporaba i zlouporaba društvenih droga danas su široko rasprostranjeni u društvu te nema dokaza da bi šport bio imun na tu pojavu. Kanabis je na popisu zabranjenih tvari u športu, iako njegov učinak na uspješnost još nije dokazan. Popularnost kanabisa kao društvene droge među mlađim naraštajima stavlja ga na vrh popisa spojeva koje otkrivaju antidopinški laboratoriji ovlašteni od strane Svjetske antidopinške agencije širom svijeta. Obrada rezultata analize mokraće prilično je teška za medicinske i disciplinske komisije, ne samo zbog društvene uporabe ove tvari, nego isto tako zbog tumačenja analitičkih podataka dobivenih iz uzoraka mokraće. Marihuana se često puši u raznim društvenim situacijama gdje ovu drogu ne puše svi nazočni. Stoga je moguće da nepušači pasivno udahnu dovoljno kanabinoida iz dima marihuane da bi izlučili takve količine metaboličnih proizvoda u mokraći koje se mogu otkriti. Apsorpcija delta-9 tetrahidrokanabinola (THC) iz zraka u prostoriji u dovoljnoj količini da stvori razine u plazmi koje se mogu otkriti te metaboliti u mokraći ovise o mnoštvu čimbenika, uključujući trajanje i učestalost izloženosti dimu, koncentraciji THC u zraku u prostoriji te pojedinačnoj osjetljivosti na marihuanu. Ako se kanabis uzima radi ublažavanja treme prije natjecanja ili nekog drugog stresnog događaja, radi poboljšanja učinkovitosti, takvu uporabu se više ne smije smatrati rekreacijskom, nego ju treba smatrati sredstvom dopinga. Otkako je 1989. godine Međunarodni olimpijski odbor (MOO) uključio kanabinoide u popis zabranjenih droga pod naslovom Vrste zabranjenih tvari u određenim situacijama, bilježi se visoka incidencija pozitivnih slučajeva na kanabinoide u analizi u okviru dopinške kontrole u športu. Prema znanstvenoj literaturi, malo je vjerojatno

S10-3

Passive marijuana smoking and positive doping control

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The use and abuse of social drugs is now widespread in society, and there is no evidence that sport is immune. Cannabis is on the list of prohibited substances in the practice of sports, although its performance enhancing effect has not yet been proved. Its popularity among younger generations as a social drug puts cannabis at the top of the list of compounds detected by the anti-doping laboratories accredited by the World Anti-Doping Agency worldwide. The management of the results of urine analysis is quite difficult for the medical and disciplinary committees not only because of the social use of the substance but also because of the interpretation of analytical data from urine samples. Marijuana is commonly smoked in social situations in which not all present smoke the drug. It is therefore possible that non-smokers can passively inhale enough of the cannabinoids from marijuana smoke to excrete detectable amounts of the metabolic products in their urine. The absorption of delta-9 tetrahydrocannabinol (THC) from room air in a sufficient quantity to produce detectable plasma levels and of its urinary metabolites would depend on a variety of factors including duration and frequency of smoke exposure, room air concentration of THC, and individual sensitivity to marijuana. If cannabis is used to manage anxiety before a competition or another stressor event, in order to increase efficiency, this use should be no longer viewed as recreational but should be considered as a doping agent. A high incidence of positive cases for cannabionids in analysis for doping control in sports has been observed since the International Olympic Committee (IOC) included them in the 1989 list of prohibited drugs under the title Classes of Prohibited Substances in

da bi pasivno udisanje dima marihuane u društvu osoba koje puše marihuanu, čak i u zatvorenom prostoru, moglo rezultirati sistemskim razinama THC koje dovode do pozitivnog testa mokraće.

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Certain Circumstances. Based on the scientific literature it is highly unlikely that passive inhalation of marijuana smoke, when in the company of marijuana smokers, even in indoor area, would result in systemic levels of THC that would produce positive urine test.

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S10-4

Regresijski model za predviđanje doze varfarina iz farmakogenetskog statusa – klinička primjena

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Varfarin je antikoagulantni lijek (racemična smjesa S- i R-enantiomera) čije se doziranje pažljivo titrira kako bi se izbjegao rizik za život ozbiljnih nuspojava poput krvarenja. Ovaj rizik barem je djelomice uzrokovan genskim polimorfizmom CYP2C9, glavnog enzima metabolizma varfarina. Uz alel divljeg tipa CYP2C9*1, najčešći mutirani aleli su CYP2C9*2 i CYP2C9*3 i oni kodiraju enzime sa svega 16%-20%, odnosno 5% aktivnosti enzima divljeg tipa. Iz genotipa se mogu procijeniti tri vrste metaboličkog fenotipa (sporometabolizirajući – PM s oba alela mutirana, srednjemetabolizirajući – IM s jednim alelom divljeg tipa i brzometabolizirajući – EM s oba alela divljeg tipa). Glavni ciljevi istraživanja bili su utvrditi višestruki regresijski model koji bi se mogao primijeniti za predviđanje optimalnog doziranja lijeka, te odrediti kliničko značenje i opravdanost genotipizacije CYP2C9 pri uvođenju i optimiranju terapije varfarinom. Metodom PCR-RFLP određen je genotip CYP2C9 u 181 bolesnika (56,4% muškaraca, srednje dobi 62 godine) koji uzimaju varfarin u dozama potrebnim za održavanje vrijednosti protrombinskog vremena unutar raspona INR 1,5-2,5). U izradu modela uključen je i doprinos drugih čimbenika (genskih polimorfizama koagulacijskih faktora II i faktora V Leiden), dobi, spola, polimorfizma gena CYP2C19 (enzim metabolizma R-varfarina), uzimanih lijekova, te različitih prisutnih dijagnoza. Ti se čimbenici u modelu nisu pokazali značajnima. Najboljim modelom višestruke linearne regresije pokazao se model koji optimalnu dozu procjenjuje iz standardne početne doze, fenotipa EM i PM, te omjera ciljne vrijednosti INR i vrijednosti INR određene 72 sata od početka terapije.

S10-4

Warfarin dose prediction regression model from pharmacogenetic status – clinical application

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Warfarin is an anticoagulant drug (racemic mixture of S- and R-enantiomers) whose dosage is carefully titrated to avoid the risk of serious side effects such as life-threatening bleeding. This risk exists at least in part due to the genetic polymorphism of CYP2C9, the major enzyme of warfarin metabolism. Besides wild type allele CYP2C9*1, the most common mutant alleles CYP2C9*2 and CYP2C9*3 code for enzymes with only 16%-20% and 5% of total wild type activity, respectively. Three types of metabolic phenotype can be derived from genotype information (poor metabolizer – PM with both alleles mutant, intermediate metabolizer – IM with one mutated allele, and extensive metabolizer – EM with both wild type alleles). The aim of the study was to find a multiple regression model that could be used for dose prediction and to assess the importance of CYP2C9 genotyping in patients receiving warfarin anticoagulant therapy. Phenotyping by PCR-RFLP method was performed in 181 patients (56.4% male, mean age 62 yrs) receiving warfarin in doses needed to maintain prothrombin time value within the INR range 1.5-2.5. The contribution of other factors (coagulation factor II (prothrombin) and factor V Leiden genetic polymorphisms), sex, age, CYP2C19 polymorphism (which is R-warfarin metabolic enzyme), influence of other medication used and of different diagnoses were also included on model assessment. These factors showed to be of no importance for the model. A model estimating optimal warfarin dose from standard dose at the beginning of therapy, patient EM and PM phenotype, and the ratio of target INR value/INR derived 72 hours of

Model unutar odstupanja ±1 mg točno procjenjuje dozu za oko 76% bolesnika. Iz modela proizlazi da prisutnost fenotipa IM doprinosi sniženju standardne doze za 6%, a fenotipa PM za 9%. Istraživanje je potvrdilo da oštećenje gena CYP2C9 značajno smanjuje optimalnu dozu održavanja terapije varfarinom u odnosu na dozu osoba genotipa 1/1: genotip 2/2 uzrokuje smanjenje doze na 66%, a genotip 3/3 na 33% (p=0,025). Fenotip IM uzrokuje smanjenje doze na 88% (p=0,008), a PM na 55%. Vjerojatnost da će osobe s alelom CYP2C9*3 uzimati doze niže od 3 mg četiri je puta veća u odnosu na osobe bez tog alela (OR=4,14; 95%CI: 1,7-10,3). Raspodjela alela CYP2C9, genotipova i fenotipova u zdravoj populaciji u skladu je s podacima drugih autora: 11,8% alela CYP2C9*2 i 4,0% alela CYP2C9*3, genotipa 1/1 (68,8%), 1/2 (22,6%), 1/3 (8,1%), 2/2 (0,5%), a genotipa 2/3 i 3/3 (0,0%). Zaključak istraživanja je da informacija dobivena genotipizacijom CYP2C9 može ukazati na povišen rizik nuspojava pri liječenju varfarinom, a upotreba predloženog regresijskog modela može značajno unaprijediti i mogućnost procjenjivanja sigurne i optimalne doze lijeka.

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therapy initiation was recognized as the best multiple linear regression model. Within ±1 mg deviation, this model was able to predict drug dose for about 76% of patients. In this model, IM phenotype contributed for 6% and PM phenotype for 9% decrement of standard dose. This study confirmed that in comparison to 1/1 genotype, CYP2C9 impairment significantly decreased optimal warfarin therapy maintenance dose: 2/2 genotype decreased the dose to 66%, and genotype 3/3 to 33% (p=0.025). IM phenotype decreased the dose to 88% (p=0.008) and PM to only 55%. The probability that CYP2C9*3 allele carriers would take doses smaller than 3 mg was four-fold that in non-carriers (OR=4.14; 95%CI: 1.7-10.3). The CYP2C9 allele, genotype and phenotype distribution in healthy population was consistent with data reported by other authors: 11.8% CYP2C9*2 alleles and 4.0% CYP2C9*3 alleles, genotype 1/1 (68.8%), 1/2 (22.6%), 1/3 (8.1%), 2/2 (0.5%), and genotype 2/3 and 3/3 (0.0%). Study conclusion is that information derived from CYP2C9 genotyping can indicate an increased risk of warfarin therapy side effects, and that the use of the regression model proposed can significantly improve assessment of the safe and optimal drug dose.

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S10-5

Uloga farmakogenetičkih varijacija u liječenju depresije

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Unatoč razvoju farmakoterapije antidepresivima 30%-40% bolesnika nema zadovoljavajući odgovor na početno liječenje, a njihova identifikacija može potrajati do 6 tjedana. Stoga bi pouzdani biološki biljezi mogli biti dragocjeni za individualizaciju terapije. Farmakogenomička istraživanja u psihijatriji pokušavaju definirati utjecaj genetičkih polimorfizama na povoljne i nepovoljne reakcije na psihotropne lijekove. Genski kandidati za farmakogenomičke studije su geni koji kodiraju proteine izravno uključene u farmakokinetiku (enzimi koji metaboliziraju lijekove, membranski transporteri lijekova itd.) i farmakodinamiku (geni koji kodiraju ciljna mjesta lijeka: elementi putova neurotransmitora kao što su transporteri i receptori). Biotransformacija antidepresivnih lijekova je raznolika i uključuje različite enzime, među kojima su naj-

S10-5

The role of pharmacogenetic variations in depression therapy

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Despite advances in antidepressant pharmacotherapy, 30%-40% of patients do not respond sufficiently to the initial treatment and their identification can take up to 6 weeks. Thus, reliable biological markers could be valuable for therapy individualization. Pharmacogenomic investigations in psychiatry are attempting to define the impact that genetic polymorphisms have on positive and adverse reactions to psychotropic drugs. Candidate genes for pharmacogenomic studies are those that encode proteins directly involved in the pharmacokinetics (drug-metabolizing enzymes, membrane drug transporters, etc.) and pharmacodynamics (genes encoding drug targets: elements of neurotransmitter pathways such as transporters and receptors) of psychoactive drugs. Biotransformation of antidepressant drugs is diverse and

važniji CYP2D6, CYP2C19 i CYP3A4. P-glikoprotein što ga kodira polimorfni MDR1 (gen rezistencije na više lijekova) prepoznat je kao ključni element u reguliranju pristupa terapijskih agenasa mozgu i drugim tkivima te je stoga od farmakogenetičkog značenja. Na farmakodinamičkoj razini, polimorfni serotoninski transporter (SERT) bi, kao glavni cilj mnogih antidepresivnih lijekova, mogao biti zanimljiv predmet farmakogenetičkog istraživanja. Cilj ove studije bio je vrednovati značajnost polimorfnih varijanata CYP2D6, CYP2C19, CYP3A4, MDR1 i SERT kod odgovora na liječenje i nepovoljnih reakcija na lijekove u bolesnika s velikim depresivnim poremećajem (MDD) koji su primali antidepresive. U studiji je sudjelovalo 114 takvih bolesnika. Farmakogenetičke analize provedene su metodama PCR-RFLP. Duplikacije CYP2D6 bile su češće u skupini rezistentnoj na lijek u usporedbi sa skupinom koja je odgovarala na lijek (p=0,02). Učestalost alela G2677- MDR1 bila je značajno viša u skupini rezistentnoj na lijek u usporedbi sa skupinom koja je odgovarala na lijek (p=0,05). Utvrđene su značajne povezanosti između alela SERTPR-L i genotipa L/L te boljeg terapijskog odgovora (p=0,03 i 0,04), izmjerene Hamiltonovom ljestvicom za ocjenjivanje depresije (*Hamilton Depression Rating Scale*, HAM-D). Zaključeno je kako genetičke varijante CYP2D6, MDR1 i SERT mogu utjecati na ishod psihofarmakoterapije.

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involves different enzymes, among which most important are CYP2D6, CYP2C19, and CYP3A4. P-glycoprotein, coded by polymorphic MDR1 (multi-drug resistance gene) is recognized as a key element in regulating access of therapeutic agents to the brain and other tissues, and thus has pharmacogenetic relevance. At pharmacodynamic level, polymorphic serotonin transporter (SERT) as the main target of many antidepressant drugs could also be an attractive candidate for pharmacogenetic study. The aim of this study was to evaluate the significance of polymorphic variants of CYP2D6, CYP2C19, CYP3A4, MDR1 and SERT for treatment response and adverse drug reactions in patients with major depression disorder (MDD) receiving antidepressant medication. The study included 114 MDD patients. Pharmacogenetic analyses were performed by PCR-RFLP methods. CYP2D6 duplications were more frequent in drug-resistant group compared to responder group (p=0.02). The frequency of G2677 alelle of MDR1 was significantly higher in drug-resistant group compared to responder group (p=0.05). Significant associations between SERTPR-L alelle and L/L genotype and better treatment response (p=0.03, and p=0.04, respectively) measured by Hamilton Depression Rating Scale (HAM-D) score were found. It is concluded that genetic variants of CYP2D6, MDR1 and SERT may have an impact on psychopharmacotherapy outcome.

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S11 – Simpozij 11 – AUTOIMUNE I ALERGIJSKE BOLESTI, S11-2

Smjernice za testiranje na antinuklearna antitijela (ANA)

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Testiranje na autoantitijela dobiva sve važniju ulogu u kliničkoj medicini, poglavito u reumatologiji. Utvrđeno je kako među različitim autoantitijelima ona koja su usmjerena prema staničnim jezgrama i citoplazmi, ovdje skupno nazvana antinuklearna antitijela (ANA), pomažu u postavljanju ispravne dijagnoze, procjeni prognoze i planiranju praćenja u bolesnika s autoimunim upalnim reumatskim bolestima (IRD). Otkriće snažno izraženih ANA u slučaju vrlo rane bolesti kad još nedostaje većina znakovitih obilježja potpuno rasplamsale bolesti, može voditi daljnju potragu za zahvaćenim organom ili tkivom te pokazati koji se tip ili podtip bolesti razvija. Neka ANA su

S11 – Symposium 11 – AUTOIMMUNE AND ALERGIC DISEASES, S11-2

Guidelines for antinuclear antibody (ANA) testing

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Testing for autoantibodies has attained an increasingly important role in clinical medicine, especially in rheumatology. Among the various autoantibodies those that are directed to cell nuclei and cytoplasm, here collectively called antinuclear antibodies (ANA), have been found to aid in setting correct diagnosis, estimating prognosis and planning follow-up of patients with autoimmune inflammatory rheumatic diseases (IRD). Discovery of a strongly expressed ANA in a case of very early disease where most of the characteristic features of the full-blown disease are still lacking can guide further search for organ or tissue involvement and indicate which type or subtype of the

specifična za bolest, dok je većina drugih udružena s bolešću, jer se mogu naći u više različitih IRD. Obvezno je temeljito ispitivanje kliničkih okolnosti u kojima je nađeno ANA, kako bi se mogla tumačiti njegova značajnost za dijagnostiku i kliničko planiranje. Stoga su podrobna klinička anamneza i fizikalni pregled neophodni za utvrđivanje potrebnih dijagnostičkih pretraga i prognozu. Razumno je započeti s dobivanjem uvida u tip bolesti pregledom rezultata jednostavnih laboratorijskih pretraga na prisutnost upale i imune aktivacije prije negoli se provede testiranje na autoantitijela kao što su ANA. Razumno je i isplativo započeti probirom na prisutnost ANA pomoću neke osjetljive i dokumentirane metode kao što je indirektna imunofluorescencija (IF) na staničnom supstratu HEp-2, te potom primijeniti postupni pristup kako bi se definiralo specifično ANA za dotični slučaj. U mnogim slučajevima može se izravno primijeniti ispravno tumačenje IF bojanja uočeno takvim probirom da bi se ukazalo na to koja je dijagnoza vjerojatnija te pomoglo u vođenju daljnje potrage za specifičnošću ANA. ANA se u osnovi mogu podijeliti na ona koja reagiraju s jezgrenom ovojnicom, nukleoplazmom, nukleolima, mitotskim vretenastim aparatusom i citoplazmom. Tek se malobrojni poznati proteini ovih odjeljaka pretvaraju u autoantigene, najvjerojatnije zbog upalom izazvane stanične smrti tijekom koje neki antigeni podliježu poslije-translacijskoj modifikaciji. Ove modifikacije započinju aktiviranjem mnogih različitih gena, ali se zna da proizvodnjom autoantitijela koja se nalazi u nekih, ali ne u svih bolesnika s IRD, upravlja aktiviranje specifičnih gena za autoantijela u kompleksu HLA klase II. Kad se u bolesnika nađe pozitivan rezultat testa IF ANA, važno je znati da daljnje pretrage na specifičnost antitijela treba provesti samo onda kad postoje izgledi da će ih ti testovi otkriti. U praktičnom smislu to znači da se samo srednje do jako izražena ANA usmjerena prema nukleoplazmi i citoplazmi mogu dalje diferencirati drugim testovima, kao što su dvostruka imunodifuzija, imunoblot, ELISA, pasivna hemaglutinacija itd. Čak se ni jako izražena ANA usmjerena ka jezgrenoj membrani, nukleolima i mitotskom vretenu ne mogu zasad dalje diferencirati u rutinskim dijagnostičkim okolnostima. Slično tome, nije vjerojatno da će se specifična ANA moći otkriti ako je test IF ANA slabo pozitivan. Čak se ni primjenom suvremenih testnih tehnologija poput autoantigenskih multi-arrays, testova s ciljnim laserskim zrakama, testova imuno-luminiscencije itd. ne može odrediti specifičnost u takvim slučajevima. Dobro se zna da se određena ANA primjenjuju kao dijagnostički kriteriji kao dio dijagnostike IRD, kao što je u slučaju sistemskog eritematoznog lupusa (SLE), miješane bolesti vezivnog tkiva (MCTD) i Sjögrenova sindroma (SjS). U slučaju skleroderme (SSc), poli- i dermatomiozitisa (PM/DM), juvenilnog kroničnog artritisa (JCA) i sekundarnog SjS, otkriće specifičnog ANA služi kao važna potpora dijagnozi i prognozi. Kod SLE prisutnost

disease is developing. Some ANA are disease-specific whereas most others are disease-associated as they can be found in several different IRD. A thorough study of the clinical setting in which an ANA is found is mandatory to be able to interpret its significance for diagnosis and clinical planning. Thus, a detailed clinical history and physical examination is indispensable to start the work-up of diagnosis and prognosis. It is rational to start by getting an impression of the disease type by looking at simple laboratory test results for the presence of inflammation and immune activation before one looks for autoantibodies such as ANA. It is rational and cost-effective to begin with screening for the presence of ANA using a sensitive and documented method such as indirect immunofluorescence (IF) on a HEp-2 cell substrate and then to use a stepwise approach to characterize the specific ANA in question. In many cases the correct interpretation of the IF staining pattern seen by such screening can be used directly to indicate which diagnosis is more likely and to help guide further search for ANA specificity. ANA can roughly be divided into those that react with the nuclear envelope, the nucleoplasm, the nucleoli, the mitotic spindle apparatus, and the cytoplasm. Only few of the known proteins of these compartments turn into autoantigens, most likely due to inflammation-induced cell death during which some antigens get post-translationally modified. These modifications are initiated by activation of many different genes, but it is also known that the autoantibody production seen in some but not all IRD patients is governed by activation of specific autoantibody genes in the HLA class II complex. When the IF ANA test has been found positive in a patient, it is important to realize that further testing for antibody specificity should be undertaken only when assays have a chance to detect them. In practical terms, this means that only intermediately to strongly expressed ANA directed to the nucleoplasm and cytoplasm can be further differentiated by other tests such as double immunodiffusion, immunoblotting, ELISA, passive hemagglutination, etc. Even strongly expressed ANA directed to nuclear membrane, to nucleoli and to mitotic spindle cannot be characterized further in a diagnostic routine setting today. Similarly, it is unlikely that a specific ANA can be detected if the IF ANA test is weakly positive. Even the use of modern test technologies, e.g., autoantigen multi-arrays, addressable laser bead assays, immuno-luminescence tests, etc. cannot determine the specificity in cases like this. It is well known that certain ANA are used as diagnostic criteria as part of an IRD diagnosis, as is the case in systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), and Sjögren's syndrome (SjS). In case of scleroderma (SSc), poly- and dermatomyositis (PM/DM), juvenile chronic arthritis (JCA) and secondary SjS the detection of a specific ANA serves as important support for diagnosis and prog-

ANA, anti-dsDNA, anti-Sm I anti-ribonukleoproteinskih P antitijela služi kao dijagnostički kriterij. Kod SjS prisutnost antitijela anti-SSA/Ro i/ili anti-SSB/La služi kao dijagnostički kriterij u američko-europskom nizu kriterija. Poznato je da se prisutnost antitijela anti-U1RNP rabi kao obvezni kriterij za MCTD. Kod SSc prisutnost antitijela anti-centromera, anti-U1RNP, anti-U3RNP, anti-RNA polimeraze 1 i anti-Scl-70 znakovita je za dijagnozu, no svako je od njih udruženo s vrlo različitim kliničkim sub-sindromima (fenotipovima), koji imaju važne korelate u smislu prognoze. Ovakvi različiti klinički fenotipovi povezani s ANA nalaze se kod SLE, PM/DM, SjS i JCA. Uvijek je predstavljalo izazov kako se baviti graničnim pozitivnim serološkim rezultatima: mogu se izvesti dvije različite vrste testova i potražiti sukladnost prije negoli se izda pozitivan rezultat; klinici se može izdati nalaz uz napomenu da se taj pozitivan rezltat ne smije rabiti s jednakom pouzdanošću kao jasno pozitivan rezultata; ili se može iznova postaviti prijelomna vrijednost (*cut-off*) za pozitivnost testa tako da se samo rezultati iznad te prijelomne vrijednosti smatraju pozitivnima. Potpuno je jasno da se rezultati dobiveni uz primjenu testova solidne faze (ELISA, testovi s kapljicama, zrakama itd.) ne mogu izravno uspoređivati s onima polučenim klasičnim tehnikama (dvostruka imunodifuzija, protuimunoelektroforeza, Farrovi testovi itd.). Stoga, kad se takvi testovi solidne faze uvode u primjenu u autoimunoj dijagnostici, obvezno je provesti poslije-marketinšku studiju uz primjenu seruma bolesnika iz vlastite klinike ili klijentele. Rezultati utvrđeni u normalnih davatelja +2 ili 3 SD iznad srednje vrijednosti imaju malo ili nikakvo značenje u ovom kontekstu. Optimalno je testirati serume iz miješane populacije bolesnika s IRD uz serume bolesnika sa zaraznim bolestima (ovi potonji za testiranje na lažno pozitivne rezultate), te tada odlučiti koju prijelomnu vrijednost (*cut-off*) postaviti kako bi razlikovala bolest prototip od svih bolesti koje mogu oponašati ovu bolest (kontrolni bolesnici s kritičnom bolešću). Nakon izrade ROC i izbora dogovorene visoke razine specifičnosti, npr. 95% prema ovim kontrolnim bolesnicima, stvoren je valjani sustav testiranja. Nakon toga osjetljivost se može vidjeti iz grafikona ROC. Testovi koji se rabe za dijagnosticiranje bolesti moraju imati visoku specifičnost. Takav postupak omogućava usporedbu rezultata između različitih laboratorija i klinika. Osjetljivost nije važna za dijagnozu, ali može biti korisna kod probira, kao u slučaju testiranja na ANA pomoću IF. Izazov bi bio dokazati da se test na neko autoantitijelo može rabiti za vrlo ranu dijagnostiku, jer to zahtijeva uzorkovanje seruma pri prvom susretu sa zdravstvenim sustavom kad se bilježe prvi simptomi i nalazi. Za dokazivanje vrijednosti treba bolesnike pratiti sve dok se ne postavi konačna dijagnoza. Kako bi se osiguralo da svi korisnici u različitim kliničkim sredinama naručuju testove na autoantitijela na osnovi istih premisa, preporuča se dogovoriti primjenu algoritma za naručivanje testova, čime

nosis. In SLE, the presence of ANA, anti-dsDNA, anti-Sm and anti-ribonucleoprotein P antibodies serve as diagnostic criteria. In SjS, the presence of anti-SSA/Ro and/or anti-SSB/La antibodies serves as diagnostic criteria in the American/European criteria set. It is well known that the presence of anti-U1RNP antibodies is used as a mandatory criterion for MCTD. In SSc, the presence of anti-centromere, anti-U1RNP, anti-U3RNP, anti-RNA polymerase 1 and anti-Scl-70 antibodies are all characteristic of the diagnosis, but each of them is associated with very different clinical sub-syndromes (phenotypes), which have important correlates in terms of prognosis. Such different ANA-related clinical phenotypes are found in SLE, PM/DM, SjS, and in JCA as well. It has always been a challenge how to deal with borderline positive serologic results: one can choose to run two different types of assays and look for agreement before a positive result is reported, one can give the clinic a report saying that this positive result cannot be used with the same confidence as a clearly positive result, or one can re-set the cut-off for positivity of the assay so that only results above this cut-off level are called positive. It is quite clear that results derived by use of solid phase assays (ELISA, bead assays, arrays,etc.) cannot be directly compared with those stemming from classic techniques (double immunodiffusion, counter-immunoelectrophoresis, Farr assays, etc.). Therefore, when such solid phase assays are introduced for use in autoimmune diagnosis, it is mandatory to do a post-marketing study using sera of patients from one's own clinic or clientele. Results found in normal donors +2 or 3 SD above mean value has little or no meaning in this context. It is optimal to test sera from a mixed population of IRD patients supplemented by infectious disease patient sera (the latter to test for false positives), and then decide which cut-off value should be set to discriminate a prototype disease from all the diseases which mimic this disease (critical disease control patients). After construction of receiver-operation curves (ROC) and choosing an agreed high level of specificity, e.g., 95% towards these control patients, a meaningful test system has been created. After doing this, the sensitivity can be seen from the ROC graph. Tests used for diagnosing disease have to have a high specificity. This procedure makes it possible to compare results between different laboratories and clinics. Sensitivity is of no importance for diagnosis but may be useful for screening as is the case with IF ANA testing. It is a special challenge to prove that a test for an autoantibody can be used for very early diagnosis, since it demands sampling of sera at the first encounter with the health system when the first symptoms and findings are recorded. To prove the value, one needs to follow up patients until a definitive diagnosis has been reached. To ensure that all users in various clinical settings order autoantibody tests based on the same premises, it is advised to agree on the use of

će se testiranje učiniti racionalnim i manje skupim, jer će se izbjeći zlouporaba testiranja. Takav algoritam može se sastaviti na više načina: 1. liječnik može testove naručivati na osnovi pretpostavljene dijagnoze, označavajući dijagnozu na koju sumnja, pa će se napraviti racionalni testovi povezani s tom bolešću; 2) ili liječnik može naručiti test probira, npr. IF ANA uz primjenu stanica HEp-2 te nastaviti s drugim testovima nakon što primi pozitivan rezultat, često prema određenom izgledu IF. Ako se testovi na specifična antitijela rade samo nakon jakih i srednje jakih seruma koji izazivaju nukleoplazmično ili citoplazmično bojenje, tada se uspješno mogu otkriti specifična ANA. U zaključku, ANA najvjerojatnije odražavaju narav tkivnog oštećenja, genetsku predispoziciju i možda etiologiju, i vjerojatno su udružena s dobro definiranim fenotipovima IRD koji mogu dovesti do procjene prognoze. Primjenjuju li se na ovaj način, kliničar može planirati praćenje i uvođenje terapijskih mjera kad je to optimalno za sprječavanje napredovanja bolesti. Suvremene platforme za testiranje možda su više ponovljive i lakše za izvođenje automatiziranim postupcima, ali nisu bolje. Za postizanje precizne dijagnoze na razini sub-sindroma važno je imati potporu bolesnika koji su voljni dati krv za kritično mjesno poslijemarketinško testiranje u takvoj suradnji koja obuhvaća laboratorijske znanstvenike i kliničke specijaliste.

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an algorithm for test ordering which will make testing rational and less expensive due to avoidance of test abuse. Such an algorithm may be constructed in several ways: 1) the doctor can order tests based on a tentative diagnosis by ticking this suspected diagnosis, and the rational tests related to it will be done, 2) or the doctor can order a screening test, e.g., IF ANA using HEp-2 cells, and go on testing after receiving a positive result, often guided by the particular IF pattern seen. If only strong and intermediately strong sera giving rise to nucleoplasmic or cytoplasmic staining are followed up by specific antibody tests, specific ANA can be detected with success. In conclusion: ANA most likely reflect the nature of tissue lesions, genetic predisposition, and perhaps etiology, and they are likely to be associated with well-defined phenotypes of IRD that can lead to an estimate of prognosis. Used in this way the clinician can plan follow-up and start therapeutic measures when it is optimal for the prevention of disease progression. Modern testing platforms may be more reproducible and easy to perform by automated procedures but are not better. To arrive at a precise sub-syndrome diagnosis it is important to have support from patients who are willing to donate their blood for critical local post-marketing testing in a collaboration that involves laboratory scientists and clinical specialists.

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S11-3

Autoantitijela u antifosfolipidnom sindromu

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Antifosfolipidni sindrom (APS) je autoimuni poremećaj koji se klinički očituje arterijskom ili venskom trombozom i/ili specifičnim opstetričkim komplikacijama te prisutnošću antifosfolipidnih antitijela (aPL) u serumu. aPL čine heterogenu skupinu antitijela različitih reaktivnosti, većinom usmjerenih na fosfolipid-vezujuće proteine, same ili u kompleksu s fosfolipidima kao što su kardiolipin, fosfatidilserin, fosfatidiletanolamin ili fosfatidilinozitol. Laboratorijski kriteriji kao dio klasifikacijskih kriterija za dijagnozu APS (kriteriji Sapporoi) trenutno uključuju prisutnost samo dvaju aPL: antikardiolipinskih antitijela (aCL) IgG i/ili IgM klase u srednjem ili visokom titru (>40 U/mL) i/ili lupus antikoagulant (LA) antitijela. aCL su

S11-3

Autoantibodies in antiphospholipid syndrome

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Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by arterial or venous thrombosis and/or specific obstetric complications in the presence of antiphospholipid antibodies (aPL). aPLs are a heterogeneous family of autoantibodies with diverse cross-reactivities mainly directed against phospholipid-binding plasma proteins, either alone or in combination with phospholipids such as cardiolipin, phosphatidyl-serine, phosphatidyl-ethanolamine and phosphatidyl-inositol. Laboratory criteria as part of the classification criteria for APS (Sapporo criteria) currently include presence of only two aPL: anticardiolipin antibodies (aCL) IgG and/or IgM isotype in medium or high titer (>40 U/mL) and/or lupus antico-

usmjerena na kompleks beta$_2$-glikoproteina I (beta$_2$-GPI) i kardiolipina, negativno nabijenog fosfolipida iz membrane mitohondrija. Reaktivnost ovih antitijela dokazuje se beta$_2$-GPI-ovisnim kardiolipinskim ELISA testom. LA antitijela najčešće su usmjerena na protrombin ili beta$_2$-GPI, a njihova prisutnost se dokazuje funkcionalnim, o fosfolipidima ovisnom koagulacijskim testom prema protokolu definiranom kriterijima Sapporo. Kliničko značenje antitijela na ostale fosfolipid-vezujuće proteine kao što su protrombin, trombin, protein C, protein S, aneksin V, trombomodulin ili kininogen nije još u potpunosti razjašnjeno. Teorija o izravnoj ulozi aPL u patogenezi APS nalazi svoje uporište u rezultatima istraživanja na životinjskim modelima. Naime, pokazalo se da pasivni transfer aPL u normalnog miša izaziva kliničke manifestacije humanog APS. Najvjerojatniji mehanizmi kojima aPL uzrokuju trombozu mogu se podijeliti na one u kojima ova antitijela interferiraju s održavanjem koagulacijske homeostaze i one u kojima izazivaju aktivaciju endotelnih stanica, monocita i trombocita. Kako mnoge osobe s visokim titrom aPL nikada ne razviju trombozu, pretpostavlja se da je potreban utjecaj dodatnog protrombotičnog čimbenika kao što je trauma, dugotrajna imobilizacija, oralni kontraceptivi, trudnoća (hiperkoagulabilno stanje) ili infekcija koja dovodi do aktivacije endotelnih stanica. Najvjerojatniji mehanizam kojima aPL uzrokuju spontani pobačaj je interakcija s placentnim aneksinom V te aktivacija komplementa. Prema novijim istraživanjima prisutnost aPL predstavlja neovisan čimbenik rizika za aterosklerozu, najvjerojatnije posredstvom vezanja za beta$_2$-GPI koji se nalazi u brojnim lipoproteinskim frakcijama uključujući i oksidirani LDL.

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agulant (LA). aCL targets complex of beta$_2$-glycoprotein-I (beta$_2$-GPI) and cardiolipin, the negatively charged phospholipid present in the mitochondrial membrane. aCL are detected by beta$_2$-GPI dependent cardiolipin ELISA test. LA activity is detected by functional phospholipid-dependent coagulation assay according to the protocol defined in Sapporo criteria. Antibodies directed against prothrombin and beta$_2$-GPI account for the majority of LA activity. Clinical relevance of a variety of other antibodies against different phospholipid-binding proteins such as prothrombin, protein C, protein S, annexin V, thrombomodulin and kininogen remains uncertain. Strong evidence for a direct role of aPL in the pathogenesis of APS has come from murine models. Passive transfer of aPL to normal mice can generally produce features resembling human APS. The most likely mechanisms involving aPL and leading to thrombosis can be classified as those by which aPL interfere with maintenance of coagulation homeostasis and those where antibodies induce cell-mediated events. The main cells involved are endothelial cells, monocytes and platelets. Since many individuals with high aPL titer do not develop thrombosis it seems clear that other prothrombotic factors may be needed, i.e. a "second hit" is required. These promoters of thrombosis include traumatic injury to the vascular bed, pregnancy (hypercoagulabile state), prolonged immobilization, oral contraceptives, or infection leading to endothelial cell activation. The most likely mechanism by which aPL can lead to fetal loss include interaction with placental annexin V and complement activation. According to recent investigations it seems that aPL could be an independent risk factor for atherosclerosis. One of the potential mechanisms include binding to beta$_2$-GPI which is present in various lipoprotein fractions, including oxidized LDL.

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S11-4

Imunodijagnostika vaskulitisa

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Antineutrofilna citoplazmatska autoantitijela (ANCA) su heterogena skupina cirkulirajućih antitijela protiv citoplazmatskih sastavnica neutrofila i monocita. Sukladno nedavno usuglašenim stajalištima ANCA pokazuju četiri različita imunofluorescentna uzorka na etanolom-fiksiranim ljudskim granulocitima: sjajna granulirana citoplazmatska fluorescencija s izrazitom fluorescencijom

S11-4

Immunodiagnosis of vasculitis

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Antineutrophil cytoplasmic autoantibodies (ANCA) are a heterogeneous group of circulating antibodies toward the cytoplasmic constituents of neutrophils and monocytes. Recent consensus statements on testing and reporting of ANCA recognize four different immunofluorescence patterns on ethanol-fixed human neutrophils: a coarse granular cytoplasmic fluorescence with accentuation

septuma između jezgrinih segmenata – klasična citoplazmatska ili C-ANCA; tipična perinuklearna fluorescencija s djelomičnom fluorescencijom jezgre – perinuklearna ili P-ANCA; izrazita perinuklearna fluorescencija sa minimalnom fluorescencijom unutar jezgre – izrazito perinuklearna ili "atipična" a/P-ANCA; i atipična ANCA, najčešće kombinacija citoplazmatske i perinuklearne fluorescencije. C-ANCA i P-ANCA opisana su u primarnim sistemskim vaskulitisima malih krvnih žila. Ta ANCA prepoznaju proteinazu 3 (PR3-ANCA) i mijeloperoksidazu (MPO-ANCA). PR3-ANCA i MPO-ANCA znakovita su za Wegenerovu granulomatozu, mikroskopski poliangiitis, idiopatski nekrotizirajući "polumjesečasti" glomerulonefritis i Churg-Straussov sindrom. Meta-analiza podataka otkrila je da su PR3-ANCA i MPO-ANCA osjetljivi dijagnostički i biomarkeri aktivnosti bolesti u bolesnika s Wegenerovom granulomatozom i mikroskopskim poliangiitisom. Isto tako, eksperimentalni podaci *in vivo* i *in vitro* podupiru izrazitu imunopatogenetsku ulogu ANCA u vaskulitisima i glomerulonefritisima. Nasuprot tomu, a/P-ANCA su nađena u kroničnim upalnim bolestima crijeva, bolestima potpornih tkiva, autoimunim bolestima jetre, raznim infekcijama i nekim vaskulitisima izazvanim lijekovima. Ta ANCA prepoznaju različite ciljne antigene. To su katepsin G, laktoferin, aktin, tropomiozin, pokretljivi nehistonski kromosomski proteini 1 i 2, baktericidni protein, lamin B1 i histon 1. Učestalost ovih antitijela varira, najčešće je niska. Osim što su korisna u diferencijalnoj dijagnozi Crohnove bolesti i ulceroznog kolitisa nemaju veće kliničko značenje. Da se izbjegne kriva klinička interpretacija od presudne je važnosti jasno razlikovati PR3-ANCA i MPO-ANCA kao pouzdane biomerkere pri dijagnozi i praćenju aktivnosti bolesti u bolesnika s vaskulitisima od a/P-ANCA ili autoantitijela specifičnih za neutrofile (NSA) koja prepoznaju mnoge antigene u bolesnika s kroničnim upalnim bolestima crijeva, jetre i bolestima potpornih tkiva.

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between the nuclear lobes – classic cytoplasmic or C-ANCA; a typically perinuclear fluorescence with some nuclear extension – perinuclear or P-ANCA; pronounced nuclear rim fluorescence with minimal nuclear extension, center of nucleus unstained – very perinuclear or "atypical" a/P-ANCA; and atypical ANCA which include all other fluorescence reactivity, most commonly a combination of cytoplasmic and perinuclear fluorescence. C-ANCA and P-ANCA have been described in primary systemic small vessel vasculitides. These ANCA recognize proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA), respectively. Both PR3-ANCA and MPO-ANCA are closely associated with Wegener's granulomatosis, microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis and Churg-Strauss syndrome. A meta-analysis of data showed that PR3- and MPO-ANCA are sensitive diagnostic and disease-activity biomarkers in patients with Wegener's granulomatosis and microscopic polyangiitis. Also, both *in vitro* and *in vivo* experimental data strongly suppport a pathogenic role of these ANCA in vasculitis and glomerulonephritis. On the contrary, a/P-ANCA were found in patients with chronic inflammatory bowel diseases, connective tissue diseases, autoimmune liver diseases, infectious diseases and some types of drug-induced vasculitis. These ANCA have multiple antigen specificities. These include cathepsin G, lactoferrin, actin, tropomyosin, high motility groups of nonhistone chromosomal proteins 1 and 2, bactericidal permeability-increasing protein, lamin B1 and histone 1. Antibody levels of these ANCA are variable, often low. Apart from the use in the differential diagnosis between Crohn's dsease and ulcerative colitis, very limited clinical significance is ascribed to them. To avoid clinical misinterpretation, it is important to clearly distinguish PR3- and MPO-ANCA as true antineutrophil cytoplasmic antibodies which are diagnostic and disease-activity biomarkers for small vessel vasculitis, from "atypical" P-ANCA that are neutrophil-specific autoantibodies (NSA) which recognize many different autoantigens and are typically found in patients with chronic inflammatory bowel and liver disease and connective tissue diseases.

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S11-5

Imunodijagnostika autoimunih bolesti jetre

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Autoimuni hepatitis (AIH) je bolest nepoznatog uzroka obilježena hipergamaglobulinemijom, cirkulirajućim serumskim autoantitijelima i najčešće dobro reagira na imunosupresivnu terapiju. Dokazivanje autoantitijela provodi se metodom indirektne imunofluorescencije (IIF), dok se za identifikaciju specifičnog antigena rabe imunokemijske metode s obilježenim reagensima. Na temelju nalaza autoantitijela u serumu AIH se danas klasificira u dva tipa. AIH I. obilježavaju antinuklearna antitijela (ANA) i/ili autoantitijela na glatku muskulaturu (AGLM), autoantitijela protiv topljivih jetrenih antigena i mikrosoma jetre i gušterače (anti-SLA/LP) te antineutrofilna citoplazmatska antitijela (ANCA) i autoantitijela protiv specifičnog asijaloglikoproteinskog receptora (anti-ASGP-R). AIH II. obilježavaju autoantitijela protiv mikrosoma jetre i bubrega (anti-LKM), autoantitijela protiv jetrenog citosolnog antigena tip 1 (anti-LC1) te anti-ASGP-R. Supstrati za dokazivanje ANA su kriostatski rezovi štakorske jetre ili osjetljiviji supstrat stanične kulture HEp2 (humane epiteloidne stanice). Metode ELISA i imunoblot (IB) se rabe za dokazivanje najčešćih antigena (histona, dsDNA, centromera). AGLM se često nalaze zajedno s ANA, a glavni ciljni antigen koji prepoznaju je F-aktin. SLA/LP autoantitijela su visoko specifična za AIH i dokazuju se metodom ELISA. Ciljni antigen za anti-LKM1 je citokrom P4502D6 (CYP2D6), a usporedbe određivanja metodom IIF na kriostatskim rezovima glodavaca s metodama koje rabe rekombinantne antigene daju dobre rezultate. Anti-ASGP-R je glikoprotein specifičan za jetru, nalazi se na staničnoj membrani u 88% bolesnika s AIH, a značajno je zastupljen i u primarnoj bilijarnoj cirozi (PBC). Zbog metodoloških nedostataka ne preporuča se u rutinskoj imunodijagnostici AIH. Standardna metoda za dokazivanje ANCA je IIF na etanolom fiksiranim ljudskim granulocitima. Atipična perinuklearna ANCA odlikuju se izrazitom perifernom fluorescencijom jezgre, nalaze se u bolesnika s primarnim sklerozirajućim kolangitisom (PSC). Antimitiohondrijska antitijela (AMA) znakovita su antitijela PBC.

AMA prepoznaju devet (M1 do M9) skupina mitohondrijskih antigena, a oko 95% oboljelih od PBC imaju pozitivna antitijela M2. AMA se dokazuju pomoću IIF na tkivnim preparatima štakorskog bubrega ili na stanicama HEp2, a

S11-5

Immunodiagnosis of autoimmune liver diseases

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Autoimmune hepatitis (AIH) is a progressive, necroinflammatory liver disorder of unknown cause, characterized by hypergammaglobulinemia, liver-specific serum autoantibodies and a positive response to immunosuppressive therapy. Testing for autoantibodies can be carried out by indirect immunofluorescence (IIF) using the appropriate substrate or if the respective target antigen is known and available, by immunologic analyses. According to the pattern of autoantibodies detected in AIH, a subclassification of the disease into two types has been proposed. AIH I is characterized by the presence of antinuclear antibodies (ANA) and/or smooth muscle autoantibodies (SMA), autoantibodies against soluble liver antigen/liver-pancreas antigen (anti-SLA/LP) which may be associated with antineutrophil cytoplasmic autoantibodies (ANCA) and autoantibodies against the asialoglycoprotein receptor (anti-ASGP-R). AIH II is characterized by the presence of autoantibodies against liver-kidney microsomal antigens (anti-LKM), against liver cytosol 1 antigen (anti-LC1) and anti-ASGP-R. ANA is readily detectable by nuclear staining of rodent tissues, HEp2 cell slides or if the respective target antigen is known and available by ELISA. SMA autoantibodies are predominantly directed against filamentary actin (F-actin). SLA/LP autoantibodies are the only antibodies which are 100% specific for AIH. The availability of cloned SLA/LP antigen now allows the development of a reliable standardized ELISA test system. Cytochrome P4502D6 (CYP2D6) has been identified as the main target of LK M1. Results of comparison between anti-LKM1 detected by IIF and anti-CYP2D6 by ELISA show a very good agremeent. Anti LC1 can be detected by IIF but LC1 pattern is usually masked by the concurrent presence of LKM. Thus, an ELISA using recombinant LC1 target antigen may be a more suitable assay. Anti ASGP-R antibodies are detected in 88% of AIH patients. It is believed that anti-ASGP-R represents a general marker of liver autoimmunity but is not recommended for routine use due to limitations in its detection. ANCA is detected by IIF using neutrophils as a substrate. The atypical perinuclear ANCA found in AIH and autoimmune sclerosing cholangitis (ASC) yields perinuclear staining irrespective of the type of fixation and probably reacts with nuclear membrane components. Antimitochondrial antibodies (AMA) are the

njihova specifičnost metodom ELISA ili IB s rekombinantnim antigenima.

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serologic hallmark of primary biliary cirrhosis (PBC). Nine AMA types (M1 to M9) can be distinguished, and some 95% of PBC patients have positive M2 antibodies. AMA is detected by IIF on tissue preparations of rat kidney or on HEp2 cells, and their specifictiy by ELISA or IB method with recombinant antigens.

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S12 – Simpozij 12 – ŠEĆERNA BOLEST, S12-1

Alotransplantacija otočića u bolesnika sa šećernom bolešću tip 1

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Presađivanje otočića odnedavno se predlaže kao alternativa inzulinskoj terapiji kod odabranih skupina bolesnika sa šećernom bolešću tip 1. Glavne indikacije za presađivanje otočića su: dijabetični bolesnici tip 1 koji već uzimaju imunosupresivne lijekove zbog drugih transplantacija (bubreg, jetra), te bolesnici s nestabilnom šećernom bolešću obilježenom nestabilnom glikemijom i hipoglikemijom koje nisu svjesni. Klinički rezultati su sad uvelike poboljšani: postotak neovisnosti o inzulinu se progresivno povećava, i do 90%, prema nedavnom izvješću godinu dana od presađivanja. Pokazana je sigurnost i učinkovitost, zajedno s njihovim pozitivnim učinkom na glikometaboličku kontrolu, te proteinski i lipidni profil. Čak je i u primatelja s djelomičnom funkcijom presatka zabilježeno poboljšanje u inzulinskoj osjetljivosti, HbA_{1c}, tkivnoj raspodjeli glukoze, jetrenom metabolizmu proteina i lipida te proizvodnji glukoze, te smanjenje preživljenja, stope kardiovaskularne smrtnosti, progresije debljine intime medije i izlučivanja albumina mokraćom. Preostaje razjasniti neke aspekte i riješiti neke probleme, čak i u svjetlu kliničke uspješnosti. Presađivanje otočića zahtijeva imunosupresivnu terapiju s mnoštvom nuspojava. Nadalje, funkcija presatka progresivno se smanjuje tijekom praćenja. Perspektive za godine koje dolaze uključuju poboljšanje presađivanja, utvrđivanje novih strategija za kontrolu aloautoodgovora na presadak i alternativne izvore beta stanica.

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S12 – Symposium 12 – DIABETES MELLITUS, S12-1

Islet allotransplantation in type 1 diabetic patients

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slet transplantation has been recently proposed as an alternative to insulin therapy in selected cohorts of type 1 diabetic patients. The main indications for islet transplantation are: type 1 diabetic patients already on immunosuppressant therapy for other transplantations (kidney, liver), and patients with brittle diabetes, characterized by unstable glycemia and unawareness hypoglycemia. Clinical results are now greatly improved: the percentage of insulin independence is progressively increased, up to 90%, as recently reported at one year after transplantation. Safety and efficacy were shown together with their positive impact on glycometabolic control, protein, and lipid profiles. Even in recipients with a partial graft function an improvement of insulin sensitivity, HbA_{1c}, tissue glucose disposal, hepatic protein and lipid metabolism and glucose production, and a decrease of patient survival, cardiovascular death rate, intima media thickness progression and urinary albumin excretion were observed. Some aspects remain to be clarified and problems to be solved, even in the light of clinical success. Islet transplantation requires immunosuppressant therapy with many side effects. Furtherrmore, graft function progressively declines during follow up. Perspectives for the next years include the improvement of engraftment, the identification of new strategies to control the alloautoresponse to the graft and alternative beta cell sources.

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S12-2

Uloga upalnih čimbenika u šećernoj bolesti i komplikacijama

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Upalni čimbenici C-reaktivni peptid (CRP), fibrinogen i homocistein (HCI) važni su za nastanak vaskularnih komplikacija u šećernoj bolesti, jer dovode do ozljede i disfunkcije endotela, što se može smatrati čimbenikom nastanka ateroskleroze. Utvrđeno je da je CRP bolji predskazatelj kardiovaskularnih (KV) bolesti od LDL kolesterola. Povišene koncentracije CRP povezane su i sa sindromom inzulinske rezistencije. Pod utjecajem rizičnih čimbenika dolazi do nastanka multipotentnih proupalnih ("primarnih") citokina IL-1-beta i TNF-alfa, koji potiču nastanak "glasničkih" citokina, primjerice IL-6, koji u jetri dovodi do ekspresije gena odgovornih za povećano stvaranja CRP i SAA.
Uz upalne čimbenike i dislipidemiju homocistein (HCI) je i značajan čimbenik u predviđanju KV bolesti: posjeduje protrombotičko djelovanje, povećava stvaranje kolagena i umanjuje učinak dušik oksida. U šećernoj bolesti utvrđena je povezanost HCI sa stupnjevima nefropatije, retinopatije, neuropatije i KV smrtnosti. U terapiji se mogu primijeniti folna kiselina, vitamini skupine B i statini.
Adiponektin (ADN) pripada u skupinu adipokina podrijetlom iz masnog tkiva. U istu skupinu ubrajaju se još visfatin, leptin, CRP, PAI-1, slobodne masne kiseline, IL-6, TNF-alfa, rezistin i angiotenzin (AT)-II. ADN dovodi do smanjenja upalnih procesa u stijenci krvnih žila. Kada su adipociti prepunjeni, dolazi do sniženja koncentracije ADN i porasta koncentracije rezistina, tumor TNF-alfa, IL-6 i leptina. Posljedice su inzulinska rezistencija, promjena metabolizma glukoze i lipida, upalni procesi. Utvrđena je negativna korelacija razine ADN i upalnih čimbenika. AT-II može uzrokovati sniženje koncentracije ADN djelovanjem na AT1 receptore (R). Blokadom AT1R i inhibicijom enzima konvertaze angiotenzina može doći do porasta koncentracije ADN. To je povezano i sa sniženjem koncentracija TNF-alfa koji moguće suprimira sekreciju ADN. Nefropatija je najučestalija mikrovaskularna komplikacija u osoba sa šećernom bolešću, a mikroalbuminurija je najjednostavniji i najosjetljiviji biljeg njezinog ranog otkrivanja te predskazatelj povećane smrtnosti od KV bolesti. Ne samo zbog djelovanja na sniženje krvnog tlaka, prevenciju albuminurije i dislipidemiju, nego i zbog značajnog učinka na upalne čimbenike RAS-inhibitori i statini smatraju se važnim čimbenicima u prevenciji nastanka vaskularnih komplikacija u osoba sa šećernom bolešću.

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S12-2

The role of inflammatory factors in diabetes mellitus and its complications

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Inflammatory factors C-reactive protein (CRP), fibrinogen and homocysteine are important in the development of vascular complications in diabetes mellitus, as they can cause endothelial dysfunction, which can be considered as an initial event in atherosclerosis. It has been established that CRP is a better predictor of cardiovascular (CV) disease than LDL cholesterol. Increased CRP concentrations are also associated with the syndrome of insulin resistance. Risk factors can stimulate the production of multipotent proinflammatory („primary") cytokines IL-1 beta and TNF-alpha. „Primary" cytokines further stimulate the production of „messenger" cytokines like IL-6, which can cause an increased expression of genes responsible for increased CRP and SAA production.
Besides inflammatory markers and dyslipidemia, homocysteine (HCY) is also important in the prediction of CV events. It has prothrombotic features, increases collagen production and reduces nitric oxide effect. In diabetes mellitus HCY association has been determined with nephropathy, retinopathy, neuropathy and CV mortality. Folic acid, vitamin B group and statins can be used in therapy for hyperhomocysteinemia. Adiponectin (ADN) belongs to a group of adipokines, originating from adipose tissue, together with visfatin, leptin, CRP, PAI-1, free fatty acids, IL-6, TNF-alpha, resistin and angiotensin (AT)-II. ADN leads to a reduction in blood vessel wall inflammation. When adipocytes are ovefilled, there is a reduction in adiponectin and an increase in resistin, TNF-alpha, IL-6 and leptin values. The consequences are insulin resistance, glucose and lipid metabolism changes, and inflammation. A negative correlation has been established between ADN and inflammatory marker levels. AT-II can cause a reduction in ADN concentration by acting on AT1 receptors (R). AT1R blockade and ACE inhibition may lead to an increase in ADN concentration. This is also associated with reduced concentrations of TNF-alpha, which possibly suppresses ADN secretion. Nephropathy is the most common microvascular complication of diabetes, whereas microalbuminuria is a sensitive marker of its early detection and a precursor of CV diseases. Not only due to their effect on blood pressure, albuminuria and dyslipidemia, but also because of the significant effect they have on inflammatory markers, RAS-inhibitors and statins are considered to be important factors in the prevention of vascular complications in diabetes mellitus.

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S12-3

Hemoglobin A1c: kamo nakon 30 godina?

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Hemoglobin A1c (HbA1c) je u proteklih 30 godina primjene postao kliničkim standardom za procjenu metaboličnе kontrole i djelotvornosti terapije šećerne bolesti. Rezultati dugogodišnjih intervencijskih studija (DCCT i UKPDS) dokazali su neprijepornu vezu između kontrole glikemije i pojave kasnih komplikacija šećerne bolesti, te postavili temelj danas globalno prihvaćenog terapijskog cilja za oba tipa dijabetesa kroz vrijednost HbA1c od < 7%. Pouzdana primjena HbA1c u kliničkoj praksi bila je opterećena raznovrsnom i nestandardiziranom metodologijom, varijabilnošću kemijskih entiteta koji nastaju glikacijom molekule hemoglobina i nepostojanjem primarnog referentnog materijala. Kombinacija navedenih čimbenika rezultirala je slabom usporedivošću rezultata što je, s obzirom na samu bit kliničke primjene HbA1c (kontinuirano, doživotno praćenje kontrole glikemije), izazvalo ozbiljne implikacije u kvaliteti dijabetološke skrbi i nametnulo potrebu za standardizacijom. Međunarodna federacija za kliničku kemiju i laboratorijsku medicinu (IFCC) je 2002. g. objavila referentnu metodu za HbA1c. Zajedno s metodom definiran je analit i proizveden primarni referentni materijal. Međutim, primjena referentne metode onemogućena je značajno nižim rezultatima HbA1c u odnosu na "konvencionalne" metode. Naime, kliničke smjernice utemeljene su na rezultatima kliničkih istraživanja u kojima se rabila precizna, ali nedovoljno specifična analitička metodologija koja je postala "dogovorni standard" za HbA1c. Zahtjev za odgovarajućom primjenom kliničkih smjernica ispunjen je usklađivanjem svih metoda s globalno prihvaćenim dogovornim standardom i izražavanjem rezultata HbA1c u obliku "ekvivalenata DCCT". Moguće snižavanje apsolutnih vrijednosti HbA1c, koje bi nastalo uvođenjem referentnog sustava IFCC prepoznato je kao ozbiljna prepreka u postizanju i održavanju dobre kontrole šećerne bolesti. Stoga dijabetološka struka energično ustrajava na zadržavanju postojećih standarda. Harmonizacija određivanja HbA1c nužno podrazumijeva kombinaciju analitičkih i kliničkih zahtjeva. Stoga je predložen "treći put", odnosno izvođenje novog parametra, "prosječne glikemije", koji bi sublimirao analitičke prednosti referentnog sustava IFCC i dragocjene kliničke podatke. Transformacija vrijednosti HbA1c u jedinstveni parametar koji bi s najvećom pouzdanošću pružio objektivni uvid u kontrolu glikemije konačni je cilj ove specifične harmonizacije.

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S12-3

Hemoglobin A1c: 30 years later

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Within the last 30 years, hemoglobin A1c (HbA1c) has become the clinical standard for both metabolic control and therapeutic efficacy assessment in diabetes mellitus. Results of the long-term intervention trials (DCCT and UKPDS) clearly demonstrated a relationship between the mean blood glucose and incidence of late diabetic complications, and provided a background for today's globally accepted therapeutic goal for both types of diabetes, defined through HbA1c of < 7%. Reliable clinical use of HbA1c has been burdened by a variable and unstandardized methodology, different chemical entities resulting from glycation of the hemoglobin molecule, and the lack of primary reference material. A combination of the aforementioned factors has resulted in poor comparability of results which, regarding the very essence of the clinical use of HbA1c (continuous, life-long monitoring of glycemic control), has created serious implications in the quality of diabetic care and posed the need for standardization. A reference method for HbA1c has been published by the IFCC in 2002. Together with the method, an analyte has been defined and a primary reference material has been developed. However, the use of the reference method has been disabled due to significantly lower HbA1c results as compared with "conventional" methods. Namely, clinical guidelines and standards are based on the results of clinical studies, where a precise but not specific enough analytical methodology was used, which has become a "designated standard" for HbA1c. The requirement of appropriate implementation of clinical guidelines was fulfilled by the harmonization of all methods towards globally-accepted designated standard, and HbA1c results exspression as "DCCT-equivalents". The possibility of lowering the absolute HbA1c values, issued by implementation of the IFCC-reference system, has been recognized as a serious hindrance in attaining and maintaining good diabetic control. Hence, diabetology specialty vigorously insists on keeping current standards. Harmonization of HbA1c determination includes implicitly a combination of both analytical and clinical requests. Thus, a "third way" has been proposed, that is, to derive a new parameter, "mean blood glucose", subliming analytical superiority of the IFCC-reference system and valuable clinical data. Transformation of the HbA1c values into a unique parameter, able to provide with utmost reliability an objective insight into glycemic control, is the ultimate goal of this specific harmonization.

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S12-4

Metabolički sindrom

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Metabolički sindrom predstavlja skup čimbenika rizika za šećernu bolest i kardiovaskularne bolesti. Iznimnim radom Reavena počinje 1988. godine moderna era metaboličkog sindroma; tada je autor opisao udruženost poremećaja tolerancije glikemije, arterijske hipertenzije, dislipidemije i debljine, što je nazvao "sindromom X", te ih povezao s postojanjem inzulinske rezistencije kao temeljnim patofiziološkim poremećajem. Svjetska zdravstvena organizacija (SZO) je 1999. godine izdala svoju definiciju metaboličkog sindroma u kojoj je kao obvezna sastavnica bila poremećena tolerancija glukoze ili inzulinska rezistencija zajedno s barem dva ili više od slijedećih poremećaja: arterijskom hipertenzijom, centralnim tipom debljine i dislipidemijom. NCEP donosi svoju definiciju dvije godine kasnije s drukčijim dijagnostičkim razinama, a na kraju je i Europska studijska grupa za inzulinsku rezistenciju (EGIR) donoseći svoju varijantu doprinjela zbrci u definiciji. IDF (*International Diabetes Federation*) je 2004. godine donijela konsenzus o novoj definiciji SZO koja bi svojom jednostavnošću bila primjenjiva u kliničkim i epidemiološkim istraživanjima diljem svijeta. Budući da se inzulinsku rezistenciju teško može jednostavno i ponovljivo određivati, zaključeno je da centralni tip debljine, osobito udružen s hipertrigliceridemijom, mora svakako biti sastavnicom definicije. Opseg struka je prihvaćen kao mjerilo centralne debljine, ali se različite granične vrijednosti moraju rabiti za različite etničke skupine (za europide je gornja granica opsega struka u muškaraca 94 cm, a u žena 80 cm). Ostali parametri uključeni u definiciju su: niska razina HDL kolesterola (< od 0,9 mmol/L u muškaraca i < 1,1 mmol/L u žena), povišen krvni tlak (> 130/85 mm Hg), te hiperglikemija natašte (glikemija natašte > 05,6 mmol/L) ili prethodno dijagnosticirana šećerna bolest. Danas ne postoji jedinstven terapijski pristup metaboličkom sindromu osim modifikacije životnih navika koje se odnose na prehranu i tjelesnu aktivnost, ali treba liječiti svaku sastavnicu metaboličkog sindroma zasebno.

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S12-4

Metabolic syndrome

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The modern era of the "metabolic syndrome", a cluster of risk factors for diabetes and cardiovascular disease, began with the seminal work of Reaven and his Banting lecture in 1988. He described the association of glucose intolerance, hypertension, dyslipidemia and obesity, and termed it syndrome X with insulin resistance suggested as the unifying underlying etiologic factor. In 1999, World Health Organization (WHO) published a suggested definition with a *sine qua non* of glucose intolerance or insulin resistance together with two or more of hypertension, central obesity and dyslipidemia (raised triglycerides, lowered HDL-cholesterol). The Nation Cholesterol Education Program came out with its own definition (ATP III) 2 years later with less primacy for glucose intolerance and different cutpoints for the variables. The European Study Group for Insulin Resistance (EGIR) also produced a variant of the WHO definition. In order to clarify the situation IDF held a Consensus Meeting in 2004 to update the WHO definition and to see whether a single common clinically and epidemiologically useful definition could be agreed for use world-wide. It was agreed that insulin resistance could not be measured simply and reproducibly, and should not be included. Central obesity, particularly when associated with raised plasma triglycerides, was set as a *sine qua non*. Waist circumference, a measure of central obesity, was ethnic-specific and different cutpoints would be needed for different ethnic groups (for male Europeans 94 cm, and for female Europeans 80 cm). The other agreed components were low HDL-cholesterol (<0.9 mmol/L for male and <1.1mmol/L for female), hypertension (>130/85 mm Hg blood pressure values) and fasting hyperglycemia or previously diagnosed diabetes (fasting glucose >5.6 mmol/L). Today we do not have specific therapy for metabolic syndrome, but we should deal with each of the component abnormalities in particular.

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P1 – Prikaz slučaja, P1-1

Neusklađeni rezultati pretraga štitnjače: prikaz slučaja

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Prikazan je slučaj dvadesetdvogodišnjeg muškarca koji je prema preporuci dermatologa upućen u naš laboratorij na hormonsku obradu štitnjače zbog vitiliga. Izmjerena je visoka koncentracija slobodnog tiroksina (FT4), povišen tirotropin (TSH), trijodtironin (TT3) unutar referentnog raspona, povišena tiroglobulinska protutijela (ATg), bez simptoma i znakova bolesti štitnjače. Kontrolni nalaz bio je isti. Zbog neusklađenih rezultata pretraga štitnjače bolesnik je upućen u KBC Rijeka, a potom u Zagreb, gdje su mu određivani hormoni za ispitivanje funkcije štitnjače, načinjen je ultrazvuk i scintigram štitnjače. Nalaz ultrazvuka i scintigrama štitnjače u obje Klinike bio je uredan, bez znakova bolesti. Bolesniku je urađen i CT hipofize; u nalazu nisu pronađene patološke promjene. Funkcija nadbubrežne žlijezde bila je uredna. U riječkoj Klinici svi laboratorijski parametri za ispitivanje funkcije štitnjače bili su unutar referentnog raspona. U Zagrebu je izmjeren povišen TSH i Atg, na osnovi čega je bolesniku uvedena nadomjesna terapija L-T4 (Eutirox) u svrhu liječenja hipotireoze. Kontrolni nalaz napravljen je u Puli: TSH unutar referentnog raspona, TT3 povišen i FT4 izrazito visok (164,4 pmol/L), a potom u Rijeci gdje su izmjerene povišene koncentracije TT3 i FT3 te TSH, TT4 i FT4 unutar referentnog raspona. Posljednja kontrola bolesnika, pod terapijom Eutiroxom, napravljena je u Puli, gdje je izmjeren TSH i TT3 unutar referentnog raspona. FT4 je izmjeren dvjema metodama: metodom RIA DPC izmjerili smo izrazito visoku koncentraciju FT4 (125,5 pmol/L), dok smo iz istog uzorka metodom LIA (IMMULITE-DPC) izmjerili FT4 (18,5 pmol/L) unutar referentnog raspona. Imunokemijska određivanja pa tako i određivanje hormona za ispitivanje funkcije štitnjače mogu biti podložna različitim interferencijama, što može biti uzrokovano prisutnošću heterofilnih protutijela životinjskog podrijetla (HAMA). Takve rezultate treba pravodobno uočiti, jer mogu dovesti do ozbiljnih poteškoća u tumačenju nalaza. Upitne rezultate treba provjeriti mjerenjem različitim metodama (razvijene su sofisticirane metode s blokirajućim protutijelima koja otklanjaju moguće interferencije).

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P1 – Case report, P1-1

Incompatible thyroid test results: case report

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A case is presented of a 22-year-old man with vitiligo, who presented to our laboratory for thyroid hormone testing upon a dermatologist's request. A high concentration of free thyroxine (FT4) was measured, along with elevated thyrotropin (TSH), triiodothyronine (TT3) within the reference range, elevated thyroglobulin antibodies (Atg), and without thyroid disease symptoms. Control results were the same. As thyroid test results were incompatible, the patient was referred to clinical hospitals in Rijeka and Zagreb. They measured hormones to determine thyroid function, performed ultrasound tests and thyroid scintigraphy. Ultrasound and scintigraphy results were normal, without signs of the disease. The patient underwent pituitary gland CT; no pathologic changes were found. Adrenal gland results were normal. In Rijeka, all laboratory parameters on thyroid function testing were within the reference range. In Zagreb, elevated TSH and Atg were measured. Consequently, L-T4 replacement therapy (Eutirox) was introduced to treat hypothyroidism. Control tests were conducted in Pula: TSH was within the reference range, TT3 was elevated and FT4 extremely high (164.4 pmol/L). On control testing in Rijeka, elevated concentrations of TT3 and FT3 were measured along with TSH, TT4 and FT4 within the reference range. The latest tests were conducted in Pula, with the patient under Eutirox therapy: TSH and TT3 were within the reference range. Two methods were used for FT4: RIA method of DPC yielded an extremely high concentration of FT4 (125.5 pmol/L), whereas LIA method (IMMULITE-DPC) showed FT4 (18.5 pmol/L) within the reference range in the same sample. Immunochemical measurements, including the hormones for thyroid function testing, may be subjected to various interferences that may be due to the presence of heterophil antibodies of animal origin (HAMA). It is necessary to observe such results on time as they can lead to serious difficulties on result interpretation. Vague results should be checked by different methods (there are sophisticated methods with blocking antibodies which eliminate the possible interferences).

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P1-2

Tromboembolija u 14-godišnjeg dječaka

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Četranaestgodišnji dječak primljen je u hitnu dječju ambulantu, a potom na odjel Klinike za pedijatriju zbog otoka desne potkoljenice. Unatrag tri tjedna dječak je bio u mirovanju kod kuće zbog infekta liječenog antibioticima. Dokazana je duboka venska tromboza (DVT) potkoljeničnih vena desne noge. Uvedena je terapija niskomolekularnim heparinom. Od petog dana boravka osjeća zadušu i bol u lijevoj strani prsišta. Dijagnosticirana je plućna embolija. Uvodi se terapija nefrakcioniranim heparinom, a potom i oralnim antikoagulansom (Marivarin(R)). Bolesnik je uspješno reagirao na terapiju, heparinska terapija je isključena i liječenje nastavljeno Marivarinom (R). Prikazuju se rezultati dijagnostičke obrade koji upućuju na mogući uzrok nastanka tromboembolije u ovog bolesnika. U bolesnika su načinjene slijedeće pretrage: SE, KKS, APTV, PV, TV, fibrinogen, test fibrinolize, D-dimeri, antitrombin III (ATIII), protein C (PC), protein S (PS), čimbenici zgrušavanja FII-FXIII, plazminogen (PG), inhibitor aktivatora plazminogena-1 (PAI-1), APC rezistencija (APCR), lupus antikoagulant (LAC), antikardiolipinska antitijela (ACA) i antitijela na dvostruku uzvojnicu DNA (anti-dsDNA) i genetski čimbenici rizika koagulacijskih poremećaja: genotipizacija polimorfizama PAI-1, FII zgrušavanja, MTHFR i mutacija FV Leiden. Određivanje APTV i PV rađeno je svakodnevno prema protokolu za praćenje dvojne antikoagulantne terapije DVT. Utvrđene su povišene vrijednosti D-dimera (1,3-1,9 mg/L), PAI-1 (4,9 U/mL), LAC (3,0 uz produljenje APTV heparinom i 2,76 nakon heparinske terapije), ACA-IgG (104,7 GPL-U/mL); snižene vrijednosti broja trombocita (90-106x10^9/L), aktivnosti FXIII (50,6%), aktivnosti PS (50,1%) i APCR (0,81). Vrijednosti unutar referentnog raspona: aktivnosti FII-FXII (73,4-117,2%), PC (93,7%), ATIII (92%), PG (89,5%), ACA-IgM (5,1 MPL-U/mL) i anti-dsDNA (16,3 IU/mL). Molekularnom dijagnostikom utvrđen je genotip 4G/5G (heterozigot za PAI-1 polimorfizam), uz uredan nalaz drugih molekularnih biljega. Dijagnostičkom obradom dokazano je više čimbenika rizika za nastanak tromboembolije u ovog bolesnika: heterozigotnost za PAI-1 polimorfizam uz povišenu koncentraciju PAI-1, smanjena APCR uz sniženu aktivnost PS, pozitivna LA i ACA autoantitijela.

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P1-2

Thromboembolism in a 14-year-old boy

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A 14-year-old boy was admitted to the emergency pediatric clinic and then transferred to the University Department of Pediatrics ward for edema of the right lower extremity. Three weeks before, the boy was at rest for infection treated by antibiotics. Deep venous thrombosis (DVT) of the lower right extremity was confirmed. Therapy with low-molecular weight heparin was introduced. From hospitalization day 5 he felt dyspnea and pain in the left chest. Pulmonary embolism was diagnosed. Therapy with unfractionated heparin was initiated, followed by oral anticoagulants (Marivarin(R)). The patient responded well to this therapy, heparin was discontinued, and treatment was continued with Marivarin (R). Results of diagnostic work-up are presented, suggesting the possible cause of thromboembolism in this patient. The following tests were performed: ESR, CBC, APTT, PT, TT, fibrinogen, fibrinolysis test, D-dimer, antithrombin III (ATIII), protein C (PC), protein S (PS), FII-FXIII, plasminogen (PG), plasminogen activator inhibitor-1 (PAI-1), APC resistance (APCR), lupus anticoagulants (LA), anticardiolipin antibodies (ACA), antibodies to double-stranded DNA (anti-dsDNA) and genetic risk factors for coagulation disorders: genotyping polymorphisms for PAI-1, coagulation FII, MTHFR and Factor V Leiden mutation. APTT and PT were determined according to the protocol for monitoring of double anticoagulant therapy of DVT. Increased values were measrued for: D-dimer (1.3-1.9 mg/L), PAI-1 (4.9 U/mL), LA (3.0 by heparin APTT prolongation and 2.76 after heparin therapy), ACA-IgG (104.7 GPL-U/mL); decreased values were measured for: platelets (90-106x10^9/L), activities of FXIII (50.6%) and PS (50.1%), APCR (0.81); values within the reference range were measured for: activities of FII-FXII (73.4-117%), PC (93.7%), ATIII (92%) and PG (89.5%), ACA-IgM (5.1 MPL-U/mL), anti dsDNA (16.3 IU/mL). Molecular diagnosis confirmed the 4G/5G genotype (heterozygous for PAI-1 polymorphism) with no detected polymorphism for other molecular markers. Thus, diagnostic work-up confirmed several risk factors for thromboembolism in this patient: heterozygosity for PAI-1 polymorphism with elevated PAI-1 concentration, decreased APCR with lower PS activity, and positive LA and ACA autoantibodies.

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P1-3

Kronična mijeloična leukemija - važnost praćenja terapije: prikaz slučaja

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U listopadu 2000. godine 49-godišnjoj bolesnici dijagnosticirana je kronična mijeloična leukemija: uz normaflan broj eritrocita i trombocita u perifernoj krvi imala je 215x10^9/L leukocita te povećanu jetru i slezenu; u koštanoj srži utvrđena je izrazita proliferacija bijele loze te dokazana translokacija t(9;22)(q34;q11)(Philadelphia kromosom) i fuzijski gen BCR-ABL. Odmah je započeta terapija hidroksiurejom i interferonom alfa te je postignuta potpuna hematološka remisija bez molekularne remisije. Nakon 2 godine citogenetičkom je analizom dokazano 67% Ph pozitivnih interfaznih jezgara (Ph+I.J.), kod bolesnice se pojavio recidiv te je započeta terapija imatinib-mesilatom u dozi od 400 mg/dan. Nakon 6 mjeseci terapije postignuta je potpuna citogenetička remisija (FISH negativan na prisutnost Ph+I.J.), ali je RT-PCR bio pozitivan na fuzijski gen BCR-ABL. Kvantitativnim PCR utvrđen je mali broj kopija fuzijskog gena (BCR-ABL/ABL<0,01) s tendencijom opadanja u slijedećih 10 mjeseci te je postignuta i djelomična molekularna remisija. Nastavljena je terapija imatinibom od 400 mg/dan. Dvadeset i tri mjeseca od početka terapije imatinibom FISH analizom pronađeno je 2% Ph+I.J., a kvantitativnim PCR porast omjera BCR-ABL/ABL 5 puta. Bolesnica je tada još uvijek bila u hematološkoj remisiji. U slijedećih 6 mjeseci prekinuta je terapija zbog nedostatka lijeka, što je vjerojatno uzrokovalo još veći porast Ph+I.J. (13%) te porast omjera BCR-ABL/ABL 20 puta. S obzirom na gubitak potpune citogenetičke remisije i porast broja kopija fuzijskog gena, uzrok treba tražiti u pojavi mutacija koje su u većini slučajeva uzrok rezistencije na terapiju imatinibom. Dio mutacija može se svladati povećanjem doze lijeka, što je u slučaju ove bolesnice i učinjeno povećanjem doze imatiniba na 600 mg/dan. Već nakon 4 mjeseca od povišenja doze smanjio se postotak Ph+I.J., ali je još uvijek bio povišen omjer BCR-ABL/ABL. U slijedećoj kontroli, nakon 5 mjeseci, zabilježen je pad Ph+I.J. (5%), a i omjer BCR-ABL/ABL je pao na vrijednosti ispod 0,01. Zadnja kontrola u svibnju 2006. pokazuje ponovni ulazak u potpunu citogenetičku remisiju s još uvijek pozitivnim RT-PCR na BCR-ABL. Prikazom ovoga slučaja pokazana je važnost praćenja terapije kvantitativnim PCR čija je dijagnostička osjetljivost između analize FISH i RT-PCR, a upravo je taj dio praćenja terapije najosjetljiviji. Osobito je to važno kod bolesnika koji ne postignu potpunu moleku-

P1-3

Chronic myelogenous leukemia – therapy monitoring: case report

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A 49-year-old female patient was diagnosed with chronic myelogenous leukemia in October 2000: hepatosplenomegaly was present along with increased white blood cell count (215x10^9/L) but normal red blood cell count and platelet count; the presence of the Philadelphia chromosome (translocation t(9;22)(q34;q11)) and BCR-ABL fusion gene was confirmed along with distinct leukocyte proliferation in the bone marrow. Initial therapy consisted of hydroxyurea and interferon alpha (Intron A), and the patient achieved complete hematologic response but no molecular response. Two years later, bone marrow cytogenetic analyses showed 67% of Ph positive interphase cells. The patient relapsed and imatinib 400 mg/day was started. Six months later, complete cytogenetic remission was achieved (FISH was negative for Ph positive interphase cells) but qualitative PCR was positive for BCR-ABL. The BCR-ABL/ABL ratio was low and decreasing in the next 10 months, and partial molecular response was achieved. Imatinib was continued at 400 mg/day. Twenty-three months of the introduction of imatinib therapy, 2% of Ph positive interphase cells werer found and BCR-ABL/ABL ratio increased five-fold but the patient was still in hematologic remission. During the following 6 months, imatinib therapy was discontinued, which was the probable cause of increase in the number of Ph positive interphase cells (13%) and BCR-ABL/ABL ratio (20x). The loss of complete cytogenetic remission, the increase in BCR-ABL copy number and resistance to therapy are the most common results of a mutation event in the BCR-ABL kinase domain. Increased drug concentrations can overcome some of these mutations so imatinib was increased to 600 mg/day. Four months later, evaluation showed a decrease in Ph positive interphase cells (5%) but the BCR-ABL/ABL ratio remained high. The same regimen of imatinib was continued, five months later bone marrow cytogenetics revealed 4% of Ph positive interphase cells and BCR-ABL/ABL ratio below 0.01. The last follow-up in May 2006 showed complete cytogenetic response but still detectable BCR-ABL fusion gene. This case report indicates the importance of the most sensitive part of therapy monitoring by quantitative PCR with diagnostic sensitivity between FISH analysis and qualitative PCR. This is extremely important for patients without

larnu remisiju, tj. ostaju pozitivni u RT-PCR, jer svaki porast broja kopija može značiti ponovnu pojavu bolesti.

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complete molecular remission (qualitative PCR positive for BCR-ABL) where the increase in BCR-ABL copy number can predict disease progression.

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P1-4

Waldenströmova makroglobulinemija i vrijednosti leukocita

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Waldenströmova makroglobulinemija prvi puta je opisana 1944. godine. Danas taj oblik ne-Hodgkinova limfoma čini 2% svih hematoloških karcinoma. Iako se javlja u svim životnim dobima i nije spolno određena, bolest je učestalija u muškaraca starijih od 50 godina. Žena u dobi od 77 godina hospitalizirana je putem hematološke ambulante zbog simptoma anemije s otežanim disanjem, slabošću i malaksalošću, te nepodnošenjem ikakvog napora uz ranije potvrđenu šećernu bolest i hipertenziju. Pri zaprimanju u bolnicu učinjene su slijedeće pretrage: sedimentacija eritrocita, glukoza, kreatinin, diferencijalna krvna slika (DKS). Pri izradi DKS na hematološkom brojaču Cell Dyn 3500 (Abbott, SAD) uz oznake upozorenja uočene su velike razlike u vrijednosti leukocita mjerene optičkim i impedancijskim načinom, pa je DKS učinjena ručno. Dobivene vrijednosti DKS uz prisutnost *rouleaux* formacija i izrazito visoku sedimentaciju, određenu elektroforezu serumskih proteina u kojoj se isticala povećana gama frakcija, izmjerene vrijednosti imunoglobulina u kojima se isticala vrijednost imunoglobulina M ukazivale su na postojanje Waldenströmove makroglobulinemije. Naknadnom obradom učinjena je imunoelektroforeza sa specifičnim protutijelima za lake i teške lance, pričem je potvrđena prisutnost monoklonskog imunoglobulina klase M tipa lakog lambda lanca. Terapija osnovne bolesti započeta je Alkeranom i prednisonom. Tijekom tromjesečne terapije vrijednosti leukocita mjerene impedancijom kretale su se unutar referentnih vrijednosti za ženski spol i navedenu dob (medijan 4,53x10^9/L), dok su vrijednosti određene optičkim načinom rasle od 39,2 do 149x10^9/L (medijan 87,2x10^9/L). Porast vrijednosti leukocita mjerene optičkim načinom korelirao je s porastom gama frakcije, odnosno imunoglobulina M, što je potvrdilo izostanak terapijskog odgovora na primijenjene lijekove te je terapija promijenjena.

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P1-4

Waldenström's macroglobulinemia and leukocyte values

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Waldenström's macroglobulinemia was first described in 1944. Today, this form of non-Hodgkin lymphoma accounts for about 2% of all hematologic cancers. Although it occurs in all age groups and is not sex-determined, it is more common in men over fifty years of age. A 77-year-old woman was hospitalized through hematologic clinic with symptoms of anemia, difficult breathing, weakness, and intolerance of even minor effort. Diabetes mellitus and hypertension were confirmed previously. On admission, the following tests were done: erythrocyte sedimentation rate, glucose, creatinine, and differential blood count. Differential blood count was done on a Cell Dyn 3500 (Abbott, USA) blood counter. During the test, there were great differences between leukocyte values measured optically and with impedance. Therefore differential blood count was done manually. Along with the presence of rouleaux formations and extremely high sedimentation, serum protein electrophoresis with considerably increased gamma fraction, the measured immunoglobulin values with marked M immunoglobulin value were indicative of Waldenström's macroglobulinemia. Additionally, immunoelectrophoresis with specific antibodies for light and heavy chains confirmed the presence of monoclonal M class immunoglobulin of the light lambda chain type. The underlying disease was treated with Alkeran and prednisone. During three months, leukocyte values measured with impedance oscillated within the reference range for women and age (median 4.53x10^9/L), while those measured optically increased from 39.2 to 149x10^9/L (median 87.2x10^9/L). The increase in optically measured leukocyte values correlated with the increase of gamma fraction, i.e. M immunoglobulin. It confirmed the lack of therapeutic response to the medications administered, and the patient's therapy was changed accordingly.

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P1-5

Intrahepatična kolestaza povezana s deficitom alfa-1-antitripsina u novorođenčeta: prikaz slučaja

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Novorođenče u dobi od 15 dana primljeno je u Gastroenterološki odjel Klinike za pedijatriju zbog aholičnih stolica i sumnje na kolestazu. Nije primijećeno žutilo kože, a mokraća je bila žute boje.
U novorođenčeta su načinjene biokemijske (ukupni i konjugirani bilirubin, AST, ALT, GGT, ALP i 5'-NU, žučne kiseline, alfa-1-antitripsin (AAT), alfa-fetoprotein, ukupni kolesterol, LDL i HDL kolesterol, trigliceridi, CRP, imunoglobulini IgG, IgA i IgM u serumu, slobodne masne kiseline u majčinom mlijeku, kloridi u znoju), hematološke (sedimentacija eritrocita, kompletna i diferencijalna krvna slika) te koagulacijske pretrage (VK, VZ, PV i fibrinogen). Od navedenih pretraga izmjerene su umjereno povišene koncentracije ukupnog (83,1 μmol/L), konjugiranog (29,5 μmol/L) i nekonjugiranog (53,6 μmol/L) bilirubina, uz izrazito povišenu koncentraciju žučnih kiselina (106,3 μmol/L). Zbog porasta nekonjugiranog bilirubina učinjena je genotipizacija na Gilbertov sindrom dječaka i njegovih roditelja. Mutacija nije pronađena. Aktivnost GGT (171 U/L) također je bila povišena, kao i koncentracije ukupnog kolesterola (5,6 mmol/L), LDL kolesterola (3,4 mmol/L) te triglicerida (3,0 mmol/L). U majčinom mlijeku jako je bila povišena koncentracija slobodnih masnih kiselina. Uz navedene pretrage izmjerena je snižena koncentracija AAT od 0,46 g/L. Ostali laboratorijski nalazi bili su uredni. Ultrazvukom abdomena nije utvrđeno postojanje urođenih malformacija žučnog mjehura i vodova, a scintigrafijom jetre (HIDA) potvrđena je potpuna intrahepatična kolestaza. Zbog snižene koncentracije AAT učinjena je fenotipizacija i genotipizacija kod dječaka i kod roditelja. Kod dječaka je dokazan fenotip PiZZ, a kod roditelja PiMZ (otac) i PiSZ (majka), pričem je M divlji, a S i Z su mutirani aleli. Rezultati su potvrđeni i genotipizacijom. Postavljena je dijagnoza potpune intrahepatične kolestaze povezane s deficitom AAT. U dobi od 4 mjeseca dječak je ponovno primljen na odjel, jer unatoč terapiji (Ursofalk, Phenobarbiton, Plivit D3) nije došlo do regresije znakova kolestaze, a bilježi se i daljnji porast koncentracije bilirubina (ukupni 94,6; konjugirani 39,3; nekonjugirani 55,3 μmol/L) te porast aktivnos-

P1-5

Intrahepatic cholestasis associated with alpha-1-antitrypsin deficiency in newborn: case report

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A 15-day-old male newborn with suspected cholestasis due to alpha-1-antitrypsin (AAT) deficiency was admitted to gastroenterology ward of the Pediatrics Department. Biochemistry tests (total and direct bilirubin, AST, ALT, GGT, ALP, 5'-NU, bile acids, ATT, alpha-fetoprotein, total cholesterol, LDL and HDL cholesterol, triglycerides, CRP, IgG, IgA and IgM immunoglobulins in serum, free fatty acids in breast milk, chlorides in sweat), hematology tests (erythrocyte sedimentation rate, differential blood count) and coagulation tests (bleeding time, clotting time, PT and fibrinogen) were determined. Increased values were found for total, direct and indirect bilirubin (83.1, 29.5 and 53.6 μmol/L, respectively) and bile acids (106.3 μmol/L). Due to the increased concentration of indirect bilirubin, genotyping for Gilbert's syndrome was performed in the infant and parents, with no mutation detected. The activity of GGT (171 U/L) and concentrations of total cholesterol (5.6 mmol/L), LDL cholesterol (3.4 mmol/L) and triglycerides (3.0 mmol/L) were also increased. Free fatty acid concentration in breast milk was extremely increased. In addition, AAT concentration was decreased (0.46 g/L). Other laboratory tests were normal. Abdominal ultrasound excluded existence of inborn malformation of the gallbladder and bile ducts but hepatobiliary scintigraphy (HIDA) identified complete intrahepatic cholestasis. Due to decreased AAT concentration, AAT phenotype and genotype testing of the infant and his parents was performed to reveal the boy to have PiZZ, the mother PiSZ and the father PiMZ phenotype (M being wild type, S and Z mutated alleles). Results were confirmed by genotyping. Taking all these into account, the diagnosis of intrahepatic cholestasis associated with AAT deficiency was made. The infant was readmitted to the gastroenterology ward at the age of 4 months. Signs of cholestasis did not disappear in spite of therapy (ursodiol, phenobarbitone, vitamins E and D3). The concentrations of total, direct and indirect bilirubin (94.6, 39.3 and 55.3 μmol/L, respectively), bile acids (151 μmol/L) and enzyme activities (AST 153, ALT 159, GGT 1790, ALP 1010 and 5'-NU

ti enzima (AST 153, ALT 159, GGT 1790, ALP 1010 U/L). Koncentracija ukupnih žučnih kiselina i aktivnost 5'-nukleotidaze također su u porastu (151 μmol/L odnosno 54,1 U/L). Ponovno je načinjen ultrazvučni pregled abdomena i scintigrafija koji, osim povećanja jetre, nisu pokazali razlike u odnosu na rezultate prvog pregleda. Bolesnik je upućen na dodatnu radiološku obradu (MRCP) kako bi se isključilo postojanje pridružene anomalije žučnih vodova. Kontrolni nalazi pred otpust bolesnika nisu izmijenjeni, a konačna dijagnoza još nije potvrđena. Fenotipizacijom i genotipizacijom dokazana je samo deficijencija AAT povezana s intrahepatičnom kolestazom koju potvrđuju patološki biokemijski nalazi pretraga enzima i bilirubina. U majke je pronađen fenotip PiSZ AAT koji u 7 godina naše prakse dosada nije zabilježen.

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54.1 U/L) showed further increase. Abdominal ultrasound and hepatobiliary scintigraphy were performed revealing no change in the results, except for liver enlargement. The patient was referred for additional radiologic studies (MRCP) to exclude inborn bile duct anomalies. Control laboratory tests before discharge from the hospital were the same. Definitive diagnosis has not yet been confirmed. Phenotype and genotype testing only confirmed AAT deficiency associated with intrahepatic cholestasis. This diagnosis was confirmed by abnormal enzyme and bilirubin test results. PiSZ phenotype discovered in the boy's mother is a very rare AAT phenotype, now detected for the first time in our 7-year experience of performing this analysis.

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P1-6

Udružena megaloblastična i AIHA (autoimuna hemolitička anemija) – prikaz slučaja

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Bolesnica M.M. u dobi od 55 godina primljena je sa simptomima slabosti, parametri vrtoglavice. Unazad 10 godina se liječi zbog shizofrenije, reumatoidnog artritisa (RA) i bilateralne gonartroze, a unazad 5 godina od hipertenzije. Bolesnica je kod prijma blijeda, adinamična, eupnoična, kardiopulmonalno kompenzirana, RR 180/100 mmHg, c/p 72/min, bez periferne limfadenopatije, adipozna; TT 117 kg, TV 162 cm, teško pokretna. Abdomen je palpatorno uredan. Nalazi kod prijma: hematološki E $1,68x10^{12}$/L, Hb 65 g/L, HTC 0,189 L/L, MCV 112,7 fL, MCH 38,9 pg, MCHC 345 g/L, RDW 32,8, Tr $87x10^{9}$/L, MPV 7,4 fL, L $5,4x10^{9}$/L. Naglašene su i oznake: dimorfna populacija eritrocita, poikilocitoza, mikroeritrociti, fragmenti eritrocita, shizociti, koje obilježavaju hemolizu. Biokemijske pretrage nakon prijma: nađen je vrlo povišen LD 26 900 U/L, dok su vrijednosti bilirubina, urobilinogena bile uredne, ali je IAT (indirektni antiglobulinski test) bio negativan. Folna kiselina je bila urednih vrijednosti, a vitamin B12 snižen (41 pg/mL). Zbog snižene vrijednosti vitamina B12 i kliničke slike bolesnice nije učinjena aspiracijska punkcija koštane srži te

P1-6

Combined megaloblastic and AIHA (immunohemolytic anemia) – case report

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A female patient aged 55 presented with weakness and vertigo. For ten years now she had been treated for schizophrenia and rheumatoid arthritis (RA). The patient was pale, adynamic, eupneic, cardiopulmonarily compensated, without peripheral lymphadenopathy, and adipose. Body weight 117 kg, height 162 cm, moving with difficulty. The abdomen normal on palpation. Blood test results on admission were as follows: RBC $1.68x10^{12}$/L, Hb 65 g/L, Hct 0.189 L/L, MCV 112.7 fL, MCH 38.9 pg, MCHC 345 g/L, RDW 32.8, Plt $87x10^{9}$/L, MPV 7.4 fL, WBC $5.4x10^{9}$/L. Red blood cell assessments showed profound alterations: dimorphic erythrocyte population, microerythrocytes, and erythrocyte fragments-schizocytes were found, whereas erythrocytes showed pronounced anisopoikilocytosis, hypochromia and polychromasia. LD showed extremely high values (26 900 U/L), while bilirubin and urobilinogen were in the normal range. IAT was negative. Serum level of folic acid was normal, whereas that of vitamin B 12 was decreased (41 pg/mL). On admission, bone marrow aspiration cytology was not performed because the clinical

je liječena kao megaloblastična anemija s 1000 μg vitamina B12 i 5 mg folne kiseline; nakon 24 sata terapije LD je značajno pao na 4715 U/L. Vrijednosti feritina i željeza su uredne, a gastrin je povišen (534 μU/mL). Gastroskopijom (EGD) je utvrđen kronični gastritis, bez infekcije *H. pylori*. Nakon 8 dana liječenja hematološki nalazi se neočekivano pogoršavaju: E 1,27x10^{12}/L, Hb 54 g/L, Htc 0,157 L/L, MCV 123,4 fL, MCH 42,1 pg, MCHC 341 g/L, RDW 30,3, Rtc 0,148, Tr 76x10^{9}/L, L 6,6x10^{9}/L. Citološki nalaz koštane srži (učinjen je nakon 21 dana terapije) nije ukazao na promjene tipične za megaloblastičnu anemiju. Kako nije došlo do očekivanog poboljšanja ponovljeni su transfuziološki testovi koji su sada bili pozitivni: IAT +, anti IgG, anti C3d, DAT + (direktni antiglobulinski test). U terapiju je uključen metilprednizolon u dozi od 16 mg i nakon 48 sati dolazi do poboljšanja. Nalazi nakon izmijenjene terapije: E 2,40x10^{12}/L, Hb 80 g/L, Htc 0,242 L/L, MCV 101,0 fL, MCH 33,4 pg, MCHC 331 g/L, RDW 22,3, Tr 441x10^{9}/L, MPV 7,4 fL, L 6,7x10^{9}/L, Rtc 0,049. Prilikom otpusta transfuziološki testovi su i dalje pozitivni. Kod naše bolesnice pojavila se iznimno rijetka udružena megaloblastična anemija s AIHA za koju smatramo da se razvila u okviru osnovne bolesti (RA i shizofrenije). Moguće objašnjenje intenziviranja hemolize kao i konverzije IAT negativnih u pozitivne je pojava nove populacije eritrocita inicirane terapijom vitaminom B12 s izmijenjenim epitopima antigena na eritrocitnoj membrani.

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picture and low vitamin B12 serum concentration were highly suggestive of megaloblastic anemia. The patient was treated with vitamin B12 and folic acid, and after 24 hours of therapy LD decreased significantly to 4715 U/L. The values of ferritin and iron were normal, while gastrin was increased (534 μU/mL). Chronic gastritis was found by gastroscopy (EGD). After eight days, the findings changed suprisingly to the worse: RBC 1.27x10^{12}/L, Hb 54 g/L, Hct 0.157 L/L, MCV 123.4 fL, MCH 42.1 pg, MCHC 341 g/L, RDW 30.3, Rtc 0,148, Plt 76x10^{9}/L, WBC 6.6x10^{9}/L. Bone marrow cytomorphology (done after 21 days of therapy) showed no changes typical of megaloblastic anemia. Since the expected improvement failed to occur, transfusion medicine tests were repeated with IAT, anti-IgG, anti-C3d, and DAT (Coombs rest) positive. Methylprednisolone in a daily dose of 160 mg was introduced and after 48 hours the patient's condition improved: RBC 2.40x10^{12}/L, Hb 80 g/L, Htc 0.242 L/L, MCV 101.0 fL, MCH 33.4 pg, MCHC 331 g/L, RDW 22.3, Rtc 0.049, Plt 441x10^{9}/L, MPV 7.4 fL, WBC 6.7x10^{9}/L. Therapy with methylprednisolone and folic acid was continued, upon which the findings improved. In conclusion, our patient with combined megaloblastic and autoimmune hemolytic anemia with two mechanisms of hemolysis (dyssynchronization of the cytoplasm and DNA maturation, and immune mechanism) is one of the rare cases described so far. Immunotransfusional conversion could be explained by change of the emerging erythrocyte population with fully expressed membrane antigens, or by primary insensitivity of the method. Apart from these parameters, very useful in recognizing hemolysis are the parameters of IRF and MRV.

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P2 – Hematologija, P2-1 (UP2-1)

Praćenje broja trombocita u bolesnika s opeklinama

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Trombociti imaju značajnu ulogu u održavanju hemostaze te sudjeluju u imunog odgovoru. U bolesnika s opeklinama dolazi do značajnog narušavanja hemostaze i imunog odgovora. Cilj ispitivanja bio je pratiti broj trombocita u vremenu s obzirom na različit postotak opečene površine tijela (%TBSA) te odrediti značenje praćenja broja trombocita s obzirom na ishod bolesti.
Ispitivanu skupinu činilo je 68 bolesnika primljenih na Odjel za opekline; ispitanici su podijeljeni na skupinu A

P2 – Haematology, P2-1 (UP2-1)

Platelet count monitoring in burn patients

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Platelets have an important role in the regulation of hemostasis and participate in immune response. Burn patients develop major disturbances in the hemostatic and immune system. The aim of the study was to monitor platelet count in burn patients with different percentage of total body surface area (%TBSA) involvement, and to estimate the value of platelet count monitoring in outcome prediction.
The study included 68 burn patients divided into group

(32 bolesnika s lakšim opeklinama, <10% TBSA) i skupinu B (36 bolesnika s umjerenim/težim opeklinama, >10% TBSA). Uzorci venske krvi vađeni su 1., 4., 7., 14., 21. i 28. dana od ozljede (ovisno o dužini hospitalizacije). Trombociti su određeni na hematološkom brojaču Sysmex XT-1800i. Za potrebe statističke obrade podataka rabili smo programski paket Medcalc. Razina statističke značajnosti bila je p<0,05. Kod prijma u bolnicu broj trombocita prema skupinama nije bio statistički značajno različit (p=0,1697). Četvrtoga dana zabilježen je pad broja trombocita u objema skupinama, ali je bio izraženiji u skupini B (p=0,0003). Od 7. do 14. dana broj trombocita raste u objema skupinama (p=0,0452; p=0,1995). Broj trombocita raste 21. dana u skupini A, dok u skupini B pada, bez razlike među skupinama (p>0,10), a 28. dana broj trombocita pada u objema skupinama (p>0,10). S obzirom na ishod bolesti nađena je statistički značajna razlika u broju trombocita praćenih 1., 4., 7., 14., 21., 28. dana između preživjelih i umrlih bolesnika (p<0,05), dok je broj trombocita bio niži u umrlih bolesnika. Rezultati pokazuju da se je 4. dana hospitalizacije broj trombocita snizio u objema skupinama bolesnika. Do pada trombocita dolazi uslijed hemodilucije i pojačanog stvaranja mikroagregata, s tim da je broj trombocita bio značajno niži u skupini B. Sedmoga dana broj trombocita raste zbog stimulacije reakcije akutne faze potaknute upalom u objema skupinama. Maksimalni broj trombocita postignut je 21. dana u skupini A, odnosno 14. dana u skupini B. U bolesnika sa smrtnim ishodom je broj trombocita bio značajno niži tijekom cijelog razdoblja praćenja.

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A (32 patients with mild burns, <10% TBSA) and group B (36 patients with moderate/severe burns, >10% TBSA). Whole blood samples were obtained on days 1, 4, 7, 14, 21 and 28 of the injury (depending on the length of hospital stay). Platelet count was determined on a Sysmex XT-1800i hematology analyzer. Data were analyzed using the Medcalc software. The level of significance was set at p<0.05. On admission, platelet count did not differ significnatly between group A and group B (p=0.1697). On day 4, the values decreased in both groups, the decline being more pronounced in group B (p=0.0003). From day 7 to day 21, platelet count continued to rise in both groups (p=0.0452; p=0.1995). Platelet count continued to rise on day 21 in group A, but declined in group B, without significant between group difference (p>0.10). On day 28, platelet count declined in both groups (p>0.10). According to outcome, a statistically significant difference in platelet count between the survived and deceased patients was recorded on days 1, 4, 7, 14, 21 and 28 (p<0.05). Platelet count was lower in patients with lethal oucome. Study results indicated a decline in platelet count on day 4 in both groups of patients, attributable to hemodilution and increased production of microaggregates, with a note that platelet count was significantly lower in group B. Platelet count rose on day 7 due to the acute phase reaction stimulation by inflammation in both groups. The highest platelet count was recorded in group A on day 21 and in group B on day 14. In patients with lethal outcome platelet count was lower throughout the monitoring period.

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P2-2

Vrijednosti hematoloških parametara u djece predškolske dobi

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Za racionalnu interpretaciju laboratorijskih nalaza potrebno je poznavanje područja referentnih vrijednosti ili onih preporučenih za određenu skupinu ispitanika. Prema dokumentu "Harmonizacija laboratorijskih vrijednosti u području hematologije" za hematološke parametre u djece do 7 godina starosti preporučena je uporaba referentnih intervala prema literaturnim izvorima. Cilj istraživanja bila je usporedba izmjerenih vrijednosti s preporučenim referentnim vrijednostima hematoloških parametara koje i inače rabimo u svakodnevnom radu. Ispitivanjem je

P2-2

Values of hematology parameters in preschool children

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For rational interpretation of laboratory test results it is necessary to know the reference range or values recommended for a particular group of subjects. The document entitled Harmonization of laboratory values in hematology recommends the use of reference intervals found in the literature to express hematology parameters in children younger than seven years of age. The aim of the study was to compare the measured values with the recommended reference range of hematology parameters used in our daily routine. The study included 462 children

obuhvaćeno 462 djece (242 dječaka i 220 djevojčica) rođene 1999. i 2000. godine s područja grada Pule i okolice, koja su u laboratorij upućena zbog redovnog sistematskog pregleda za upis u prvi razred osnovne škole. Svoj djeci određena je krvna slika na hematološkom brojaču Sysmex XT2000i (Sysmex Inc., Japan). Izmjerene vrijednosti leukocita, eritrocita, hemoglobina, hematokrita, MCV, MCH, MCHC, RDW, trombocita, MPV iskazane su srednjom vrijednosti, medijanom, standardnom devijacijom, koeficijentom varijacije. Odnos prema referentnim vrijednostima iskazan je značajnošću razlike (p).
Statističkom obradom podataka dobivene su slijedeće vrijednosti izražene medijanom: E=4,74x10^{12}/L, Hb=131 g/L, Hct=0,376 l/L, MCV=794 fL, MCH=27,7 pg, MCH=347 g/L, L=6,97x10^{9}/L, Tr=307x10^{9}/L, RDW=13,1%, MPV=9,7 fL. Utvrđena je statistički značajna razlika za sve izmjerene parametre u odnosu na referentne vrijednosti za ovu dobnu skupinu (p<0,001). Vrijednosti svih izmjerenih hematoloških parametara kretale su se unutar u literaturi preporučenih referentnih intervala za ovu dobnu skupinu, te iako postoji statistički značajna razlika, klinički značajna razlika nije potvrđena.

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(242 boys and 220 girls) from Pula and its surroundings, born in 1999 and 2000. The children were referred to the laboratory for the regular medical check-up prior to school enrolment. Blood count was done on a Sysmex XT2000i (Sysmex Inc., Japan) blood counter. The measured values of leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets and MPV were expressed as mean, median, standard deviation and coefficient of variation. The relation to reference values was shown by the significance of difference (p). On statistical data processing, the following values were obtained (as expressed in median): E=4.74x10^{12}/L ,Hb=131 g/L, Hct=0.376 l/L, MCV=794 fL, MCH=27.7 pg, MCH=347 g/L, L=6.97x10^{9}/L, Plt=307x10^{9}/L, RDW=13.1%, and MPV=9.7 fL. A statistically significant difference from reference values for this age group was found for all measured parameters (p<0.001).
The values of all measured hematology parameters were within the recommended reference intervals for this age group. In spite of the statistically significant differences, no clinically significant difference was confirmed.

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P3 – Koagulacija, P3-1 (UP1-1)

Određivanje agregacije trombocita u djece na Behringovom instrumentu Coagulation Timer i referentni intervali

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Trombociti imaju važnu ulogu u primarnoj hemostazi u procesu adhezije, agregacije i stvaranja trombocitnog ugruška. Agregacija trombocita *in vitro* izaziva se različitim trombocitno-stimulirajućim agonistima. Cilj ispitivanja je bio odrediti referentne vrijednosti inducirane agregacije trombocita kod djece pomoću ADP (3 μM), adrenalina (1,5 μM), kolagena (0,2 mg/mL) i ristocetina (1,5 mg/mL) na Behringovom instrumentu Coagulation Timer (BCT). Analiza se zasniva na spektrofotometrijskom određivanju promjene ekstinkcije u standardiziranom uzorku plazme bogate trombocitima (200x10^{9}/L trombocita) kroz 10 min na 405 nm. Promjenu ekstinkcije moguće je pratiti pomoću ispisa krivulje, a proporcionalna je brzini i veličini agregacije trombocita u odnosu na plazmu siromašnu trombocitima (PRP). Izražava se u postotcima kao mak-

P3 – Coagulation, P3-1 (UP1-1)

Determination of platelet aggregation in children on a Behring Coagulation Timer and reference intervals

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Platelets have an important role in the mechanism of primary hemostasis in the process of adhesion, aggregation and platelet clot formation. Aggregation inductors can induce platelet aggregation. The aim of the study was to determine reference intervals of induced platelet aggregation in children by ADP (3 μM), adrenaline (1.5 μM), collagen (0.2 mg/mL) and ristocetin (1.5 mg/mL) on a Behring Coagulation Timer (BCT). The assay is based on spectrophotometric measurement of extinction change in the standardization sample of platelet-rich plasma (PRP) for 10 min at 405 nm. The change of extinction can be determined by the curve recording, which is proportional to the rate and magnitude of aggregation (V_{max}). Our study included 76 normal children aged 2-12 years freee from hemostasis disorder, with normal

simalna agregacija (V_{max}). U ispitivanje je bilo uključeno 76 zdrave djece u dobi od 2 do 12 godina bez poremećaja hemostaze, s normalnim brojem trombocita (raspon 210-425x10^9/L) i bez uzimanja lijekova koji bi inhibirali funkciju trombocita. Referentni intervali (percentili 0,025 -0,975) za induciranu agregaciju trombocita su bili za ADP 68,6–92,3%, adrenalin 67,6–95,0%, kolagen 72,1–89,0% i ristocetin 79,8–98,7%. Određivanje inducirane agregacije trombocita na BCT s različitim agonistima je potpuno automatizirana i vrlo precizna analiza, izvodi se s malim volumenom plazme i standardiziranim brojem trombocita. Važan je dijagnostički parametar za utvrđivanje uzroka krvarenja, procjenu disfunkcije trombocita i utjecaja lijekova na primarnu hemostazu.

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platelet count (range 210-425x10^9/L) and not taking any antiaggregation drugs or drugs that can cause inhibition of platelet function. The reference intervals (0.025-0.975 percentiles) for induced aggregation were: ADP 68.6-92.3%, adrenaline 67.6-95.0%, collagen 72.1-89.0%, and ristocetin 79.8-98.7%. Accordingly, determination of induced platelet aggregation on BCT through aggregation inductors is a fully automated and high precision analysis. The analysis is performed on a small sample volume with standardized platelet count. Induced platelet aggregation is an important analysis for detecting the risk of bleeding, platelet dysfunction and effects of antiplatelet drugs on primary hemostasis.

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P3-2

Modifikacija testa STA-STACLOT APC-R za dokazivanje Faktora V Leiden uporabom omjera RAPC

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Zbog visoke učestalosti Faktora V Leiden (FVL) ispitivanje rezistencije na aktivirani protein C (RAPC) sastavni je dio analiza probiranja na trombofiliju. Pouzdani test za ispitivanje RAPC u rutinskom radu mora biti osjetljiv i specifičan kako bi se smanjio broj molekularnih analiza za utvrđivanje prisutnosti FVL. U testu STA-STACLOT APC-R (Diagnostika Stago, Asnieres, Francuska) uzorci se analiziraju samo u prisutnosti aktiviranog proteina C (APC), a rezultati se izražavaju kao vrijeme zgrušavanja u sekundama. Cilj ovoga rada bio je prilagoditi izvorni test STA-STACLOT APC-R za koagulacijski analizator BCT (Dade Behring), uz istodobno analiziranje uzoraka s i bez APC, te ispitati može li izražavanje rezultata kao omjer RAPC omogućiti bolje razlikovanje osoba s FVL od onih bez mutacije. U istraživanje je uključeno 353 uzoraka plazme ispitanika koji su bili upućeni u koagulacijski laboratorij zbog probiranja na trombofiliju, od kojih je bilo 171 uzoraka plazme trudnica, 20 ispitanika na oralnoj antikoagulacijskoj terapiji (INR-raspon: 1,36-4,66) i 15 ispitanika s lupus antikoagulantom. Rezultati omjera RAPC uspoređeni su s rezultatima analize DNA za FVL. Dodatno je ispitan mogući utjecaj različitih aktivnosti proteina C (12,4-218,5%), proteina S (7,1-193,9%) i FVIII:C (96-580%) na rezultate omjera RAPC. Analizom

P3-2

Improvement of STA-STACLOT APC-R test for detection of Factor V Leiden by use of APCR ratio

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Activated protein C resistance (APCR) has become a well-established part of the thrombophilia screening profile in most laboratories due to the high frequency of Factor V Leiden (FVL). The goal of every coagulation laboratory is to find a reliable screening test for the detection of activated protein C resistance (APCR) that will be suitable for routine work and that will at the same time reduce the need of genetic testing for Factor V Leiden (FVL). In the original STA-STACLOT APC-R Test (Diagnostika Stago, Asnieres, France) samples are only analyzed in the presence of APC and results are expressed as clotting time in seconds. The objectives of our study were to adapt the original STA-STACLOT APC-R Test for the BCT (Dade Behring) coagulation analyzer by testing the samples simultaneously with and without APC, and to investigate whether the expression of results as APCR ratio can better discriminate FVL carriers from non-carriers. We tested 353 plasma samples from patients who were referred to our laboratory for thrombophilia screening. Among them, we analyzed plasma samples from 171 pregnant women, 20 patients receiving oral anticoagulants (INR range: 1.36-4.66) and 15 lupus anticoagulant positive patients. Results of APCR ratios were compared with DNA analysis

DNA FVL je dokazan u 47 ispitanika (42 heterozigota i 5 homozigota). Utvrđenom graničnom vrijednošću od 1,81 za omjer RAPC (osjetljivost i specifičnost 100%), omogućeno je potpuno razlikovanje heterozigota za FVL (omjer RAPC: 1,20–1,81) od zdravih ispitanika (omjer RAPC: 1,90-5,42), dok su izvornom metodom dobivena 2 lažno pozitivna i 8 lažno negativnih rezultata (osjetljivost 95,2% i specifičnost 97,2%). Nije utvrđena interferencija oralne antikoagulacijske terapije, različitih aktivnosti proteina C, proteina S i FVIII:C te lupus antikoagulanta na rezultate omjera RAPC.

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for FVL. Additionally, we tested the possible influence of different protein C levels (12.4–218.5%), protein S levels (7.1–193.9%) and FVIII:C levels (96–580%) on the APCR ratio. By DNA analysis, we identified 47 carriers of FVL (42 heterozygotes and 5 homozygotes). Using a cut-off value of 1.81 for APCR ratio (100% sensitivity and specificity), we detected no overlaps between heterozygotes for FVL (APCR ratio: 1.20-1.81) and normal subjects (APCR ratio: 1.90-5.42), as compared to 2 false positive and 8 false negative results obtained by using the original method (95.2% sensitivity and 97.2% specificity). No interference of oral anticoagulant therapy, lupus anticoagulant and different levels of protein C, protein S and FVIII:C with APCR ratio was observed.

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P3-3

Korelacija APTV određenog reagensima Pathromtin SL i Dade Actin FS na analizatoru Berichrom Coagulation System i reagensom STA Cephascreen na analizatoru STA Compact

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Aktivirano parcijalno tromboplastinsko vrijeme (APTV), probirni je test za praćenje unutarnjeg puta zgrušavanja krvi, kao i za praćenje heparinskog liječenja. Reagensima Pathromtin SL i Dade Actin FS na analizatoru Berichrom Coagulation System (BCS) i reagensom STA Cephascreen na analizatoru STA Compact usporedno je određen APTV u 100 različitih uzoraka svježe plazme te ispitana korelacija vrijednosti određenih u istom uzorku između reagensa Pathromtin SL i Dade Actin FS na analizatoru BCS, reagensa Pathromtin SL na analizatoru BCS i STA Cephascreen na STA analizatoru Compact, te reagensa Dade Actin FS na analizatoru BCS i STA Cephascreen na analizatoru STA Compact. Cilj je bio ispitati koliko su vrijednosti određene u istim uzorcima reagensima Pathromtin SL i Dade Actin FS (Dade Behring) i reagensom STA Cephascreen (Diagnostica Stago) međusobno usporedive. Ispitano je 100 uzoraka svježe plazme zdravih osoba i osoba izloženih liječenju heparinom ili njegovim derivatima. Vrijednosti APTV određene su koagulometrijskom metodom usporedno pomoću sva tri reagensa i na oba analizatora neposred-

P3-3

Correlation of aPTT determined with Pathromtin SL and Dade Actin FS reagents on Berichrom Coagulation System analyser and STA Cephascreen reagent on STA Compact analyser

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Activated partial thromboplastin time (aPTT) is a screening test of the intrinsic coagulation pathway and also a test to monitor heparin therapy. Using Pathromtin SL and Dade Actin FS reagents on a Berichrom Coagulation System (BCS) analyzer and STA Cephascreen reagent on a STA Compact analyzer we measured aPTT in parallel in 100 different fresh plasma samples. We investigated correlation between the values obtained with Pathromtin SL and Dade Actin FS reagents on a BCS analyzer, Pathromtin SL on a BCS analyzer and STA Cephascreen reagent on a STA Compact analyzer, and with Dade Actin FS on a BCS analyzer and STA Cephascreen reagent on a STA Compact analyzer. Our aim was to investigate whether the aPTT values obtained with Pathromtin SL and Dade Actin FS (Dade Behring) and with STA Cephascreen reagent (Diagnostica Stago) were comparable. The study included 100 fresh plasma samples from healthy individuals and patients on heparin or heparin derivative therapy. The values of aPTT were analyzed in parallel with all three reagents on both analyzers immediately

no nakon donošenja materijala u koagulacijski laboratorij. Usporedbom rezultata dobivenih pomoću Pathromtin SL i Dade Actin FS dobiven je koeficijent korelacije 0,9507 (p<0,05), za Dade Actin FS i STA Cephascreen 0,9325 (p<0,05), a za rezultate dobivene pomoću Pathromtin SL i STA Cephascreen 0,8848 (p<0,05). Linearnom regresijom dobiveni su slijedeći koeficijenti determinacije: 0,9035 za Pathromtin SL i Dade Actin FS, 0,8672 za Dade Actin FS i STA Cephascreen, te 0,7788 za Pathromtin SL i STA Cephascreen. Ispitivanje je pokazalo da najbolje međusobno koreliraju vrijednosti dobivene reagensima Pathromtin SL i Dade Actin FS, dok najslabije koreliraju vrijednosti dobivene reagensima Pathromtin SL i STA Cephascreen, iako je korelacija i dalje jaka. Unatoč dobivenim rezultatima, odnosno visokim korelacijama i jakoj linearnoj povezanosti, iz praktičnog iskustva ne preporuča se bolesnike izložene heparinskom liječenju istodobno pratiti različitim reagensima zbog različite osjetljivosti reagensa prema heparinu i heparinskim derivatima.

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upon the receipt in coagulation laboratory. The method of determination was coagulometry. The correlation coefficient for the results obtained was 0.9507 (p<0.05) for Pathromtin SL and Dade Actin FS, 0.9325 (p<0.05) for Dade Actin FS and STA Cephascreen, and 0.8848 (p<0.05) for Pathromtin SL and STA Cephascreen. The coefficients of regression obtained by linear regression analysis were: 0.9035 for Pathromtin SL and Dade Actin FS, 0.8672 for Dade Actin FS and STA Cephascreen, and 0.7788 for Pathromtin SL and STA Cephascreen. Our study showed the best correlation between Pathromtin SL and Dade Actin FS reagents. The lowest yet rather strong correlation coefficient was obtained on comparing Pathromtin SL and STA Cephascreen reagents. Despite the results obtained, i.e. strong correlations and strong linear connection, from practical experience it is not recommendable to monitor patients on heparin therapy simultaneously with different reagents because of the different reagent sensitivity to heparin and heparin derivatives.

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P3-4

Ispitivanje poremećaja primarne hemostaze uporabom analizatora funkcije trombocita PFA-100

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Analizator funkcije trombocita PFA-100 (Dade Behring) je specifični instrument za jednostavno, brzo i kvantitativno ispitivanje kapaciteta primarne hemostaze *in vitro*. Načelo rada instrumenta se temelji na adheziji i agregaciji trombocita oponašanjem *in vivo* uvjeta fiziološke brzine protoka krvi u arterijama u prisutnosti kolagena i adrenalina (CEPI), te kolagena i ADP (CADP), pričem se mjeri vrijeme nastanka trombocitnog ugruška (CT). Ciljevi ovoga istraživanja bili su utvrđivanje referentnih intervala za CEPI-CT i CADP-CT te usporedba rezultata CEPI-CT i CADP-CT s uobičajenim metodama za ispitivanje primarne hemostaze: vremenom krvarenja po Ivyju, određivanjem aktivnosti von Willebrandova faktora (VWF:RCo) i antigene komponente (VWF:Ag), te agregacijom trombocita (PA) pomoću ADP, adrenalina i ristocetina. Mjerenjem na PFA-100, koje je provedeno u uzorcima pune citratne krvi (0,105 M Na citrat) 44 zdrava ispitanika utvrđeni su referentni intervali: 80-160 s za CEPI-CT i 60-120 s za CADP-CT. Ukupno je

P3-4

Laboratory evaluation of primary hemostasis on a PFA-100 platelet function analyzer

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Platelet Function Analyzer PFA-100 (Dade Behring) has recently been developed as a quantitative, simple and rapid method for the investigation of primary hemostasis *in vitro*. It simulates *in vivo* platelet adhesion and aggregation by measuring high shear stress dependent platelet plug formation which occurs on collagen/epinephrine (CEPI) or collagen/ADP (CADP) membranes and is expressed as closure time (CT). The aims of the present study were to establish the reference intervals for CEPI-CT and CADP-CT, and to compare the performance of PFA-100 CTs with traditional methods for the investigation of primary hemostasis: bleeding time, measurement of VW-Factivity (VWF:RCo), VWF antigen (VWF:Ag), and platelet aggregation (PA) with ADP, epinephrine, and ristocetin. Whole blood anticoagulated with buffered 0.105 M sodium citrate was obtained from 44 normal volunteers and from 197 patients undergoing evaluation for primary hemostasis disorders. The reference interval (mean±SD)

laboratorijski obrađeno 197 ispitanika zbog sumnje na poremećaj primarne hemostaze, te je napravljena korelacija PFA-100 s VWF:RCo (r=-0,5091 za CEPI; r=-0,5649 za CADP) i s VWF:Ag (r=-0,4003 za CEPI; r=-0,5223 za CADP). Logaritamskom transformacijom dobivena je jača korelacija s VWF:RCo (r=-0,6423 za CEPI; r=-0,7403 za CADP), kao i s VWF:Ag (r=-0,4879 za CEPI; r=-0,6117 za CADP). Ispitivanjem korelacije vremena krvarenja po Ivyju s PFA-100 dobiveni su bolji rezultati (r=-0,4291 za CEPI; r=-0,5614 za CADP) nego s VWF (r=-0,2216 za VWF:RCo; r=-0,1590 za VWF:Ag). Potpuno podudaranje rezultata između PFA-100 i ispitivanja PA (oba rezultata patološka ili oba normalna) dobiveno je u 94/162 (58%) ispitanika. U skupini od 96 ispitanika s normalnim nalazom PA, u 72 (75%) je dobiven normalan rezultat za CEPI-CT i CADP-CT, u 13 (13,5%) ispitanika dobiven je barem jedan patološki nalaz na PFA-100, dok su oba patološka rezultata dobivena u 11 (11,5%) ispitanika. Od 66 ispitanika s patološkim vrijednostima PA s najmanje jednim agonistom, oba patološka rezultata na PFA-100 nađena su u 22 (33,3%) ispitanika, barem jedan normalan rezultat dobiven je u 15 (22,7%) ispitanika, a oba normalna nalaza su dobivena u 29 (44,0%) ispitanika. U zaključku, normalan nalaz CEPI-CT i CADP-CT ne može isključiti sve poremećaje primarne hemostaze zbog izrazite složenosti primarne hemostaze te velikog broja različitih poremećaja funkcije trombocita.

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was found to be 80-160 s for CEPI-CT, and 60-120 s for CADP-CT. The PFA-100 CTs were correlated to VWF:RCo (r=-0.5091 for CEPI; r=-0.5649 for CADP), and to VWF:Ag (r=-0.4003 for CEPI; r=-0.5223 for CADP). With logarithmic transformation of data, we obtained an even stronger correlation to VWF:RCo (r=-0.6423 for CEPI; r=-0.7403 for CADP), as well as to VWF:Ag (r=-0.4879 for CEPI; r=-0.6117 for CADP). Bleeding times were correlated much better to PFA-100 CTs (r=0.4291 for CEPI; r=0.5614 for CADP) than to plasma VWF levels (r=-0.2216 for VWF:RCo; r=-0.1590 for VWF:Ag). The overall agreement (both normal or both abnormal) between PFA-100 and PA was 94/162 (58%). In the group of 96 patients with normal PA results, both normal CTs were found in 72 (75%), at least one abnormal CT in 13 (13.5%), and both abnormal CTs in 11 (11.5%) patients. Among 66 patients with at least one abnormal PA result, both abnormal CTs were observed in 22 (33.3%), at least one normal CT in 15 (22.7%) and both normal CTs in 29 (44.0%) patients. In conclusion, normal CTs could not exclude all primary hemostasis disorders, probably due to the large number and variety of platelet defects.

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P3-5

Predviđanje stupnja deficita FVIII analizom reakcijskih krivulja za APTV

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Na modernim koagulacijskim analizatorima APTV ne predstavlja samo broj, nego skup foto-optičkih podataka u obliku reakcijske krivulje. U ovom radu napravljena je kvantitativna analiza reakcijskih krivulja za APTV na analizatoru Behring Coagulation Timer (BCT) te je pomoću dobivenih parametara ispitana mogućnost predviđanja stupnja deficita FVIII. U uzorcima plazme 38 zdravih ispitanika i 87 bolesnika s hemofilijom A izmjeren je APTV (Actin FS) uporabom dviju metoda procjene reakcijske krivulje: zadana promjena apsorpcije (*fixed absorbance*, APTV-FA) i točka infleksije (*point of inflexion*, APTV-PI). Zatim su izračunati slijedeći parametri: delta-APTV (DaPTT) kao razli-

P3-5

Is it possible to predict the degree of FVIII deficiency from aPTT waveform analysis on a Behring Coagulation Timer (BCT)?

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With modern coagulation instruments, every aPTT result is not just a number but a collection of photo-optical data in the form of reaction curve. We performed a quantitative aPTT waveform analysis to see whether it was possible to detect the degree of FVIII deficiency using parameters from this analysis. We measured aPTT (Actin FS) in 38 normal subjects and 87 hemophilia A patients on a Behring Coagulation Timer, with two different evaluation modes: fixed absorbance (FA) and point of inflexion (PI). Additionally, we calculated delta-aPTT (DaPTT) as aPTT-PI minus aPTT-FA and aPTT-slope ratio (aPTTSR) as the ratio between DaPTT and aPTT-FA. FVIII activity was measured

ka između APTV-PI i APTV-FA, i APTV-omjer (aPTTSR) kao omjer između DaPTT i APTV-FA. U uzorcima bolesnika s hemofilijom izmjerena je aktivnost FVIII, izvršen probir na prisutnost inhibitora, te je u slučaju pozitivnog rezultata određen njihov titar. Bolesnici s hemofilijom su prema aktivnosti FVIII podijeljeni u 4 skupine (H1-H4) te prema rezultatu probira na prisutnost inhibitora u skupinu s negativnim inhibitorima (I1) i skupinu s pozitivnim inhibitorima (I2). Nađena je statistički značajna razlika ($p<0,05$) u vrijednostima DaPTT i aPTTSR između zdravih ispitanika i bolesnika s hemofilijom, kao i između skupina H1-H4, te I1 i I2. U zdravih ispitanika dobivene su slijedeće vrijednosti (srednja vrijednost ± SD): DaPTT=4,24±1,11; aPTTSR=0,16±0,03, a u bolesnika s hemofilijom DaPTT=20,03±11,47, aPTTSR=0,33±0,14. Slijedeći rezultati dobiveni su u pojedinačnim skupinama: H1 (n=41, FVIII <0,01) DaPTT=29,48±8,62, aPTTSR=0,44±0,13; H2 (n=12, FVIII=0,01-0,05) DaPTT=17,51±5,87, aPTTSR=0,29±0,09; H3 (n=24, FVIII=0,05-0,30) DaPTT=10,25±4,18, aPTTSR=0,23±0,05; H4 (n=10, FVIII=0,30-0,50) DaPTT=7,75±1,19, aPTTSR=0,19±0,04; I1 (n=48) DaPTT=17,83±12,19, aPTTSR=0,30±0,14, I2 (n=21) DaPTT=28,06±6,61, aPTTSR=0,42±0,13. Prema ovim rezultatima vrijednosti DaPTT >6,5 omogućavaju razlikovanje između zdravih ispitanika i bolesnika s hemofilijom (osjetljivost 94,7%, specifičnost 100%), dok vrijednosti DaPTT >17,7 razlikuju bolesnike s inhibitorom i bez njega (osjetljivost 95,5%, specifičnost 64,7%).

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by one-stage assay. FVIII inhibitor screen was performed and inhibitor concentrations were determined. Hemophilia patients were further divided into 4 groups (H1-H4) according to FVIII activity, and into 2 groups according to the presence (I2) or absence (I1) of the inhibitor. Significant differences ($p<0.05$) were found in DaPTT and aPTTSR values between normal and hemophilia patients as well as between H1 to H4 and I1 and I2 groups. In normal and hemophilia patients the obtained results (mean ± SD) for DaPTT were 4.24±1.11 and 20.03±11.47, and for aPTTSR 0.16±0.03 and 0.33±0.14, respectively. Results in specific groups were as follows: group H1 (n=41, FVIII <0.01) DaPTT=29.48±8.62, aPTTSR=0.44±0.13; group H2 (n=12, FVIII=0.01-0.05) DaPTT=17.51±5.87, aPTTSR=0.29±0.09; group H3 (n=24, FVIII=0.05-0.30) DaPTT=10.25±4.18, aPTTSR=0.23±0.05; group H4 (n=10, FVIII=0.30-0.50) DaPTT=7.75±1.19, aPTTSR=0.19±0.04; group I1 (n=48) DaPTT=17.83±12.19, aPTTSR=0.30±0.14, group I2 (n=21) DaPTT=28.06±6.61, aPTTSR=0.42±0.13. Based on these results, DaPTT values >6.5 and >17.7 allowed for discrimination between normal subjects and hemophilia patients (sensitivity 94.7%, specificity 100%), and between patients with and without inhibitors (sensitivity 95.5%, specificity 64.7%).

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P3-6

Usporedivost rezultata D-dimer testova s referentnom metodom VIDAS D-Dimer Exclusion AS

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Rezultati D-dimera određeni različitim komercijalnim testovima u istom uzorku mogu se značajno razlikovati, što vjerojatno proizlazi iz različite reaktivnosti antitijela prema različitim monoklonskim antigenima. Cilj studije bio je usporediti komercijalne D-dimer testove s automatskom metodom ELISA VIDAS D-Dimer Exclusion kao referentnom metodom. D-dimer određivan je VIDAS D-Dimer Exclusion (bioMerieux) ELISA testom na Mini VIDAS, NycoCard D-Dimer New testom na NycoCard Reader

P3-6

Comparison of D-dimer assays with the VIDAS D-Dimer Exclusion AS reference method

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The numerical values obtanied with different D-dimer assays vary widely. The variation is probably due to differences in reactivity of various monoclonal antibodies. The aim of the study was to compare D-dimer assays with Vidas D-Dimer Exclusion as a reference method. All D-dimer assays were performed according to the manufacturers' instructions: VIDAS D-Dimer Exclusion (BioMerieux) ELISA assay on a Mini VIDAS, NycoCard D-Dimer New assay on a NycoCard Reader II, Stratus CS D-Dimer assay

II, Stratus CS D-Dimer testom (Dade Behring) na Stratus CS i D-Dimer Plus (Dade Behring) testom na Sysmex CA 500. Kalibratori i kontrolni uzorci bili su od istog proizvođača kao i reagensi.
Rezultati dobiveni pomoću VIDAS D-Dimer Exclusion:
za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji bila je 2,8-6,2, a iz dana u dan 3,9-5,3.
Netočnost
Bias = 0,024 mg/L, deklarirano 0,597 mg/L.
Bias = 0,35 mg/L, deklarirano 4,51 mg/L.
NycoCard D-Dimer New test
Nepreciznost iz dana u dan (KV %) bila je 9,9, a netočnost je do 12%.
Stratus CS D-Dimer test
Za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji bila je 2,3-3,2.
D-Dimer Plus
Za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji je 0,65–0,84, a iz dana u dan 1.0.
Netočnost
Bias = 0,018 mg/L, deklarirano 0,61 mg/L.
Bias = 0,027 mg/L, deklarirano 4,20 mg/L.
Usporedba rezultata
Obrada rezultata učinjena je pomoću statističkog programa SigmaStat. Rezultati pokazuju statistički značajan stupanj linearne korelacije do koncentracije 5,1 mg/L.
VIDAS D-Dimer Exclusion – NycoCard D-Dimer New test rs = 0.93, p<0,001, n = 30
VIDAS D-Dimer Exclusion – Stratus CS D-Dimer test rs = 0,91, p<0,001, n = 30
VIDAS D-Dimer Exclusion – D-Dimer Plus rs = 0.92, p<0,001, n = 36
Korelacija do koncentracije 2,0 mg/L se pokazala također statistički značajnom.
VIDAS D-Dimer Exclusion – NycoCard D-Dimer New test rs = 0,78, p<0,001, n = 18
VIDAS D-Dimer Exclusion – Stratus CS D-Dimer test rs = 0,77, p<0,001, n = 21
VIDAS D-Dimer Exclusion – D-Dimer Plus rs = 0,79, p<0,001, n = 26
Dobivena je dobra podudarnost rezultata različitih D-dimer testova prema referentnoj metodi, naročito u nižem koncentracijskom području, što ih čini prihvatljivim u rutinskom radu.

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(Dade Behring) on a Stratus CS and D-Dimer Plus (Dade Behring) assay on a Sysmex CA 500.
Results obtained by VIDAS D-Dimer Exclusion:
reproducibility (CV%) in low and high concentration within runs 2.8–6.2 and between runs 3.9–5.3.
Accuracy
Bias = 0.024 mg/L, target value 0.597 mg/L
Bias = 0.35 mg/L, target value 4.51 mg/L
NycoCard D-Dimer New test
Reproducibility (CV%) between runs 9.9 and accuracy 12%.
Stratus CS D-Dimer test
Reproducibility (CV%) in low and high concentration within runs 2.3–3.2.
D-Dimer Plus
Reproducibility (CV%) in low and high concentration within runs 0.65–0.84 and between runs 1.0.
Accuracy
Bias = 0.018 mg/L, target value 0.61 mg/L.
Bias = 0.27 mg/L, target value 4.20 mg/L
Data comparison
Statisticai analysis was done by SigmaStat statistical software.
Results showed a statistically significant level of linear correlation of up to 5.1 mg/L.
VIDAS D-Dimer Exclusion – NycoCard D-Dimer New assay rs = 0.93, p<0.001, n = 30
VIDAS D-Dimer Exclusion – Stratus CS D-Dimer assay rs = 0.91, p<0.001, n = 30
VIDAS D-Dimer Exclusion – D-Dimer Plus rs = 0.92, p<0.001, n = 36
A significant correlation was found even in lower concentratios of up to 2.0 mg/L.
VIDAS D-Dimer Exclusion – NycoCard D-Dimer New assay rs = 0.78, p<0.001, n = 18
VIDAS D-Dimer Exclusion – Stratus CS D-Dimer assay rs = 0.77, p<0.001, n = 21
VIDAS D-Dimer Exclusion – D-Dimer Plus rs = 0.79, p<0.001, n = 26
D-dimer assays demonstrated performances comparable with those of VIDAS D-Dimer as a reference method, and were found suitable for emergencies and individual determination.

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P3-7

Utjecaj niskomolekularnog heparina na globalne koagulacijske testove kod bolesnika na hemodijalizi

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U liječenju tromboembolijskih bolesti niskomolekularni heparini (LMWH) imaju niz prednosti u odnosu na visokomolekularni nefrakcionirani heparin (UFH). Zbog manjeg nespecifičnog vezanja na proteine plazme i endotel LMWH imaju bolju bioraspoloživost, ujednačeniju farmakokinetiku i predvidljiviji terapijski odgovor, što smanjuje potrebu za laboratorijskim ispitivanjem. Primarno se izlučuju putem bubrega pa se kod bolesnika s kroničnim zatajivanjem bubrega njihov eliminacijski poluživot može povećati nekoliko puta, a time time i rizik za pojavu krvarenja. Cilj studije je bio ispitati farmakokinetiku LMWH fragmina i njegov utjecaj na rezultate globalnih koagulacijskih testova PV, APT omjera i fibrinogena. U ispitivanje je uključeno 10 bolesnika (3 Ž i 7 M) na kroničnoj hemodijalizi (HD). Prije HD bolesnici su primili fragmin intravenski u dozi od 40 IU/kg. Uzorci krvi vađeni su četiri puta tijekom 48 sati: prije HD i injekcije fragmina, 2 sata nakon početka HD, nakon 4 sata HD i nakon 48 sati prije slijedeće HD. Aktivnost PV, APT omjera i koncentracija fibrinogena određeni su koagulacijskom metodom, a koncentracija fragmina kao anti-faktor Xa aktivnost izražena u IU/mL kromogenom metodom Dade Behringovim testovima na instrumentu Behring Coagulation Timer Version 1,7 (BCT). Vrijednosti fragmina prije HD su bile 0,08 IU/mL (raspon 0,07-0,17), nakon 2 sata 0,43 IU/mL (raspon 0,16-0,82), nakon 4 sata 0,20 IU/mL (raspon 0,08-0,60) i prije slijedeće HD 0,08 IU/mL (raspon 0,06-0,10). Smanjenje aktivnosti PV za 12% i porast APT omjera za 22,7% nakon 2 sata HD u odnosu na početne vrijednosti bili su još uvijek unutar referentnih intervala. Koncentracija fibrinogena je bila veća za 20,4% i povećavala se tijekom HD. Vrijednosti PV, APT omjera, fibrinogena i anti-faktor Xa nakon 48 sati odgovaraju početnim vrijednostima prije injekcije fragmina. Zaključuje se kako primijenjena doza fragmina ne predstavlja rizik za pojavu krvarenja za vrijeme i nakon HD, a sigurno sprječava trombozu u izvantjelesnom protoku krvi.

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P3-7

Impact of low molecular weight heparin on the results of global coagulation assays in hemodialysis patients

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Low molecular weight heparins (LMWH) have several advantages over unfractionated heparin (UFH) in the treatment of thrombosis. Unlike UFH, LMWHs have excellent bioavailability as the result of reduced nonspecific binding to plasma proteins and endothelium. Their pharmacokinetics is more uniform leading to a predictable response in most patients without the need of laboratory monitoring. LMWHs are cleared primarily by renal excretion. In patients with chronic renal failure, the elimination half-life may increase several-fold, thus also the risk of bleeding. The aim of the study was to evaluate the pharmacokinetics of the LMWH fragmin after a bolus dose as an anticoagulant during a 48-h period and its impact on the results of global coagulation assays PT, APT R and fibrinogen. We examined 10 patients (3 female and 7 male) on chronic hemodialysis (HD). The patients received a single bolus dose of fragmin of 40 IU/kg before HD. Blood samples were collected four times during a 48-h period: before HD, and at 2 h, 4 h and 48 h of HD. PT, APT R and fibrinogen were determined by the coagulation method, and anti-factor Xa activity was determined by the chromogenic method. Anti-factor Xa levels: before HD 0.08 IU/mL (range 0.07-0.17); at 2 h of HD 0.43 IU/mL (range 0.16-0.82); at 4 h of HD 0.20 IU/mL (range 0.08-0.60), and at 48 h of HD 0.08 IU/mL (range 0.06-0,.0). At 2 h of HD, the activity of PT decreased from initial levels by 12%; APT R increased by 22.7%; fibrinogen concentration increased by 20.4% and stayed so throughout HD. PT, APT R, fibrinogen and anti-factor Xa activities returned to initial levels after 48 h of HD. The dose of fragmin used in the study was found to be safe considering the risk of bleeding during and after HD, and effective in preventing extracorporeal circuit thrombosis.

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P3-8

Funkcija trombocita u trudnoći: usporedba analizatora PFA-100 i standardne metode agregacije trombocita

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Trudnoća je stanje u kojem je fiziološki prisutna povećana reaktivnost trombocita kao rezultat pojačanog lučenja trombocita i opće hiperkoagulabilnosti krvi. U ovom smo ispitivanju kao ispitanike ciljano odabrali trudne žene u svrhu procjene kliničke primjene PFA-100 u stanju hiperagregabilnosti trombocita. Analizator funkcije trombocita (PFA-100, Dade Behring) procjenjuje *in vitro* primarnu hemostazu u punoj krvi upotrebom test kaseta obloženih agonistima trombocita: kolagen/epinefrin (KOL/EPI) ili kolagen/adenozin difosfat (KOL/ADP). Cilj je bio usporediti rezultate funkcije trombocita ispitane analizatorom PFA-100 (puna krv) i standardnom metodom agregacije trombocita prema Bornu (plazma) u trudnica. U ispitivanje je bilo uključeno 47 trudnica životne dobi 19-44 godina i gestacijske dobi 33-41 tjedan. Uz pretragu agregacije trombocita u svih trudnica određeni su: broj trombocita, APTV, PV, fibrinogen, TV, test fibrinolize. Rezultati su izraženi kao medijan i raspon. Zabilježeni su slijedeći rezultati: broj trombocita 198x10^9/L (134-399x10^9/L), APTV 26 s (23-35 s), PV 137% (105-150%), TV 17 s (13-19 s), fibrinogen 6,5 g/L (3,5-8,9 g/L), fibrinoliza 210 min (90-210 min). PFA-100: KOL/EPI 101,5 s (66-237 s), KOL/ADP 79 s (61-127 s). Standardna metoda agregacije trombocita: ADP 72,8% (19-88%), KOL 72,4% (23-95%), EPI 74,9% (23-86%). Podudarnost rezultata dviju metoda iznosila je 91,5% (43/47), a 8,5% (4/47) rezultata pokazalo je neslaganje. U dva od 47 rezultata pokazana je smanjena agregabilnost trombocita mjerena pomoću PFA-100 (KOL/EPI 147 s,237 s; KOL/ADP 102 s,127 s) uz pojačanu agregabilnost trombocita standardnom metodom agregacije (ADP 86%,88%; KOL 86%,88%; EPI 83%,85%). U dva nepodudarna rezultata vrijednosti PFA-100 bile su normalne (KOL/EPI 82 s,110 s; COL/ADP 70 s,76 s), a agregacija trombocita u plazmi smanjena (ADP 19%,33%, KOL 30%,36%, EPI 23%,33%). Usporedba rezultata dviju metoda potvrdila je kliničku primjenjivost PFA-100 u procjeni funkcije trombocita u stanjima njihove pojačane agregabilnosti. U usporedbi sa standardnom metodom agregacije trombocita u plazmi PFA-100 je tehnički manje zahtjevna metoda, puna krv bolje odražava *in vivo* uvjete, a rezultati su dostupni u kraćem vremenu.

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P3-8

Platelet function in pregnancy: comparison of PFA-100 analyzer and standard method of platelet aggregation

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Pregnancy is a state in which physiologically increased platelet reactivity is present as the result of enhanced platelet secretion and general blood hypercoagulability. In this study, pregnant women were included to evaluate the clinical utility of PFA-100 in the state of platelet hyperaggregability. Platelet function analyzer (PFA-100, Dade Behring) evaluates *in vitro* primary hemostasis in whole blood using test cartridges coated with platelet agonists: collagen/epinephrine (COL/EPI) or collagen/adenosin diphosphate (COL/ADP). The aim was to compare results of platelet function as assessed by the PFA-100 (whole blood) and standard method of platelet aggregation according to Born (plasma) in pregnant women. The study included 47 pregnant women aged 19-44, gestational age 33-41 weeks. In addition to platelet aggregation tests, platelet count, aPTT, PT, fibrinogen and fibrinolysis test were also performed. Results are expressed as median and range. The following results were recorded: platelet count 198x10^9/L (134-399x10^9/L), APTT 26 s (23-35 s), PT 137% (105%-150%), TT 17 s (13-19 s), fibrinogen 6.5 g/L (3.5-8.9 g/L), fibrinolysis 210 min (90-210 min). PFA-100: COL/EPI 101.5 s (66-237 s), COL/ADP 79 s (61-127 s). Standard method of platelet aggregation: ADP 72.8% (19-88%), COL 72.4% (23-95%), EPI 74.9% (23-86%). The agreement between the two methods was 91.5% (43/47), while 8.5% (4/47) of the results showed discrepancies. Two of 47 results showed reduced platelet aggregation by PFA-100 (COL/EPI 147 s,237 s; COL/ADP 102 s,127 s) and increased platelet aggregation by standard method of aggregation (ADP 86%,88%; COL 86%,88%; EPI 83%,85%). Two discordant results had normal PFA-100 values (COL/EPI 82 s,110 s; COL/ADP 70 s,76 s) and reduced standard platelet aggregation (ADP 19%,33%; COL 30%,36%; EPI 23%,33%). Accordingly, comparison of the results obtained by the two methods confirmed the clinical utility of the PFA-100 in the assessment of platelet function in conditions of their increased aggregability. In comparison with standard method of platelet aggregation in plasma, PFA-100 is technically easer to perform, whole blood better reflects *in vivo* conditions, and results are accessible in a shorter period of time.

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P3-9

Vrijednost određivanja D-dimera kod bolnički liječenih bolesnika

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D-dimer je kao osjetljiv biljeg uključen u dijagnostički algoritam tromboembolija. Samo negativan rezultat D-dimera dobiven visoko ili umjereno osjetljivim testom može se uz kliničku vjerojatnost rabiti za isključenje tromboembolije, ali je specifičnost takvih testova za plućnu emboliju niska. Kako je samo negativan rezultat koristan u donošenju kliničke odluke, udio bolesnika s negativnim rezultatom u skupini kod koje je isključena tromboembolija (tj. specifičnost) određuje kliničku vrijednost testa.
Cilj rada bio je ispitati vrijednost određivanja D-dimera u bolničkoj skupini bolesnika. Ispitivanje je provedeno u okviru uobičajene obrade bolesnika koji su bili na bolničkom liječenju. Ispitali smo 76 bolesnika, 46 muškaraca i 30 žena u dobi od 22 do 85 godina. Koncentracija D-dimera određivana je Vidas D-dimer testom. Od ukupnog broja samo je 16 (21%) bolesnika imalo koncentracije D-dimera ispod granične vrijednosti (0,5 mg/L FEU), dok je kod 60 (79%) bolesnika nađena povišena koncentracija D-dimera. Tromboembolija je potvrđena kod 13 bolesnika, dok su ostali bolesnici imali povišene vrijednosti zbog maligne bolesti (n=21), pneumonije (n=14), KOPB (n=4), politraume (n=1). Najviše koncentracije zabilježene su u skupini politraume (16,5 mg/L). Kod bolesnika s embolijom raspon vrijednosti je bio od 0,96 do 12,99 mg/L, kod maligne bolesti od 0,3 do 5,72 mg/L, a kod pneumonije od 0,64-3,73 mg/L. Zamijetili smo velik broj bolesnika s lažno povišenim vrijednostima D-dimera u bolničkoj populaciji. Razlog je vjerojatno veća učestalost onih bolesti kod kojih se nalaze povišene vrijednosti, kao i neprepoznate tromboembolije. Stoga je njegova uporaba u ovoj populaciji ograničena te određivanje D-dimera ne treba rabiti kao test probiranja, odnosno upitno je njegovo određivanje kod visoko rizičnih bolesnika – starije populacije, bolesnika s malignom bolesti ili hospitaliziranih bolesnika.

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P3-9

Usefulness of D-dimer measurement in hospitalized patients

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D-dimer is a sensitive marker incorporated into the diagnostic algorithm of venous thromboembolism (VTE). Only normal D-dimer concentration measured with a high sensitivity or moderate sensitivity can rule out VTE with low to intermediate clinical probability. Therefore, the specifity of highly sensitive D-dimer assays for pulmonary embolism is low. Since only negative D-dimer results are useful for clinical decision making, the proportion of patients with negative results among those without a thromboembolic disease (i.e. specificity) determines the clinical usefulness of the test. We assessed the potential utility of D-dimer testing in hospitalized patients. The study was performed as part of regular investigations in our hospital inpatients. We examined 78 hospitalized patients, 48 male and 30 female, age range 22-85. D-dimer was analyzed by a sensitive ELISA (Vidas D-dimer). Of 78 study patients, only 16 (21%) had D-dimer concentration below the predefined cutoff value (0.5 mg/L FEU). In 60 (79%) patients we found elevated D-dimer concentrations. Thromboembolism (PE or DVT) was present in 13 patients, and the rest had elevated levels of D-dimer due to cancer (n=21), pneumonia (n=14), COPD (n=4) and polytrauma (n=1). The highest value was found in the group with polytrauma (16.5 mg/L). In patients with pulmonary embolism the values were 0.96-12.99 mg/L. In the subgroup with cancer the D-dimer level ranged from 0.3 to 5.72 mg/L, and in patients with pneumonia from 0.64 to 3.73 mg/L. There were a high number of patients with false-positive D-dimer tests, most likely due to the an increased prevalence of comorbid diseases and inconspicuous thrombosis in these patients. Thus, the usefulness of this clinical test in this patient population is limited, suggesting that D-dimer should not be used as a screening test and is of questionable value in patients at a high risk of false-positive results, i.e. in the elderly, cancer patients and hospitalized patients.

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P3-10

Procjena vrijednosti D-dimera u bolničkih i ambulantnih bolesnika upotrebom dviju metoda

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Testovi koji mjere D-dimere u plazmi primjenjuju se zadnjih deset-petnaest godina i spadaju u neinvazivnu dijagnostiku venskog tromboembolizma (VTE). Noviji testovi na osnovi metode ELISA imaju visoku negativnu prediktivnu vrijednost u dijagnostici VTE. Točnije rečeno, niski D-dimeri isključuju duboku vensku trombozu (DVT). Nasuprot tome, visoka koncentracija D-dimera često može zbuniti u dijagnozi. Cilj ovoga rada bio je usporediti visoke vrijednosti D-dimera (>500 ng/mL; >246 μg/L) i kliničko-patološko stanje bolesnika upotrebom dviju metoda: VIDAS D-Dimer Exclusion test i D-Dimer Plus (Dade Behring). U 37 bolesnika D-dimeri u plazmi mjereni su dvjema navedenim metodama (12 muškaraca i 25 žena). Kliničku dijagnozu bolesnici su već imali u trenutku određivanja D-dimera ili je naknadno dobivena nakon daljnje kliničke i laboratorijske obrade. Bolesnici sa sumnjom na DVT upućeni su na obojeni dopler UZV ili CT. Šestoro (16%) bolesnika s visokim D-dimerima imalo je DVT, četvoro (11%) bolesnika s visokim D-dimerima imalo je akutni površinski tromboflebitis, desetoro (27%) bolesnika s visokim D-dimerima imali su uznapredovalu malignu bolest, u šestoro (16,2%) bolesnika bio peti dan poslije operacije zbog maligne bolesti, troje (8,1%) bolesnika je primalo je adjuvantnu kemo- i radioterapiju, a četvoro (10,8%) bolesnika je bilo u stanju neposredno nakon operacije zbog nemaligne bolesti. Četvoro (10,8%) bolesnika je imalo niske D-dimere upotrebom obiju metoda. Jedan bolesnik je imao niske D-dimere mjereno jednom metodom, a visoke mjereno drugom metodom i DVT dokazanu pomoću CT. Od ukupno 33 bolesnika koji su imali visoke D-dimere 22 (66%) ih je imalo malignu bolest. Zaključeno je kako niski D-dimeri uglavnom isključuju DVT, dok visoki D-dimeri trebaju usmjeriti daljnju kliničku obradu u smjeru maligne bolesti, a potom za DVT.

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P3-10

Evaluation of D-dimer value in inpatients and outpatients by use of two methods

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Plasma D-dimer tests as used for the last ten to fifteen years are noninvasive tests for diagnosing venous thromboembolism (VTE). Recent tests based on the ELISA method are a powerful tool with a high negative predictive value in the diagnostic workout for VTE. More precisely, low D-dimer levels exclude deep vein thrombosis (DVT). On the other hand, a high D-dimer concentration may cause diagnostic confusion. The study was aimed at comparing high D-dimer levels (>500 ng/mL; >246 μg/L) and patient clinicopathologic status using the following tests: VIDAS D-Dimer Exclusion Test and D-Dimer Plus (Dade Behring). Using the above tests, plasma D-dimers were measured in 37 patients (12 male and 25 female). At the time of performing D-dimer measurement, the patients' underlying disease had already been diagnosed or it was defined later upon subsequent clinical and laboratory testing. Patients suspected of DVT were referred for either color Doppler US or CT.
Among 33 patients showing high D-dimer levels, six (16%) had DVT, four (11%) had acute superficial thrombophlebitis, ten (27%) had advanced malignant disease, in six (16.2%) patients it was day 5 after operation for malignant disease, three (8.1%) were undergoing adjuvant chemo- and radiotherapy, and four (10.8%) patients underwent testing immediately after operation for a nonmalignant disease. Both methods showed low D-dimer levels in four (10.8%) patients. In one patient, one of the tests showed low D-dimers, whereas the other showed high D-dimer levels with DVT confirmed by CT. Of 33 patients showing high D-dimer levels, 22 (66%) had a malignant disease. In conclusion, low D-dimer levels may be sufficient to exclude DVT on the one hand, whereas on the other hand high D-dimer levels should primarily prompt further diagnostic steps towards a malignancy, and then towards DVT.

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P3-11

Procjena epruveta tvrtke Terumo u izvođenju pretraga zgrušavanja krvi

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Staklene epruvete tvrtke Terumo rabe se za laboratorijsko uzorkovanje krvi za koagulacijske analize. Cilj studije bio je usporediti plastične epruvete tvrtke Terumo i staklene epruvete istog proizvođača u izradi pretraga zgrušavanja krvi. Uzorci za koagulacijske pretrage podijeljeni su u 4 skupine s obzirom na terapiju bolesnika: bolesnici bez terapije, bolesnici liječeni oralnom antikoagulacijskom terapijom, bolesnici na niskomolekularnoj, te oni na visokomolekularnoj heparinskoj terapiji. Metode za određivanje PV, APTV i heparina postavljene su prema propisima proizvođača i prilagođene radu na analizatoru ACL8000 tvrtke Instrumentation Laboratory. Rezultati pokazuju visok stupanj korelacije kod bolesnika liječenih oralnom antikoagulacijskom terapijom (R=0,99), niskomolekularnom (R=0,93), te visokomolekularnom heparinskom terapijom (R=0,97), dok bolesnici bez terapije pokazuju zadovoljavajući stupanj korelacije za testove PV (R=0,86) i APTV (R=0,80). Na temelju dobivenih rezultata za navedene parametre u ispitivanim uzorcima, kao i njihove statističke obrade zaključujemo da nema značajnih razlika između staklenih i plastičnih epruveta tvrtke Terumo. Prethodne su studije ukazale na važnost vremenskog razmaka između uzorkovanja krvi i izrade pretrage. Dulja odgoda dovodi do znatne razlike u rezultatima pretraga na uzorcima iz staklenih ili plastičnih epruveta. U našoj je studiji najveća odgoda između uzorkovanja i izrade pretrage bila ograničena na 180 minuta.

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P3-11

Evaluation of Terumo test tubes for coagulation tests

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Glass test-tubes produced by Terumo are used to collect blood samples for coagulation analyses.
The aim of the study was to compare Terumo plastic and glass test-tubes used to perform coagulation tests. Patient samples for coagulation tests were divided into four groups according to therapy administered to patients: no therapy, oral anticoagulant therapy, low-molecular heparin therapy, and high-molecular heparin therapy. Methods for PT, aPTT and heparin determination were introduced according to the manufacturer's instructions and adapted for the Instrumentation Laboratory ACL8000 analyzer. Results showed a high degree of correlation in patients on oral anticoagulant therapy (R=0.99), low-molecular- (R=0.93) and high-molecular heparin therapy (R=0.97), while subjects without therapy showed a satisfactory degree of correlation for PT (R=0.86) and aPTT (R=0.80) tests.
Based on the results of these tests and subsequent statistical processing, it was concluded that there were no significant differences between the glass and plastic test-tubes manufactured by Terumo.
Previous studies have shown the importance of the delay between blood sampling and test performance. A prolonged delay leads to a substantial difference in test results between glass and plastic tubes. In our study, the maximum delay was limited to 180 minutes.

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P3-12

Usporedba PV, APTV, TV i fibrinogena na koagulacijskim analizatorima Berichrom Coagulation System i STA Compact

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Uspoređena su četiri koagulacijska parametra: protrombinsko vrijeme (PV, % i INR), aktivirano parcijalno tromboplastinsko vrijeme (APTV), trombinsko vrijeme (TV) i fibrinogen na koagulacijskim analizatorima Berichrom Coagulation System (BCS) proizvođača Dade Behring GmbH, Marburg, Njemačka i STA Compact proizvođača Roche Diagnostics GmbH, Mannheim, Njemačka, koji se rabe u svakodnevnom radu Kliničkoga zavoda za laboratorijsku dijagnostiku Kliničke bolnice Dubrava. BCS i STA Compact višekanalni su automatski analizatori koji rabe metode koagulometrije, fotometrije i imunoturbidimetrije. Cilj je bio ispitati odnos vrijednosti navedenih parametara usporedno određenih reagensima Dade Behring i Diagnostica Stago na analizatorima BCS i STA Compact. PV, APTV, TV i fibrinogen određeni su u 50 različitih uzoraka svježe plazme zdravih osoba i osoba izloženih oralnom antikoagulantnom ili heparinskom liječenju usporedno na oba analizatora, neposredno nakon donošenja materijala u koagulacijski laboratorij. Reagensi upotrebljeni na BCS bili su Thromborel S, Pathromtin SL, BC Thrombin Reagent i Multifibren U. Reagensi upotrebljeni na STA Compact bili su STA Neoplastin Plus, STA Cephascreen, STA Thrombin i STA Fibrinogen. Svi parametri određeni su koagulometrijskom metodom, osim trombinskog vremena na BCS, koje je određeno fotometrijski pomoću BC Thrombin Reagent. ISI vrijednost za Thromborel S bila e 1,0 a za STA Neoplastin Plus 1,28. Usporedbom rezultata dobiveni su koeficijenti korelacije 0,9868 ($p<0,05$) za % PV; 0,9715 ($p<0,05$) za INR; 0,8821 ($p<0,05$) za APTV; 0,8307 ($p<0,05$) za TV i 0,9674 ($p<0,05$) za fibrinogen. Linearnom regresijom dobiveni su slijedeći koeficijenti determinacije: 0,9730 za % PV, 0,8622 za INR, 0,7734 za APTV, 0,5841 za TV i 0,9350 za fibrinogen. Korelacije za PV (%, INR) i fibrinogen visoko su značajne. Korelacija za APTV je jaka, kao i povezanost ispitana linearnom regresijom. Uspoređivanjem vrijednosti TV korelacija je također bila jaka, a linearnom regresijom povezanost se pokazala umjerenom, no statistički prihvatljivom. Uzrok tome mogla bi biti različitost metoda za određivanje trombinskog vremena na analizatorima BCS i STA Compact ili pak različita osjetljivost reagensa. Ispitivanje je pokazalo da je za navedene pretrage moguća usporedna uporaba obaju analizatora u svakodnevnom radu.

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P3-12

Comparison of PT, APTT, TT and fibrinogen on the Berichrom Coagulation System and STA Compact coagulation analyzers

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Four coagulation parameters, i.e. prothrombin time (PT, % and INR), activated partial thromboplastin time (aPTT), thrombin time (TT) and fibrinogen, were compared on the Berichrom Coagulation System (BCS), Dade Behring GmbH, Marburg, Germany and STA Compact, Roche Diagnostics GmbH, Mannheim, Germany, coagulation analyzers. Both analyzers are used routinely at Clinical Department of Laboratory Diagnosis, Dubrava University Hospital. Both instruments are multi-channel automatic analyzers that use coagulometric, photometric and immunoturbidimetric methods. The aim was to compare the results of PT, aPTT, TT and fibrinogen determined in paralle by use of Dade Behring and Diagnostica Stago reagents on BCS and STA Compact analyzers. PT, aPTT, TT and fibrinogen were determined in 50 fresh plasma samples of healthy individuals and patients on oral anticoagulant or heparin therapy immediately upon sample receipt in coagulation laboratory. The reagents used on BCS were Thromborel S, Pathrombin SL, BC Thrombin Reagent and Multifibren U. The reagents used on STA Compact were STA Neoplastin Plus, STA Cephascreen, STA Thrombin and STA Fibrinogen. All parameters were determined by coagulometric method except for TT on BCS that was determined photometrically with BC Thrombin Reagent. The ISI value for Thromborel S was 1.0 and for STA Neoplastin plus 1.28. The correlation coefficient for compared values was 0.9868 ($p<0.05$) for % PV, 0.9715 ($p<0.05$) for INR, 0.8821 ($p<0.05$) for aPTT, 0.8307 ($p<0.05$) for TT, and 0.9674 ($p<0.05$) for fibrinogen. The coefficients of determination obtained by linear regression analysis were: 0.9730 for % PT, 0.8622 for INR, 0.7734 for aPTT, 0.5841 for TT, and 0.9350 for fibrinogen. Correlation analysis yielded significant correlations for all of the study parameters. Correlations for PT (%, INR) and fibrinogen were very strong. Correlation for aPTT was strong as well as the relationship tested with linear regression. Comparison of TT values showed a strong correlation and a moderate degree of association that was statistically acceptable. The cause for this could be the different methods of TT determination on the BCS and STA Compact analyzers or different sensitivity of the reagents. This study showed it to be possible to use both analyzers in parallel for the mentioned analyses.

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P3-13

Tromboelastografija i globalni koagulacijski testovi

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Tromboelastografija (TEG) je metoda koja ima mogućnost praćenja svih faza hemostatske aktivnosti iz jednog uzorka krvi. Ona prati trombodinamski status krvi, a isto tako mjeri viskoelastična i mehanička svojstva stvorenog ugruška. Tromboelastogram je mali analizator priključen na računalo koji prati električne signale. Osnovni parametri koji definiraju stvaranje ugruška su reakcijsko vrijeme (R); K vrijeme; kut alfa i maksimalna amplituda (MA). Globalni koagulacijski testovi (protrombinsko vrijeme (PV), aktivirano parcijalno tromboplastinsko vrijeme (APTV), broj trombocita, koncentracija fibrinogena) mjere izolirano pojedine sastavnice hemostaze i završavaju stvaranjem ugruška.
Cilj studije bio je pronaći korelaciju između parametara tromboelastografa i globalnih koagulacijskih testova. Obrađeno je ukupno 50 bolesnika (muškaraca i žena). Za TEG je rabljena puna citratna krv, a plazma za globalne koagulacijske testove (PV, APTV, koncentracija fibrinogena). Globalni koagulacijski testovi rađeni su na uređaju BCS (Dade Behring, Njemačka), a tromboelastografija je rađena na uređaju TEG Analyzer 5000 (Haemoscope, SAD). MA, parametar tromboelastograma, značajno korelira s koncentracijom fibrinogena (r=0,77; p=0), dok ostali parametri tromboelastograma i globalni koagulacijski testovi nisu korelirali ili korelacija nije bila značajna (MA i broj trombocita (r=0,54; p=0); R i APTV (r=0,411; p=0)). Tromboelastograf rabi male količine uzorka pune krvi, mjeri interakciju trombocita s faktorima koagulacije, brzinu ugruška, čvrstoću ugruška, kao i vezivanje trombocita i fibrinogena i moguću lizu ugruška. Vrijeme, brzina, čvrstoća i stabilnost ugruška pokazuju ima li bolesnik normalan, hipokoagulabilan ili hiperkoagulabilan koagulacijski profil i prikazuje se kao graf. TEG nudi mogućnost pretrage uz bolesnika i jednostavna je i brza koagulacijska metoda.

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P3-13

Thromboelastography and global coagulation tests

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Thrombelastography (TEG) is a technology capable of monitoring all phases of hemostatic activity from a single blood sample. It monitors the thrombodynamic status of the blood as it measures the viscoelastic and mechanical properties of a developing clot. TEG analyzer is a small instrument connected to a computer for electrical signal monitoring. The major parameters of the clot formation are reaction time (R); K time (K); alfa angle; and maximum amplitude (MA). The global coagulation tests (protrombin time (PT), acivated partial thromboplastin time (APTT), platelet count and fibrinogen concentration) measure various components of hemostasis in isolation and end with the formation of fibrin strands. The aim of the study was to assess the correlation between TEG parametars and global coagulation tests. A total of 50 patients of both sexes were included in the study. Whole citrate blood samples were obtained for TEG and plasma samples for global coagulation tests (PT, APTT, fibrinogen concentration). Global coagulation tests were performed on a BCS (Dade Behring, Germany) and TEG on a TEG 5000 Analyzer (Haemoscope, USA). The TEG parameter MA significantly correlated with fibrinogen concentration (r=0.77; p=0), whereas other TEG parameters showed no or nonsignificant correlation with global coagulation tests (MA and platelet count (r=0.54; p=0); R and APTT (r=0.411; p=0)). Using a small sample of whole blood, TEG analyzer measures the interaction of platelets with coagulation factors, clot rate, clot strength as well as platelet and fibrinogen binding and the possible clot lysis. The time, rate, strength and stability of the clot indicate whether the patient has a normal, hypocoagulable or hypercoagulable coagulation profile, and is shown as TEG tracing. So, TEG offers an option of point-of-care testing, and is a simple and rapid coagulation monitoring technique.

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P4 – Srce i srčani biljezi, P4-1 (UP8-1)

Procjena analitičke i dijagnostičke vrijednosti kolorimetrijskog testa vezanja kobalta na albumin kod bolesnika sa sumnjom na akutni koronarni sindrom

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U novije vrijeme razvijen je novi test za otkrivanje rane ishemije miokarda, kolorimetrijski test vezanja kobalta na albumin (ACB-test, engl. *albumin cobalt binding test*), koji se temelji na smanjenom vezanju kobalta na ishemijom izmijenjeni serumski albumin. Procijenjena je analitička i dijagnostička vrijednost testa ACB kao biljega rane ishemije miokarda kod bolesnika sa sumnjom na akutni koronarni sindrom. Kod dijagnostičke procjene određivane su vrijednosti testa ACB u 66 zdravih osoba (referentna skupina) i 110 bolesnika (ispitivana skupina) sa sumnjom na akutni koronarni sindrom, uz mjerenje troponina I kao biljega akutnog infarkta miokarda. Analitička procjena obuhvatila je nepreciznost unutar serije, nepreciznost iz dana u dan, linearnost testa i stabilnost uzorka. Vrijednosti testa ACB bile su statistički značajno više u ispitivanoj skupini od vrijednosti u referentnoj skupini ($p<0,001$). Vrijednosti testa u skupini bolesnika s konačnom dijagnozom infarkta miokarda nisu bile statistički značajno različite od vrijednosti u skupini bolesnika sa sumnjom na akutni koronarni sindrom bez infarkta miokarda. Vrijednosti kod bolesnika s infarktom miokarda nisu bile značajno različite s obzirom na normalne ili povišene vrijednosti troponina I kod prijma u bolnicu. Uz optimalnu graničnu vrijednost od 0,566 dobivena je osjetljivost testa za ishemiju miokarda od 0,89 i specifičnost od 0,66. Analitičkom procjenom dobivena je niska nepreciznost iz dana u dan i unutar serije, dobra linearnost i stabilnost uzorka. Zaključeno je kako bolesnici s dokazima ishemije imaju smanjeno vezanje kobalta na albumin. Iako pokazuje slabu specifičnost za ishemiju miokarda kolorimetrijski test ACB mogao bi biti korisnim biljegom rane ishemije miokarda.

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P4 – Heart and cardial markers, P4-1 (UP8-1)

Analitycal and diagnostic assessment of serum albumin-cobalt binding assay in patients suspect of acute coronary sindrome

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Recently, a novel assay for detecting early myocardial ischemia has been developed which measures reduced cobalt binding to ischemia modified albumin. The aim of this study was analytical and diagnostic assessment of the colorimetric albumin-cobalt binding (ACB) assay in patients with suspected acute coronary sindrome (ACS). We evalueted 66 healthy individuals as a reference group and 110 patients with suspected ACS. Samples were tested by the standard coronary disease marker, troponin I, and ACB test. Analytical evaluation consisted of determination of the within-run and between-run imprecision, linearity, and sample stability. Test values were significantly higher in the study group as compared to the reference group ($p<0.001$). There was no singificant difference in test values between patients with definitive diagnosis of myocardial infarction and other patients with suspected ACS, or beetwen patients with normal and elevated troponin levels in the group of patients with definitive diagnosis of myocardial infarction. Using a cut-off value of 0.566 ABSU selected from ROC analysis, the sensitivity was 0.89 and specificity 0.66 (for myocardial ischemia). Analytical evaluation yielded a low within-run and between-run imprecision, good linearity, and analyte stability. Accordingly, patients with evidence of myocardial ischemia and acute coronary syndrome have a reduced cobalt binding capacity to albumin. Although the assay shows poor specifity for myocardial ischemia, we conclude that this assay may prove to be an early and useful biochemical marker of miocardial ischemia.

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P4-2

Seroprevalencija *Helicobacter pylori* kod koronarne arterijske bolesti

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Helicobacter pylori ima važnu ulogu u gastritisu i peptičkom ulkusu u općoj populaciji. Uz to, utvrđena je povećana seroprevalencija Helicobacter pylori u bolesnika s koronarnom arterijskom bolešću. Na prvi pogled može se činiti kako nema očitog objašnjenja za to osim činjenice da su i Helicobacter pylori i koronarna arterijska bolest udruženi s niskim socioekonomskim statusom. Međutim, promjene koagulacijskog statusa prepoznate su kao posljedica infekcije bakterijom Helicobacter pylori. To bi pak moglo neizravnog utjecaja na prirodnu povijest koronarne arterijske bolesti. U ovoj smo studiji željeli istražiti seroprevalenciju Helicobacter pylori u bolesnika s koronarnom arterijskom bolešću te ustanoviti postoji li udruženost između infekcije Helicobacter pylori i koronarne arterijske bolesti. U studiju je bilo uključeno 79 bolesnka s elektrokardiografski dokazanom koronarnom arterijskom bolešću i 50 zdravih kontrolnih osoba izjednačenih po dobi i spolu. IgG antitijela specifična za Helicobacter pylori mjerili smo testom dostupnim na tržištu. Serumske razine IgG antitijela specifičnih za Helicobacter pylori bile su značajno više u bolesnika s koronarnom arterijskom bolešću nego u kontrolnih osoba (p<0,01). Rezultati ovoga ispitivanja ukazuju na pojačanu seroepidemiološku udruženost infekcije Helicobacter pylori s koronarnom arterijskom bolešću.

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P4-2

Helicobacter pylori seroprevalence in coronary artery disease

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Helicobacter pylori plays an important role in gastritis and peptic ulcer disease in general population. Also, there is an increased seroprevalence of *Helicobacter pylori* in patients with coronary artery disease. At first glance, there would seem to be no obvious explanation for this other than the fact that both *Helicobacter pylori* infection and coronary artery disease are associated with low socioeconomic status. However, alterations in coagulation status have been identified as a consequence of *Helicobacter pylori* infection. This might have some indirect influence on the natural history of coronary artery disease. In this study we aimed to investigate the seroprevalence of *Helicobacter pylori* in patients with coronary artery disease and to find out whether there is an association between *Helicobacter pylori* infection and coronary artery disease. The study included 79 patients with electrocardiography evidence of coronary artery disease and 50 healthy age- and sex-matched control subjects. *Helicobacter pylori* specific IgG antibodies were measured with a commercially available kit. Serum levels of *Helicobacter pylori* specific IgG antibodies were significantly higher in patients with coronary artery disease than in control subjects (p<0.001). Study results suggested an increased seroepidemiological association of *Helicobacter pylori* infection with coronary artery disease.

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P4-3

Biokemijsko dijagnosticiranje akutnog infarkta miokarda: srčani troponin I ili izoenzim MB kreatin kinaze

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Biokemijski biljezi nekroze miokarda imaju bitnu ulogu u dijagnosticiranju akutnog infarkta miokarda. Cilj ove prospektivne studije je bio evaluirati relativni porast i dija-

P4-3

Biochemical diagnosing of acute myocardial infarction: cardiac troponin I or creatine kinase MB isoenzyme

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Biochemical markers of myocardial necrosis have an essential role in the diagnosis of acute myocardial infarction (AMI). The aim of this prospective study was to evaluate

gnostičko značenje izoenzima MB kreatin kinaze i srčanog troponina I (cTnI) u krvi bolesnika s akutnim infarktom miokarda. U studiju je bilo uključeno 36 bolesnika s akutnim infarktom miokarda (20 muškaraca,16 žena, starosti 61±14 godina). Koncentracija cTnI i aktivnost CK-MB u serumu je određivana u tri vremenska razdoblja (6-9 sati, 24 sata i 6-7 dana) od početka bolova u prsištu. Srčani TnI je određivan fluoroenzimometrijski na analizatoru AxSYM (Abbott Laboratories). CK-MB aktivnost je određivana metodom inhibicije enzima (Chronolab) na analizatoru Flexor. Oba biljega su imala maksimalne vrijednosti u krvi 24 sata od početka simptoma infarkta. U usporedbi sa CK-MB, cTnI je imao bolju osjetljivost u sva tri razdoblja određivanja. Dakle, visok sadržaj cTnI u srcu čini ga idealnim biljegom za otkrivanje minimalnog oštećenja miokarda. Dugo trajanje povećanih vrijednosti cTnI čini ga idealnim biljegom za kasno dijagnosticiranje akutnog infarkta miokarda.

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the relative increase and diagnostic significance of blood creatine kinase MB isoenzyme (CK-MB) and cardiac troponin I (cTnI) in AMI patients. Thirty-six AMI patients (20 male and 16 female, aged 61±14 years) were included in the study. We measured serum concentrations of cTnI and activities of CK-MB in three time periods (6-9 h, 24 h and 6 -7 days) from chest pain onset. Cardiac troponin I was measured by the fluoroenzymometric method on an AxSYM analyzer (Abbott Laboratories). The enzyme inhibition method (Flexor analyzer) was used for CK-MB activity (Chronolab). Both markers showed maximal blood level at 24 h of the onset of infarction symptoms. As compared with CK-MB, cTnI showed higher sensitivity in all three periods of measurement. Thus, high heart tissue content of cTnI makes it an ideal marker for the detection of minimal myocardial damage, whereas prolonged elevation of cTnI level makes it an ideal marker for late AMI diagnosing.

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P4-4

Vrijednost koncentracije cTnI i Myo u ranoj procjeni srčanog zatajenja u hitnoj službi

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Bol u prsima je jedan od najčešćih razloga zbog kojeg bolesnici dolaze u hitnu medicinsku službu i od velike je važnosti pravodobno prepoznati znakove srčanog zatajenja kako bi se moglo reagirati na vrijeme. Mjerenjem kombinacije ranog i kasnog biljega moglo bi se lakše i brže isključiti srčano zatajenje i tako omogućiti otpuštanje onih bolesnika koji ne zahtijevaju prijam u bolnicu ili duže promatranje. Cilj studije je bio ocijeniti vrijednost serumske koncentracije mioglobina (Myo) i srčanog troponina I (cTnI) u ranoj procjeni bolesnika koji su imali simptome boli u prsima kada su došli u hitnu medicinsku službu. Uzorci krvi su vađeni odmah nakon dolaska bolesnika i koncentracija srčanih biljega mjerena je u hitnom laboratoriju. Odredili smo osjetljivost, specifičnost i površinu ispod krivulje ROC za cTnI i Myo kod 916 bolesnika koji su pristupili u hitnu službu. Osjetljivost za serumsku koncentraciju cTnI i Myo bila je 30% i 42% za svaki pojedinačno, specifičnost je bila viša, tj. 96% i 83%, pozitivna prediktivna vrijednosti 77% i 52%, a negativna prediktivna vrijednost 77% za oba biljega. Površina ispod krivulje ROC s graničnim vrijednostima od 0,2 µg /L za cTnI i 92 µg /L za Myo je bila slična, 0,674 i 0,671. Ne postoji značajna razlika

P4-4

The value of cTnI and Myo concentration in the early assessment of heart failure at emergency department

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Chest pain is one of the most common complaints presented to emergency department (ED) and rapid decision depends on several factors including values of biochemical cardiac markers. The value of biochemical markers in diagnostic procedu re lies in its ability to provide more information to the clinician within short time, when prompt decision is necessary. The use of a combination of an early and late marker may facilitate rapid exclusion of heart failure and enable discharge of patients who do not require hospital admission or prolonged observation. The aim of study was to evaluate the value of serum myoglobin (Myo) and cardiac troponin I (cTnI) in the early assessment of patients presenting to ED with the symptoms of chest pain. Blood samples were obtained immediately upon patient arrival to ED. Serum concentrations of the two cardiac markers were determined at emergency laboratory. We determined the sensitivity, specificity, receiver operating characteristics (ROC) curve for cTnI and Myo in 916 patients upon their admission to ED. Serum concentrations of cTnI and Myo demonstrated a sensitivity of 30% and 42%, higher specificity of 96% and 83%, positive predictive value of 77% and 52%, respectively, and nega-

u površini ispod krivulje za ova dva biljega (p=0,893). U zaključku, naši rezultati pokazuju kako oba biljega imaju visoku specifičnost i dobru negativnu prediktivnu vrijednost u procjeni stanja bolesnika. Prema našim podacima na osnovi biokemijskih biljega, isključenje srčanog zatajenja je pouzdanije od njegova otkrivanja.

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tive predictive value of 77% for both markers. The areas under the ROC curve with cut-off values of 0.2 µg/L for cTnI and 92 µg/L for Myo were similar, 0.674 and 0.671, respectively. There was no significant difference between the areas under the curve for either marker (p=0.893). In conclusion, our data indicate that both markers have high specificity and good negative predictive value on patient assessment. According to our results, exclusion of heart failure on the basis of these biochemical markers is more reliable than its detection.

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P4-5

BNP – biljeg ishemijske bolesti srca

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B-tip natriuretskog peptida (BNP) je novi biljeg koji se pokazao korisnim u procjeni težine ishemjskog oštećenja srca. To je mali srčani hormon koji se oslobađa izravno srazmjerno povećanju ventrikularnog volumena. Nakon višemjesečnog određivanja BNP u bolesnika s otežanim disanjem cilj je bio utvrditi koje vrijednosti BNP i dijagnoze bolesti su najčešći bili praćeni zahtjevom za određivanjem BNP. BNP se je određivao testom BNP na analizatoru AxSYM tehnologijom MEIA. Rezultati su podijeljeni u skupine prema vrijednostima BNP (0-100 pg/mL, 100-500 pg/mL i >500 pg/mL). Vrijednosti BNP su se dobro slagale s dijagnozama bolesnika. BNP je svakako koristan kao pomoć u procjeni je li došlo do srčane dekompenzacije i jesu li potrebni daljnji dijagnostički postupci.

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P4-5

BNP – a marker of ischemic heart failure

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B-type of natriuretic peptide (BNP) is a new marker, helpful in the assessment of ischemic heart failure severity. It is a small hormone which is released from the heart in direct proportion to ventricular volume expansion. After several months of testing patients with dispnea, we wanted to review the connection between test results and requests for BNP testing. Testing was perfomed with the BNP test on an AxSYM analyzer using MEIA technology. Results were divided into groups according to BNP level (0-100 pg/mL, 100-500 pg/mL and >500 pg/mL). The levels of BNP showed good correlation with the disease diagnosis. Thus, BNP is a valuable tool to assess whethere there is a pending risk of congestive heart failure and the need of additional diagnostic work-up.

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P4-6

Dijagnostička vrijednost određivanja BNP i NT-proBNP

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Povećane koncentracije BNP i NT-proBNP u krvi nalazimo kod bolesnika s različitim kardiovaskularnim bolestima. Cilj ove pilot studije bio je usporediti korisnost dvaju NP

P4-6

Diagnostic value of BNP and NT-proBNP

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Brain natriuretic peptide (BNP) and NT-proBNP are widely recognized markers for the diagnosis and prognosis of adverse outcome and treatment in patients with heart

testova (BNP i NT-proBNP) u dijagnostici i procjeni rizika bolesnika sa srčanim insuficijencijama prema dobi, spolu i klasifikaciji New York Heart Association (NYHA). Odredili smo koncentraciju BNP (MEIA, Abbott) i NT-proBNP (ECLIA, Roche) u 40 bolesnika sa srčanim bolestima (20 žena i 20 muškaraca starosti 71,2±12,0 godina) i u 80 zdravih ljudi (39 žena i 41 muškarac starosti 64,6±8,2 godina). Rezultate smo obradili Studentovim t-testom i Pearsonovim ili Spearmanovim koeficijentom korelacije s odabranom razinom značajnosti p<0,05. Statistički značajne razlike koncentracija BNP i NT-proBNP pronađene su kod bolesnika sa srčanim bolestima u usporedbi s koncentracijama kod kontrolne skupine (BNP 104,1-6022,0; srednja vrijednost 1128,6 pg/mL prema BNP 0-95,6, srednja vrijednost 14,7 pg/mL; NT-proBNP 143,0-35000,0; srednja vrijednost 5989,5 pg/mL prema NT-proBNP 5,6-191,0; srednja vrijednost 66,4 pg/mL; p<0,001 za oba). U skupini bolesnika nisu pronađene statistički značajne razlike prema spolu u koncentracijama BNP (muškarci u usporedbi sa ženama: srednja vrijednost 1335,0 pg/mL prema 966,8 pg/mL; p=0,37) i NT-proBNP (muškarci u usporedbi sa ženama: 7076,3 pg/mL prema 4309,9 pg/mL; p=0,36). Isto je zabilježeno u kontrolnoj skupini zdravih ispitanika (BNP muškarci u usporedbi sa ženama: 17,1 pg/mL prema 12,3 pg/mL; p=0,37; i NT-proBNP muškarci u usporedbi sa ženama: 69,1 pg/mL prema 63,1 pg/mL; p=0,36). U skupini bolesnika sa srčanim bolestima prema klasifikaciji NYHA pronađena je statistički značajna pozitivna korelacija BNP (rho=0,708; p<0,001) i NT-proBNP (rho=0,887; p<0,001). Nije pronađena statistički značajna razlika s obzirom na dob u skupini bolesnika sa srčanim bolestima: BNP (r=0,101; p=0,277) i NT-proBNP (r=0,140; p=0,239), za razliku od ispitanika u kontrolnoj skupini gdje je dob pokazala statistički značajnu korelaciju s BNP (r=0,240; p=0,016) i NT-proBNP (r=0,312; p=0,003). BNP, a osobito NT-proBNP, su korisni biljezi za dijagnozu i procjenu rizika u srčanih bolesnika neovisno o dobi i spolu. Određivanje koncentracija BNP i NT-proBNP u krvi dobar je prognostički pokazatelj kod ovih bolesnika.

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failure (HF). Both peptides are products of proteolytic processing of the precursor molecule preproBNP, which is synthesized in cardiac myocytes. The aim of this pilot study was to compare the efficiency of two NP tests (BNP i NT-proBNP) in the diagnosis and risk stratification of HF patients according to age, sex and New York Heart Association (NYHA) classification. The sample consisted of 40 HF patients (20 female and 20 male, mean age 71.2±12.0 years) and 80 healthy subjects (39 female and 41 male, mean age 64.6±8.2 years). The output measures were BNP (MEIA, Abbott) and NT-proBNP (ECLIA, Roche) values in all subjects. Differences in independent data were tested by Student's t-test, and correlations by Pearson's or Spearman's coefficient. The level of significance was set at p<0.05.

Significantly higher values of both BNP and NT-proBNP tests were recorded in HF patients as compared with control group (BNP range 104.1-6022.0, mean 1128.6 pg/mL *vs*. BNP 0-95.6, mean 14.7 pg/mL; and NT-proBNP range 143.0-35000.0, mean 5989.5 pg/mL *vs*. NT-proBNP range 5.6-191.0, mean 66.4 pg/mL; p<0.001 both). In HF group, there were no sex differences in BNP (male to female: mean 1335.0 pg/mL *vs*. 966.8 pg/mL; p=0.37) and NT-proBNP (male to female: mean 7076.3 pg/mL *vs*. 4309.9 pg/mL; p=0.36). The same applied to the control group of healthy subjects (BNP male to female: 17.1 pg/mL *vs*.12.3 pg/mL; p=0.37; and NTproBNP male to female: 69.1 pg/mL *vs*.63.1 pg/mL; p=0.36). In HF group, a significant positive correlation was found between NYHA class, BNP (rho=0.708; p<0.001) and NT-proBNP (rho=0.887; p<0.001). In HF group, there was no significant correlation between age, BNP (r=0.101; p=0.277) and NT-proBNP (r=0.140; p=0.239), in contrast to healthy subjects where age yielded a significant positive correlation with BNP (r=0.240; p=0.016) and NT-proBNP (r=0.312; p=0.003). BNP and especially NT-proBNP are useful markers in the diagnosis and risk stratification of HF patients, independently of age and sex. BNP and NT-proBNP are also good predictors of prognosis in these patients.

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P4-7

Usporedba metoda za određivanje srčanog troponina I

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U posljednje vrijeme srčani troponin I (cTnI) postao je vodeća pretraga u dijagnostici infarkta miokarda te kod procjene rizika u bolesnika s akutnim koronarnim sindromom. Nakon oštećenja miokarda cTnI se oslobađa u obliku kompleksa s troponinom C(I-C kompleks), kompleksa s troponinom T i troponinom C(I-T-C kompleks) te kao slobodni oblik. U cirkulaciji se mogu naći i drugi oblici cTnI (fosforilirani, defosforilirani, reducirani, oksidirani i/ili proteolitički razgrađeni). Cilj je bio usporediti vrijednosti cTnI dobivene dvjema različitim metodama. Analizirano je ukupno 67 usporednih uzoraka. Analize su izvršene na analizatoru Dimension RxL (Dade Behring) i na analizatoru Access (Beckman-Coulter). Na analizatoru Dimension RxL cTnI se određivao enzim-imunokemijskom metodom (EIA), a na analizatoru Access kemiluminiscentnom metodom. Dobivena je jednadžba regresije (Passing i Bablok regresija):
y(Access)=0,5465 *x (Dimension RxL) – 0,0019 uz koeficijent korelacije r=0,9637.
Wilcoxon testom nađena je statistički značajna razlika između dobivenih vrijednosti (p<0,0001).
Zaključeno je kako se različitim metodama dobiju različite brojčane vrijednosti za cTnI. Postoji više čimbenika koji pridonose tim razlikama:
- u testovima se rabe različita monoklonska antitijela koja ne prepoznaju iste epitope na molekuli cTnI
- različita osjetljivost metoda na oblike cTnI prisutne u cirkulaciji
- nepostojanje internacionalnog standarda za kalibraciju

Svi dobiveni rezultati su usporedivi samo primjenom graničnih vrijednosti za pojedini test (Access AccuTnI 0,06 μg/L; Dimension CTNI 0,1 μg/L).

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P4-7

Comparison of methods for determination of cardiac troponin I

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Over recent years cardiac troponin I (cTnI) has become the leading assay in the diagnosis of myocardial infarction and for risk stratification of patients with acute coronary syndromes. Following myocardial damage cTnI is released as a complex with troponin C (I-C complex), with troponin T and troponin C (I-C-T complex), and as free cTnI. Other forms of cTnI (phosphorylated, dephosphorylated, reduced, oxidized and/or proteolytically degraded) may also exist in the circulation. The aim was to compare cTnI values obtained by two different cTnI methods. A total of 67 parallel samples were analyzed. Assays were preformed on a Dimension RxL analyzer (Dade Behring) and on an Access analyzer (Beckman-Coulter). On Dimension RxL analyzer cTnI was determined by use of enzyme-immunoassay (EIA), while on Access analyzer chemiluminiscent immunoassay was used. Passing and Bablok regression line was:
(Access)=0.5465 *x (Dimension RxL) – 0.0019, with a correlation coefficient r=0.9637. There was a statistically significant difference between the results (Wilcoxon test, p<0.0001). Accordingly, different methods for cTnI yielded numerically different results. The following factors contribute to these discrepancies:
- the antibodies used in each method do not react with the same epitops on cTnI,
- different sensitivity of the methods to the cTnI forms in the circulation, and
- the lack of international standard for calibration.

Thus, results could only be compared according to cut-off values of the assays (Access AccuTnI 0.06 μg/L; Dimension CTNI 0.1 μg/L).

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P7 – Šećerna bolest, P7-1 (UP12-1)

Učinak trajanja šećerne bolesti tip 1 na katalitičnu koncentraciju N-acetil-beta-D-glukozaminidaze u mokraći djece i adolescenata

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N-acetil-beta-D-glukozaminidaza (NAG) (EC 3.2.1.30) je lizosomski enzim. Enzim koji se izlučuje mokraćom podrijetlom je iz bubrega i potječe pretežito iz stanica bubrežnih tubula. NAG je rani pokazatelj razvoja dijabetične nefropatije. Kod šećerne bolesti tip 1 izlučivanje NAG mokraćom povećava se prije pojave minimalne albuminurije. Neka istraživanja pokazuju da izlučivanje NAG mokraćom ovisi o trajanju šećerne bolesti tip 1. Cilj je bio ispitati ovisi li izlučivanje NAG u mokraći djece i adolescenata sa šećernom bolešću tip 1 o trajanju bolesti. Katalitične koncentracije NAG određivale su se spektrofotometrijski u slučajnim uzorcima mokraće 66 djece i adolescenata sa šećernom bolešću tip 1 i 68 ispitanika iz kontrolne skupine. Bolesnici iz skupine dijabetičara bili su podijeljeni s obzirom na trajanje bolesti: I. skupina manje od tri godine (n=17); II. skupina tri do pet godina (n=19); III. skupina pet do deset godina (n=19); IV. skupina više od deset godina (n=11). Vrijednosti NAG izražene su u odnosu na koncentraciju kreatinina u mokraći radi isključivanja razlika u koncentraciji mokraće. Izlučivanje NAG mokraćom kod dijabetičara bilo je statistički značajno povećano u odnosu na kontrolnu skupinu (p<0,001). U sve četiri skupine dijabetičara izlučivanje NAG mokraćom bilo je statistički značajno povećano u odnosu na kontrolnu skupinu (I. skupina p=0,001; II. i III. skupina p<0,001 i IV. skupina p=0,004), no nije nađena statistički značajna razlika među skupinama dijabetičara (I. *vs*. II. skupina p=0,716; I. *vs*. III. skupina p=0,899; I. *vs*. IV. skupina p>0,10; II. *vs*. III. skupina p=0,549; II. *vs*. IV. skupina p>0,10; III. *vs*. IV. skupina p>0,10). Nije nađena korelacija između vrijednosti NAG u mokraći dijabetičara i trajanja bolesti (r=-0,017; p=0,892). Izlučivanje NAG mokraćom povećano je kod djece i adolescenata sa šećernom bolešću tip 1, ali ne ovisi o trajanju bolesti.

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P7 – Diabetes mellitus, P7-1 (UP12-1)

Effect of diabetes mellitus type 1 duration on urinary N-acetyl-beta-D-glucosaminidase excretion in children and adolescents

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N-acetyl-beta-D-glucosaminidase (NAG) (EC 3.2.1.30) is a lysosomal enzyme. Urinary NAG is of renal origin and is distributed mainly in renal proximal tubules. It is an early predictor of the development of diabetic nephropathy. It increases before the occurrence of microalbuminuria in diabetes mellitus type 1, and some studies have shown that there is a relationship between the level of urinary NAG excretion and duration of diabetes. The aim of the study was to assess urinary NAG NAG in children and adolescents with diabetes mellitus type 1 as compared to healthy subjects and according to the disease duration. NAG levels were determined spectrophotometrically in random urine samples from 66 children and adolescents with type 1 diabetes mellitus and 68 control subjects. Diabetic patients were divided according to the duration of diabetes into four groups: group I, less than three years (n=17); group II, three to five years (n=19); group III, five to ten years (n=19); and group IV, more than ten years (n=11). To exclude the influence of urine concentration differences, urinary NAG levels were referred to the level of urinary creatinine. Urinary NAG excretion in diabetic patients was significantly increased as compared to controls (p<0.001). All four groups had a significantly higher excretion of urinary NAG as compared to controls (group I p=0.001, groups II and III p<0.001, and group IV p=0.004) but there was no significant difference among diabetic subgroups (group I *vs*. II p=0.716; group I *vs*. III p=0.899; group I *vs*. IV p>0.10; group II *vs*. III p=0.549; group II *vs*. IV p>0.10 and group III *vs*. IV p>0.10). There was no correlation between urinary NAG level and duration of diabetes (r=-0.017; p=0.892). Urinary NAG excretion was significantly higher in children and adolescents with diabetes mellitus type 1 but it was not dependent of the disease duration.

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P7-2 (UP12-2)

Aktivnost alkalne fosfataze u bolesnika sa šećernom bolešću tip 1 i 2: razlike prema dobi i spolu

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Rast aktivnosti enzima alkalne fosfataze (ALP) kod bolesnika s dijagnozom šećerne boelsti objavljen je u više radova posljednjih godina, ali razlozi koji dovode do ovog porasta su još uvijek nepoznati. Neki autori ovaj rast aktivnosti pripisuju koštanim izoenzimima, a taj porast nastupa nakon uzimanja oralnih antidijabetičnih lijekova. Puno je spekulacija vezano za značajnost ovoga enzima u dijabetesu, pogotovo ako se ima u vidu uloga enzima u razvoju jetrenih bolesti i koštanih abnormalnosti kod ove skupine bolesnika. Najnovije studije ukazuju na povezanost ovoga enzima i pretilosti, pa to samo po sebi nameće nova pitanja i nove odgovore vezane za njegovu ulogu u dijabetesu. U ovom radu je 40 bolesnika Dijebetološkog savjetovališta Opće bolnice Sarajevo bilo neposredno uključeno u studiju. Kontrolnu skupinu (n=20) su činili bolesnici bez evidencije šećerne bolesti i kroničnih bubrežnih bolesti. Dijabetični bolesnici također nisu imali kroničnu bolest jetre, kao ni dokaza za značajniju dijabetičnu nefropatiju. U svim testiranim uzorcima aktivnost ALP i koncentracija glukoze su određeni uz primjenu standardnih protokola IFCC. Statistička obrada podataka provedena je programu SPSS za Windows. Dobiveni rezultati ukazuju na značajne razlike u koncentraciji glukoze i aktivnosti ALP između kontrolne skupine i populacije bolesnika s dijagnozom dijabetesa. U bolesnika s dijagnozom dijabetesa tip 1 aktivnost enzima se približila gornjoj razini referentnih vrijednosti i bila je veća za 61,5% od one u kontrolnoj skupini. U bolesnika s dijagnozom dijabetesa tip 2 ovaj rast je bio na razini od 32,43%. Dvije skupine bolesnika sa šećernom bolešću nisu se razlikovale prema aktivnosti ALP. U radu nisu primijećene značajnije razlike prema spolu i dobi bolesnika. U ovoj studiji nisu nađene korelacije vezane za aktivnost enzima i koncentraciju glukoze.

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P7-2 (UP12-2)

Alkaline phosphatase activity in patients with diabetes mellitus type 1 and 2: age and sex differences

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Elevation of serum alkaline phosphatase (ALP) activity in patients with diabetes mellitus has been reported for several years now, but the cause underlying this elevation remains unknown. Some authors attribute this finding to the increased activity of bone isoenzymes after the administration of oral antidiabetic agents. Speculations still exist about the role of this enzyme in diabetes, especially considering the development of liver disease or bone abnormalities. Recent studies have pointed to the association of this enzyme with central adiposity, thus posing even more questions to be answered related to its role in diabetes. Forty patients from Diabetic Counseling Service of the Sarajevo General Hospital were included in the study. Twenty patients with no evidence of diabetes or chronic liver disease were included as control group. In diabetic patients, there was no evidence of chronic liver disease or diabetic nephropathy. ALP activity and glucose concentration were determined in all serum samples using standard approved IFCC protocols. Statistical analysis of the results was performed by use of SPSS for Windows. Study results showed a significant difference in glucose concentration and ALP activity between the control and diabetic populations. In the group of patients with type 1 diabetes, ALP activity approached the upper limit of the reference range and exceeded the activity recorded in the control group by 61.5%. In the group of patients with the diagnosis type 2 diabetes, the respective increase was at the level of 32.43%. The two groups of patients did not differ according to the level of ALP activity. No sex or age differences were recorded in the study parameters between diabetic patients and respective controls. Study results revealed no correlations related to ALP activity and glucose concentration.

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P7-3

Direktna automatska imunoturbidimetrijska metoda za određivanje HbA_{1c}

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Cilj rada bio je analitička procjena reagencija za direktno imunoturbidimetrijsko određivanje hemoglobina A_{1c} (Pointe Scientific, Inc., MI, SAD) u punoj krvi na analizatoru Olympus 2700 (Olympus, Optical Co., Japan). Uzorci venske krvi dobiveni su od dijabetičnih bolesnika uzetih s antikoagulansom K3EDTA. Određivanje glikiranog hemoglobina (HbA_{1c}) metodom turbidimetrijskog imuno testa temelji se na izravnom određivanju HbA_{1c} u punoj krvi. Ukupni hemoglobin i HbA_{1c} imaju istu nespecifičnu brzinu apsorpcije na lateks čestice. To je temelj prve reakcije, gdje se miješa uzorak i R1 (lateks čestice u glicin puferu). Hemoglobin A_{1c} se veže na lateks IgG poliklonska antitjela. Aglutinacijski kompleks se formira u interakciji HbA_{1c} vezanog za lateks čestice s određenim antitjelima. Jačina apsorpcije je proporcionalna HbA_{1c} vezanom na lateks čestice odnosno % HbA_{1c} u uzorku. Reagens je kalibriran s četiri kalibratora (prema NGSP). Ispitivana je nepreciznost u seriji za 3 koncentracijske razine, nepreciznost iz dana u dan (10 dana za dvije koncentracijske razine), netočnost prema kontrolnom uzorku, netočnost procijenjena usporedbom s reagensom tvrtke Roche Diagnostics, Njemačka (Tina-Quant) uz primjenu statističke metode po Passing Bablocku (n= 50), te procjena prijenosa (carry-over). Za nepreciznost u seriji koeficijenti varijacije CV (%) iznosili su od 1,12 do 1,87 (n=20), a za nepreciznost iz dana u dan 1,23 i 2,00. Odstupanja od deklarirane koncentracije kontrolnih seruma iznosile su 0,638% i 1,584%. Usporedbom uzoraka s reagensom Roche statistička analiza je pokazala korelaciju y=0,1224x+0,9310 s koeficijentom korelacije r=0,9680, p>0,1, što znači da su rezultati s oba reagensa usporedivi (Passing-Bablock). Konstanta K za procjenu prijenosa uzoraka iznosila je 0% do1,81%, što je manje od najviše dopuštene dvostruke vrijednosti CV za nepreciznost iz dana u dan. Kalibraciju reagensa treba provoditi svakih 7 dana. Uzorci krvi stabilni su 7 dana u hladnjaku na 2-8 °C. Pripremljeni hemolizat uzorka stabilan je do 10 dana na temperaturi 2-8 °C. Rezultati ove procjene pokazuju da reagens u potpunosti zadovoljava sve analitičke kriterije standarda za kvalitetu te da je direktno određivanje HbA_{1c} koje ne uključuje postupak određivanja ukupnog hemoglobina i matematički zahvat izračuna HbA_{1c} (gdje se isključuje mogući izvor pogrješke i potrošnja reagensa) uz osiguranu preciznost i točnost metodološki napredak.

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P7-3

A novel simplified automated immunoturbidimetric assay for HbA_{1c} determination (direct HbA_{1C})

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The aim of the study was to adapt a direct immunoassay (Pointe Scientific, Inc., MI, USA) for *in vitro* determination of hemoglobin A_{1C} in whole blood on an Olympus 2700 autoanalyzer (Olympus Optical Co., Japan). Venous blood samples from diabetic patients are collected into K3EDTA containing vacutainer tubes. This direct determination of HbA_{1c} utilizes the interaction of antigen and antibody to directly determine HbA_{1C} in whole blood. Total hemoglobin and HbA_{1c} have the same nonspecific absorption rate to latex particles. When mouse antihuman HbA_{1c} monoclonal antibody is added (R2), the latex-HbA_{1c}-mouse anti human HbA_{1c} antibody complex is formed. Agglutination occurs when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA_{1c} absorbed onto the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA_{1c} value is obtained from calibration curve. Calibration of the procedure was performed using calibrators referenced to the NGSP value. Within-run imprecision (at three different HbA_{1c} concentrations), between-run imprecision (in triplicate for two control hemolysates measured for 10 days), and accuracy (with control material), and by comparison with the Tina-Quant HbA_{1c} turbidimetric inhibition immunoassay (Roche Diagnostics, Germany; n=50) were assessed. The within-run and between-run imprecision (n=20) CV (%) was 1.12-1.87 and 1.23 and 2.00, respectively. Results of the comparison study showed no statistical difference according to the Passing & Bablok regression analysis (y= 0.1224+0.9310x, r=0.9680, p>0.1). Calibration of the assay was stable for at least 7 days. Refrigerated samples remained stable for HbA_{1c} analysis for 7 days. Stability of the hemolysate was up to 10 days at 2-8 °C. Results of the study indicate that the new automated immunoturbidimetric procedure directly determines percentage of HbA_{1c} adapted for routine chemistry analyzer using only one channel (a separate channel for the determination of total hemoglobin fraction for the sample is not necessary), providing a precise and accurate determination of HbA_{1c}.

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P7-4

Vrijednosti IGF-I i IGFBP3 u očnoj vodici i serumu dijabetičnih bolesnika

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Cilj je bio kvantitativno odrediti IGF-I i IGFBP3 u očnoj vodici i serumu dijabetičnih bolesnika i u kontrolnoj skupini bolesnika, te odrediti povezanost tih parametara u očnoj vodici sa stupnjem dijabetične retinopatije. IGF-I mjeren je modificiranom metodom RIA, a IGFBP3 metodom RIA. U istraživanje smo uključili 24 bolesnika s dijagnozom šećerne bolesti tip 2. U kontrolnoj skupini ispitali smo 9 nedijabetičnih bolesnika. Bolesnike sa šećernom bolešću podijelili smo u 3 skupine: bolesnici bez dijabetične retinopatije (NDR, n=7); bolesnici s neproliferativnom dijabetičnom retinopatijom (NPDR, n=10); i bolesnici s proliferativnom dijabetičnom retinopatijom (PDR, n=7).
Očna vodica uzorkovana je tijekom operacije katarakte. Rezultati nisu pokazali povezanost između vrijednosti IGF-I i IGFBP3 u serumu ni u kojoj bolesničkoj skupini. Sinteza unutar oka povezana je s porastom vrijednosti IGF-I i IGFBP3 u očnoj vodici. Zaključili smo kako su IGF-I i IGFBP3 važni lokalni parametri u razvoju proliferativne dijabetične retinopatije.

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P7-4

Vitreous and serum levels of IGF-I and IGFBP3 in patients with diabetes mellitus

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The study was aimed at quantitative determination of IGF-I and IGFBP3 in aqueous humor and serum of diabetic patients and control subjects, and determination of the relationship of IGF-I and IGFBP3 levels in aqueous humor with the stage of diabetic retinopathy. IGF-I was measured by a modified RIA technique and IGFBP3 by RIA technique. The study included 24 diabetic patients (diabetes mellitus type 2) and 9 nondiabetic patients as control subjects. The patients with diabetes were classified into three groups: no diabetic retinopathy (NDR, n=7), non-proliferative diabetic retinopathy (NPDR, n=10) and proliferative diabetic retinopathy (PDR, n=7). Aqueous humor was aspirated during cataract surgery. No significant differences were found between serum contrentration of IGF-I and IGFBP3 in any of the patient groups. Intraocular synthesis is associated with increased vitreous levels of IGF-I and IGFBP3. It is concluded that IGF-I and IGFBP3 are important local factors in the development of proliferative diabetic retinopathy.

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P7-5

Koncentracije ukupnih proteina i albumina u serumu dijabetičnih bolesnika

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Dijabetes melitus je veoma često praćen hipoalbuminemijom i hipoproteinemijom, što upućuje na važnost određivanja ovih analita u serumu bolesnika s ovom dijagnozom. Promjene koncentracije navedenih parametara kod dijabetičnih bolesnika se mogu javiti kao posljedica albuminurije, proteinurije, upalnih procesa, hemodijalize itd. Uslijed toga se preporučuje povremeno kvalitativno ispitivanje istih u mokraći i serumu radi planiranja liječenja i utvrđivanja učinkovitosti terapije. U ovom eksperimental-

P7-5

Serum total protein and albumin concentrations in diabetic patients

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Diabetes mellitus (DM) is quite frequently accompanied by hypoalbuminemia and hypoproteinemia, which points to the importance of determination of these analytes in serum of diabetic patients. In DM patients, changes in the concentration of these parameters can be due to albuminuria, proteinuria, inflammatory process, hemodialysis, etc. Therefore, periodical qualitative determination of these analytes in serum and urine is advised for treatment planning and therapeutic efficacy assessment. The pres-

nom radu populaciju ispitanika činile su dvije skupine bolesnika. Kontrolnu skupinu činilo je 20 bolesnika s normalnom koncentracijom glukoze, albumina i ukupnih proteina u krvi, a eksperimentalnu 40 bolesnika s dijagnozom šećerne bolesti (20 bolesnika tipa 1 i 20 boelsnika tip 2). U kontrolnoj skupini, kao i u skupinama dijabetičnih bolesnika tip 1 i tip 2 bilo je po 10 muškaraca i 10 žena. Ciljevi rada su bili odrediti koncentracije glukoze, ukupnih proteina i albumina u serumu svih ispitanika, usporediti razlike u koncentraciji ispitivanih parametara kod svih ispitivanih grupa te utvrditi korelacije između praćenih parametara, spola i dobi u pojedinim skupinama te korelacije između praćenih parametara i tipa dijabetesa. Dobiveni rezultati ukazuju na visoko statistički značajane razlike srednjih vrijednosti koncentracija ispitivanih parametara između skupine dijabetičnih bolesnika tip 1, te dijabetičnih bolesnika tip 2 i kontrolne skupine. Razlike nisu zabilježene u koncentraciji albumina kod dijabetesa tip 2. Usporedbom bolesnika s dijabetesom tip 1 i tip 2 jedino je ustanovljena statistički značajna razlika u koncentraciji albumina (p=0,032). U kontrolnoj grupi ustanovljene su korelacije: između koncentracije glukoze i koncentracije albumina; koncentracije ukupnih proteina i koncentracije albumina; koncentracije glukoze, albumina i spola ispitanika. Kod bolesnika s dijabetesom tip 1 nađena je korelacija između koncentracije albumina i spola ispitanika. Druge vrste korelacija nisu zabilježene osim korelacije između tipa dijabetesa i koncentracije albumina.

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ent study included two patient groups. Control group consisted of 20 patients with normal blood concentration of glucose, albumin and total protein; experimental group consisted of 40 diabetic patients (20 patients with type 1 and type 2 DM each); thus, each study group included 10 patients. The aims of the study were to determine serum concentration of glucose, total protein and albumin in all study groups, to compare differences in the concentration of these parameters across the groups, and to identify correlations between these parameters, age and sex within particular study group as well as between the parameters and type of DM. The results obtained pointed to statistically highly significant differences in the mean concentrations of the study parameters between type 1 DM and type 2 DM patients, and control group. No such differences were recorded for albumin concentration in type 2 DM patients. Comparison of type 1 DM and type 2 DM patients only yielded a statistically significant difference in albumin concentration (p=0.032). In the control group, correlations were observed between glucose and albumin concentration, between total protein and albumin concentration; and between glucose and albumin concentrations, and sex. In the group of patients with type 1 DM, a correlation was found between albumin concentration and sex. No correlation other than that between the type of DM and albumin concentration was observed.

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P7-6

Procjena promjena metaboličkih procesa ovisno o primjeni hipoglikemičnih lijekova

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P7-6

Assessment of changes in the metabolic pathway depending on hypoglycemic medicaments

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Bolesnici sa šećernom bolešću uglavnom uzimaju lijekove koji različito utječu na različite stadije metabolizma. Raznovrsni su podatci potakli ovo ispitivanje. Imena proizvođača se ne navode zbog etičkih razloga te razlika u pojedinačnim odgovorima. Ispitivanje je obuhvatilo bolesnike koji uzimaju ove lijekove radi kontrole glukoze u krvi i metabolizam. Pretrage su u bolesnika provedene prije i nakon liječenja kroz razdoblje od mjesec dana. Određivali smo parametre koje smatramo najvažnijima u smislu utjecaja terapije na metabolizam, tj. razine glukoze, triglicerida, kolesterola, HDL/LDL kolesterola, aktivnosti AST, ALT

Patients with diabetes mellitus (DM) are usually exposed to therapy with medicaments which have different effects on different metabolic stages. The study was prompted by the variety of data reported. The names of drug manufacturers are excluded for ethical reasons and because of the variable individual response. The study included DM patients on oral hypoglycemics for the control of blood glucose level and metabolism. DM patients were tested before and after therapy over a one-month period. In our opinion, the parameters estimated in the study are most relevant for the metabolic effect of this therapy. So, we

i GGT te razinu laktata u krvi. Ovi biokemijski parametri određivali su se na automatskom analizatoru Intergra 700 standardnom metodom. Rezultati izmjerenih parametara prije i nakon liječenja pokazali su različite učinke. Razina glukoze bila je očito povišena u većine bolesnika. U nekih su bolesnika razine lipidnih sastavnica bile promijenjene, uz različitu razinu statističke značajnosti. Aktivnosti AST, ALT i GGT su se snizile nakon terapije. Najzanimljiviji rezultat je bila povišena razina laktata nakon liječenja, utvrđena u većine bolesnika. Rezultati ove studije navode na zaključak kako svaki lijek izaziva različite učinke u svakog bolesnika. Laktat je proizvod metabolizma ugljikohidrata koji završava u citoplazmi, međutim, bez dokaza da je uključen u daljnje stadije metabolizma ili čini njegov dio. Povišene razine glukoze i laktata zabilježene kod naših bolesnika navode na pretpostavku da se uz ove lijekove proces glikolize nastavlja samo u anaerobnim uvjetima, bez konačne koristi za bolesnika.

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determined blood levels of glucose, triglycerides, cholesterol, HDL/LDL cholesterol, AST, ALT and GGT activities, and lactate. Biochemical parameters were determined on an automatic analyzer Intergra 700 using the standard procedure. Results obtained before and after treatment indicated different effects. Glucose level was overtly elevated in most patients. In some patients, the level of lipid components showed changes, with a varying statistical significance. The acivities of AST, ALT and GGT decreased upon therapy administration. The most interesting result was that the majority of patients showed increased lactate levels after treatment. Study results pointed to a conclusion that every medicament caused different effects in all patients. Lactate is a product of carbohydrate metabolism that ends in the cytoplasm, however, still with no evidence that it is included or a part of further metabolic stages. The increased levels of glucose and lactate in our patients suggested that on this kind of therapy, the process of glycolysis proceeds only in anaerobic conditions, without eventual benefit for the patient.

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P8 – Koštani metabolizam i bolesti, P8-1 (UP9-1)

Biljezi koštane pregradnje u terapiji osteoporoze selektivnim modulatorima estrogenskih receptora

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Osteoporoza je metabolička koštana bolest obilježena smanjenom mineralnom gustoćom, promjenama koštane mikroarhitekture i smanjenim biomehaničkim svojstvima kosti koje mogu imati za posljedicu prijelome i deformitete. Veliki je problem kod žena u postmenopauzi gdje nastaje kao posljedica nedostatka estrogena i loših životnih navika. Biokemijski biljezi u terapiji osteoporoze su od velike važnosti, jer promjena njihove koncentracije ili aktivnosti odražava dinamičko stanje koštanog metabolizma, a informaciju o odgovoru na terapiju možemo dobiti već nakon 3 mjeseca djelotvorne terapije. Cilj ovoga rada bio je ispitati terapijski odgovor biokemijskih biljega osteoporoze kod žena u postmenopauzi podvrgnutih terapiji selektivnim modulatorima estrogenskih receptora. Ispitane su 22 bolesnice u dobi od 52 do 76 godina. Na osnovi vrijednosti mineralne gustoće kosti kralježnice (L1-L4) i kuka mjerene metodom DEX postavljena im je

P8 – Bone metabolism and diseases, P8-1 (UP9-1)

Bone turnover markers in osteoporosis therapy with selective estrogen receptor modulators

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Osteoporosis is a metabolic bone disease characterized by decreased mineral density, microarchitectural deterioration of bone tissue, and decreased biomechanical properties of bone, which can lead to bone fractures and deformities. Osteoporosis is a big problem in postmenopausal women, where it occurs as the result of decline in estrogen concentration and unhealthy lifestyle.
Biochemical markers are very important in osteoporosis therapy because changes in their concentration or activity reflect the dynamic state of bone metabolism and can provide information on therapeutic response as early as at three months of therapy introduction. The aim of this study was to investigate therapeutic response of biochemical osteoporosis markers in postmenopausal women on therapy with selective estrogen receptor modulators. The study included 22 patients aged 52-76. Osteoporosis was diagnosed on the basis of bone mass density of the

dijagnoza osteoporoze. Uzorci krvi i druge jutarnje mokraće uzeti su prije terapije i tri mjeseca nakon terapije. Izmjerene su vrijednosti ALP (Olympus, Olympus AU 640), BAP (Metra, ETI-MAX 3000), osteokalcina (Metra, ETI-MAX 3000), beta-*crosslapsa* (Roche Modular E170) i TP1NP (Roche Modular E170) u serumu i DPD (Metra, ETI-MAX 3000) u drugoj jutarnjoj mokraći. Prosječno sniženje biljega bilo je: 5% za ALP, 38% za osteokalcin, 11% za beta-*crosslaps* i 21% za TP1NP, dok su BAP (2%) i DPD (38%) pokazali porast vrijednosti.
Osteokalcin i TP1NP su se pokazali pogodnim biljezima za praćenje odgovora na terapiju selektivnim modulatorima estrogenskih receptora u relativno kratkom vremenu, jer su pokazali statistički značajan (p=0,006 za osteokalcin i p=0,005 za TP1NP) pad vrijednosti već tri mjeseca nakon početka terapije.

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lumbar spine (L1-L4) and hip measured with DEX method. Blood samples and second morning urine sample were collected from patients before and three months after therapy initiation. Biochemical markers measured in serum samples were: ALP (Olympus, Olympus AU 640), BAP (Metra, ETI-MAX 3000), osteocalcin (Metra, ETI-MAX 3000), beta-crosslaps (Roche Modular E170) and TP1NP (Roche Modular E170), whereas DPD (Metra, ETI-MAX 3000) was determined in urine samples. The mean reduction in serum markers after three-month therapy was: 5% for ALP, 38% for osteocalcin, 11% for beta-crosslaps and 21% for TP1NP, whereas BAP (2%) and DPD (38%) showed increased values. Osteocalcin and TP1NP were found to be suitable markers for monitoring selective estrogen receptor modulator therapy in a relatively short time, because they showed a statistically significant (p=0.006 for osteocalcin and p=0.005 for TP1NP) decrease.

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P8-2 (UP9-2)

Tartarat-rezistentna kisela fosfataza i PINP u dijagnostici osteoporoze

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Koštani biljezi tartarat-rezistentna kisela fostfataza (TRAP) i PINP određeni su u serumima ispitanika s verificiranom osteoporozom i osteopenijom. S ozbirom na različite podatke u literaturi, željeli smo utvrditi imaju li navedeni koštani biljezi dijagnostičko značenje. Prva skupina ispitanika (n=60) imala je T-*score* mineralne gustoće kostiju (BMD) između -0,4 i -1,7, što prema kriterijima SZO odgovara smanjenoj koštanoj masi (osteopeniji). Druga skupina (n=60) imala je T-*score* BMD ne veci od -2.3, što se prema standardima SZO definira kao osteoporoza. Katalitična aktivnost TRAP je mjerena kinetičkom metodom uz upotrebu reagensa tvrtke BioMerieux na analizatoru Mira Cobas Plus. BMD je određen ultrazvučnom tehnikom, a koncentracija PINP je određena tehnikom ECL i reagensima tvrtke Roche na analizatoru Elecsys 2010. Kod ispitanika s osteopenijom dobivene su katalitične aktivnosti TRAP u serumu od 3,51±1.14 U/L, dok je aktivnost istoga enzima u skupini ispitanika s osteoporozom iznosila 5,49±1,63 U/L. Utvrđene razlike su statistički značajne (p<0,001). Razlike

P8-2 (UP9-2)

Tartarate-resistant acid phosphatase (TRAP) and N-terminal propeptide of type I procollagen (PINP) in the diagnosis of osteroporosis

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The bone markers tartrate resistant-acid phosphatase (TRAP) and N-terminal propeptide of I type procollagen (PINP) were measured in the sera of patients with confirmed osteoporosis and osteopenia. The aim of the study was to establish whether the two bone markers have a diagnostic usefulness, considering the discordant data found in the literature. In the first group of patients (n=60) bone mineral density (BMD) T-score was found to be between -0.4 and -1.7, defined by WHO as osteopenia. The BMD T-score in the second group was not above -2.3, defined by WHO as osteoporosis. The TRAP catalytic activity was measured by the kinetic BioMerieux method on a Mira Cobas Plus analyzer. BMD was determined by ultrasound technique. The PINP concentration was measured by the ECL technique using Roche reagents on an Elecsys 2010 analyzer. In patients with osteoporosis, serum TRAP catalytic activities were 3.51±1.14 U/L, while the activity of the same enzyme in the group of patients with osteoporosis was 5.49±1.63 U/L. The differences were

dobivenih koncentracija PINP u ispitanika s osteopenijom u odnosu na one kod ispitanika s osteoporozom nisu bile statistički značajne. Smatramo da bi određivanje katalitične aktivnosti TRAP u serumu, uz druge biokemijske biljege koštane pregradnje, unaprijedilo dijagnostički potencijal za osteoporozu. Potrebno je utvrditi vlastiti referentni raspon za PINP, jer su sve dobivene vrijednosti u objema skupinama ispitanika imale maksimalne vrijednosti znatno ispod gornje granice referentnog raspona koji je preporučila tvrtka Roche. Tek bi usporedbom s vlastitim referentnim vrijednostima bilo moguće donijeti ispravne zaključke o dijagnostičkom značenju određjivanja PINP u serumu.

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statistically significant ($p<0.001$). The differences in PINP concentrations between the two groups of patients were not statistically significant. We conclude that the TRAP catalytic activity in serum, along with other biochemical markers of bone turnover, could improve the diagnostic potential for osteoporosis. It is necessary to establish a reference range for PINP, as the results found in both patient groups had their maximum values by far below the maximum value of the reference range suggested by Roche. So, correct conclusions about the diagnostic significance of PINP determination in serum could only be made upon comparison with one's own reference values.

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P8-3

Analitička procjena P1NP – biokemijskog biljega koštane pregradnje

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Oko 90% koštanog matriksa koji sintetiziraju osteoblasti sastavljen je od kolagena tipa I. Sintetizira se u obliku predkursorske molekule prokolagena I koji sadrži N-(amino) i C-(karboksi) terminalnu produženu domenu. Prije sazrijevanja kolagenskih fibrila ovi tzv. N- i C-propeptidi se cijepaju specifičnim proteazama. Slobodni propeptidi kolaju krvlju i svjedoče o aktivnoj sintezi kolagena te se mogu definirati kao pravi biljezi koštane pregradnje. Cilj studije je bila analitička procjena amino-terminalnog propeptida tipa I prokolagena (PINP) elektrokemiliuminescentnom imunometodom, te usporedba rezultata sa svojstvima testa koje je deklarirao proizvođač. Analitička procjena izvedena je prema standardiziranom protokolu baziranom na konceptima dokumentacije ECCLS. Netočnost je određena određivanjem analita u kontrolnim uzorcima kroz 10 dana. Nepreciznost u seriji određena je na 30 uzastopnih mjerenja u dva skupna seruma. Nepreciznost iz dana u dan određena je mjerenjem koncentracije kontrolnih seruma u triplikatu kroz 10 dana. Ispitivane su interferencije hiperbilirubinemije, lipemije i hemolize. Odstupanja od kontrolnih uzoraka pokazala su zadovoljavajući stupanj točnosti, a mjerenja nisu ni u jednom mjerenju prelazila granicu od 1 SD. Kod ispitivanja nepreciznosti u seriji dobiveni su koeficijenti varijacije 2,14% i 2,31% (2,22%), a kod ispitivanja nepreciznosti iz dana u dan dobiveni su koeficijenti varijacije 1,44%, 1,71% i 2,04% (=1,73%). Ispitivanje

P8-3

Analytical evaluation of P1NP – a biochemical marker of bone turnover

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More than 90% of organic bone matrix consists of type I collagen. It is derived from type I procollagen synthesized by fibroblasts and osteoblasts. Type I procollagen contains both N-(amino) and C-(carboxy) terminal extensions which are removed by specific proteases during the conversion of procollagen to collagen and its subsequent incorporation into bone matrix. Therefore, these extensions are a specific indicator of type I collagen deposition and thus may be defined as a true bone formation marker. The aim of the study was analytical evaluation of the total procollagen 1 amino-terminal propeptide (P1NP) electrochemiluminescence immunoassay, and comparison of the results with the assay characteristics declared by the manufacturer. Analytical evaluation was performed according to the standardized protocol based on the concepts of ECCLS documents. Inaccuracy was tested using control material for 10 days. Intra-assay imprecision was tested on 30 replicates *per* analysis in two pool sera. Inter-assay imprecision was tested using control material in triplicate for 10 days. Interferences were investigated for hyperbilirubinemia, lipemia and hemolysis. Deviation from control specimens showed satisfactory accuracy, and the measurements did not exceed the limit of 1 SD. Imprecision studies yielded intra-assay CVs of 2.14% and 2.31% (2.22%) and inter-assay CVs of 1.44%, 1.71% and 2.04% (=1.73%). Inaccuracy showed bias from target val-

netočnosti pokazalo je odstupanje od ciljnih vrijednosti i to kod niskih koncentracija PINP iznosi 9,28%, kod srednje visokih koncentracija PINP postotak odstupanja iznosi 6,19%, a kod niskih koncentracija PINP postotak odstupanja iznosi 8,53%. Dobivene vrijednosti odstupanja od ciljnih vrijednosti prihvatljive su pri rutinskom određivanju PINP. Rezultati su pokazali da je pouzdan rezultat isključivo u diluciji 1:2. Odnos izmjerene i očekivane vrijednosti s velikom je proporcionalnom i konstantnom pogrješkom (a=-19,5 i 26,5). Dakle, dilucijom većom od 1:2 ne bismo mogli jednostavnim umnoškom s faktorom dilucije dobiti točan rezultat. Koncentracije bilirubina (<610 µmol/L), triglicerida (<15 µmol/L) te koncentracije hemoglobina (<1,8 mmol/L) ne interferiraju s rezultatima mjerenja. Dakle, nova elektrokemiluminescentna imunometoda tvrtke Roche za P1NP daje precizne i točne rezultate, uglavnom bez ujecaja interferencija. P1NP može poslužiti kao vrlo koristan dijagnostički parametar u procjeni pregradnje kosti kod bolesnika na hemodijalizi i nakon prijeloma kosti, te kao biljeg procjene terapije kod osteoporoze.

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P8-4

Oksidativni status u osteoporozi

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Osteoporoza je multifaktorni poremećaj uzrokovan smanjenjem koštane mase. Najviše pogađa starije ljude, a žene u postmenopauzi predstavljaju najrizičniju skupinu. Uzroci osteoporoze su različiti i uključuju utjecaj načina života, okoliša, ali i nasljednih čimbenika. Mnogi od spomenutih čimbenika mogu uzrokovati ili su uzrokovani oksidativnim stresom. Cilj ovoga rada bio je ispitati oksidativni status i elemente u tragovima kod žena u postmenopauzi s osteoporozom. Ispitane su 22 bolesnice u dobi od 52 do 76 godina kojima je na osnovi vrijednosti mineralne gustoće kosti kralježnice (L1-L4) i kuka mjerene metodom DEX dijagnosticirana osteoporoza. Bolesnicama su uzeti uzorci krvi u kojima su izmjerene vrijednosti ukupnog antioksidativnog statusa - TAS (Randox, Olympus AU 400), glutation reduktaze – GR (Randox, Olympus AU 400), cinka (Sentinel, Olympus AU 640) i bakra (Randox, Olympus AU 640). Kontrolnu skupinu je činilo 35 zdravih žena u dobi od 23 do 58 godina.
Rezultati su pokazali prosječno sniženje za TAS 16% (p<0,001) i cink 11% (p=0,035) u odnosu na kontrolnu sku-

P8-4

Oxidative status in osteoporosis

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Osteoporosis is multifactorial disease and is the result of the loss in skeletal mass. It mostly involves the elderly, with postmenopausal women being at the highest risk. A number of factors contribute to and cause osteoporosis, including the effects of lifestyle, environmental factors as well as genetic factors. Many of these can cause or are caused by oxidative stress. The aim of this study was to investigate oxidative status and trace elements in postmenopausal women with osteoporosis. The study included 22 female patients aged 52-76. Osteoporosis was diagnosed on the basis of bone mass density of the lumbar spine (L1-L4) and hip measured by DEX method. The parameters measured in serum were: total antioxidative status - TAS (Randox, Olympus AU 400), glutathione reductase - GR (Randox, Olympus AU 400), zinc (Sentinel, Olympus AU 640) and copper (Randox, Olympus AU 640). Control group consisted of 35 healthy women aged 23-58. Study results revealed a mean TAS decrease by 16% (p<0.001) and zinc decrease by 11% (p=0.035) as compared to control group, suggesting an impaired anti-

pinu, što ukazuje na oštećeni antioksidativni potencijal koji bi mogao doprinijeti staničnom oštećenju i posljedično razvoju osteoporoze.

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P8-5

Koncentracija specifične alkalne fosfataze (BAP) u bolesnica na terapiji metimazolom

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Hipertireoza utječe na pojačanu osteoblastičnu i osteoklastičnu aktivnost, što može dovesti do smanjenja mineralizacije kostiju i pojačanog metabolizma kosti. Klinički podaci su potvrdili da je koštana alkalna fosfataza (BAP) osjetljiv i pouzdan pokazatelj izgradnje kosti i rani pokazatelj osteoblastične diferencijacije. Cilj je bio istražiti utjecaj metimazolske terapije na razinu BAP u serumu bolesnica s hipertireozom, u kojih se zbog premenopauze ne očekuje poremećaj u koštanom metabolizmu ili pojava osteoporoze te usporediti serumske koncentracije BAP i tireotropina (TSH). U istraživanje je bilo uključeno 56 žena u premenopauzi (dob: 22-40 god.) koje su zbog hipertireoze bile na terapiji metimazolom (Athyrazole; doza:1,25-100 mg). Koncentracije BAP i TSH određene su imunometrijskim kompletima Ostase BAP EIA (IDS Ltd., Engleska) i Immulite Third Generation TSH (DPC, SAD). Koncentracija BAP određena je u 121 uzorku seruma 56 bolesnica koje se liječe zbog hipertireoze metimazolom i uspoređena s odgovarajućim vrijednostima TSH. Povišene koncentracije BAP nađene su u 70 (58%) uzoraka seruma, od kojih je u 50 (41%) vrijednost TSH bila izvan referentnog raspona. Povišen BAP i snižen TSH utvrđen je u 36 (51%) uzoraka, a istodobno povišeni BAP i TSH u 14 (20%) uzoraka, dok je povišeni BAP i uredan TSH nađen u 20 (29%) uzoraka seruma. U 18 bolesnica je tijekom praćenja (3-5 posjeta) BAP bio povišen u 38 od 70 (54%) seruma, od kojih je TSH bio snižen u 14 (37%), povišen u 9 (24%) i normalan u 15 (39%) uzoraka. Povišena razina BAP nađena je u 14 od 44 (32%) uzoraka seruma bolesnica koje su uzimale dozu metimazola manju od 10 mg, u 30 od 47 (64%) seruma bolesnica s dozom metimazola od 10-20 mg i u 24 od 28 (86%) uzoraka seruma bolesnica s dozom metimazola višom od 20 mg (p=0,0532, p=0,0076 odnosno p=0,0000). Izvedeni su slijedeći zaključci: 1. Hipertireoza pojačava koštani metabolizam i utječe na porast razine BAP u serumu; viša doza

P8-5

Concentration of serum bone specific alkaline phosphatase (BAP) in patients on methimazole therapy

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Hyperthyroidism is associated with enhanced osteoblastic and osteoclastic activity, and patients frequently have low bone mineral density and high bone turnover. Clinical data demonstrate that bone alkaline phosphatase (BAP) is a sensitive and reliable indicator of bone formation and an early marker of osteoblast differentiation. It reflects overall bone turnover when the bone resorption and formation processes remain coupled. The aim of the study was to evaluate the effect of the antithyroid drug methimazole on BAP concentration in serum of hyperthyroid premenopausal patients (in whom osteoporosis is not expected) and to compare BAP with thyrotropin (TSH) concentrations. The study included 56 premenopausal women (aged 22-40 yrs) with hyperthyroidism, on methimazole therapy (Athyrazole; dose range 1.25-100 mg). Immunometric assays for serum BAP and TSH determination were used: Ostase BAP EIA (IDS Ltd., UK) and Immulite Third Generation TSH (DPC, USA). BAP concentration was measured in 121 serum samples of 56 patients on methimazole therapy and these values were compared with the respective TSH values. Increased BAP was found in 70 (58%) samples of which 50 (41%) had TSH beyond the reference range. Increased BAP and decreased TSH were detected in 36 (51%) samples, increased BAP and TSH in 14 (20%) samples, and increased BAP and normal TSH in 20 (29%) samples. Eighteen patients were followed up on three to five occasions; BAP was increased in 38 (54%) of 70 serum samples. Of these, TSH was also increased in 9 (24%), decreased in 14 (37%) and normal in 15 (39%) samples. An increased BAP level was recorded in 14 of 44 (32%) samples of patients on a methimazole dose lower than 10 mg, in 30 of 47 (64%) samples of patients on a dose of 10-20 mg, and in 24 of 28 (86%) samples of patients with a dose higher than 20 mg (p=0,0532, p=0,0076 and p=0,0000, respectively). Study

metimazola korelirala je s većom proporcijom povišenih vrijednosti BAP, što je moguća posljedica jače aktivnosti bolesti u tih bolesnica; 2. Budući da su povišene koncentracije BAP zabilježene u velikom postotku bolesnica s urednim i povišenim vrijednostima TSH bilo bi uputno da se u bolesnica s hipertireozom mjeri neki od biljega odgovornih za izgradnju i razgradnju kosti i nakon postizanja zadovoljavajućeg kliničkog statusa hipertireoze.

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results pointed to the following conclusions: 1) hyperthyroidism speeds up bone turnover and causes elevation of BAP level; a higher dose of methimazole correlated with a higher proportion of increased BAP values. The increased bone turnover may persist in patients on methimazole therapy even after normalization of TSH values; and 2) as an increased BAP level was recorded in a significant number of samples with normal and elevated TSH concentration, it is necessary to measure some of the bone formation/absorption markers even after normalization of the clinical signs of hyperthyroidism in these patients.

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P8-6

Uloga biokemijskih biljega u dijagnostici osteoporoze

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Biokemijski biljezi koštane pregradnje su molekule koje izravno proizlaze iz strukture i funkcije koštanog tkiva. Iako nisu specifični za određenu bolest, njihovo je uvođenje u kliničku praksu značajno poboljšalo dijagnostički potencijal, pa primijenjeni zajedno s denzitometrijom kosti značajno olakšavaju odluku o tome kada početi liječenje. Cilj ovoga rada bio je ispitati neke biokemijske biljege u osteoporozi i usporediti ih s nalazom mineralne gustoće kosti kralježnice (BMD) (L1-L4) i kuka mjerene metodom DEX te tako procijeniti njihovo značenje u postavljanju dijagnoze. Ispitali smo 24 bolesnice u postmenopauzi u dobi od 52 do 76 godina. Svima je napravljena denzitometrija, a zatim su uzeti uzorci krvi u kojima su izmjerene vrijednosti osteokalcina (Metra, ETI-MAX 3000), TP1NP (Roche Modular E170) i beta-*crosslaps* (Roche Modular 170) u serumu, te uzorak druge jutarnje mokraće u kojem je izmjeren DPD (Metra, ETI-MAX 3000). Kontrolnu skupinu činilo je 35 zdravih žena u dobi od 23 do 58 godina. Prosječna BMD kosti kralježnice (L1-L4) bila je 0,746; T-vrijednost -2,7; Z-vrijednost -1,1; a kuka BMD 0,79; T-vrijednost -1,2; Z-vrijednost -0,09. Rezultati su pokazali statistički značajnu razliku u odnosu na kontrolnu skupinu za TP1NP (p=0,026) i beta-*crosslaps* (p=0,005), te su se oni pokazali dobrim i osjetljivim biljezima u procjeni rizika za osteoporozu.

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P8-6

The role of biochemical markers in the diagnosis of osteoporosis

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Biochemical markers of bone turnover are related primarily to bone structure and function. Although not related specific to any disease, they have significantly upgraded diagnostic potential in clinical practice. In combination with DEX, they are of great help to physician on deciding when to start therapy. The aim of this study was to investigate some biochemical markers in osteoporosis and to compare them to bone mass density (BMD) of lumbar spine (L1-L4) and hip measured with DEX method, thus evaluating their role in the diagnosis of osteoporosis. The study included 24 postmenopausal women aged 52-76. Bone mass densitometry was performed in all women. Blood samples and second morning urine were also collected. The following biochemical markers were measured in serum: osteocalcin (Metra, ETI-MAX 3000), beta-crosslaps (Roche Modular E170) and TP1NP (Roche Modular E170), while DPD (Metra, ETI-MAX 3000) was measured in urine samples.
Control group consisted of 35 healthy women aged 23-58. The mean BMD of lumbar spine (L1-L4) was 0.75; T-score -2.7; Z-score -1.1, and the mean BMD of hip was 0.79; T-score -1.2; Z-score -0.1.
Results showed a statistically significant difference between TP1NP (p=0.026) and beta-crosslaps (p=0.005) levels, so they seem to be good and sensitive markers to assess the risk of osteoporosis.

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P9 – Upala, P9-1

Plazmatske razine PMN-elastaze i neopterina u ranom stadiju sepse

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Makrofazi, limfociti i granulociti su glavne sastavnice staničnog obrambenog sustava. Razmjeri aktiviranja upalnog staničnog sustava pod septičnim uvjetima mogu se mjeriti za makrofage pomoću razina neopterina, a za granulocite pomoću razina PMN-elastaze u plazmi. Neopterin je proizvod puta tetrahidrobiopterina, što ga oslobađaju makrofazi potaknuti interferonom gama. Fiziološki zadatak PMN-elastaze je razgradnja fagocitoziranog materijala. Cilj ovoga ispitivanja je bio istražiti pomažu li neopterin i PMN-elastaza u razlikovanju između neseptičnih i septičnih bolesnika, te u njihovoj procjeni prema težini septikemije. Ispitano je ukupno 63 bolesnika (u dobi od 32-56 godina): 16 bez simptoma septikemije (kontrolna skupina), 33 sa septikemijom i 14 s teškom septikemijom. Razine u plazmi mjerene su kod prijma u bolnicu te nakon 24 i 72 sata. PMN-elastaza se je mjerila u uzorcima plazme s EDTA turbidimetrijskim imuno testom tvrke Merck na automatskom analizatoru Cobas Mira Plus. Razine neopterina mjerile su se kompetitivnim enzimskim imuno testom na mikrotitarskim trakama tvrtke Brahms. Apsorbancija na 405 nm očitavala se je na instrumentu Bio-Rad Microplate Reader 550. Rezultati za sve skupine u razdoblju od 72 sata od prijma u bolnicu bili su za PMN-elastazu 188±32 μg/L, 226±26 μg/L i 276±120 μg/L, a za neopterin 43±18 nmol/L, 66±13 nmol/L i 120±99 nmol/L. Cirkulirajuća PMN-elastaza nije izravno povezana s bijelom krvnom slikom i mogla je razlikovati kontrolnu skupinu i tešku septikemiju tijekom 72-satnog razdoblja promatranja nakon prijma. Neopterin je pokazao značajne razlike među skupinama u svakom razdoblju mjerenja. Najviše razine bile su udružene s lošijom prognozom u bolesnika sa septikemijom. Zaključujemo kako ova dva bijega predstavljaju korisne parametre za ranu dijagnostiku septikemije i motrenje kliničkog tijeka u septičnih bolesnika.

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P9 – Inflammation, P9-1

Plasma PMN-elastase and neopterin levels in the early stage of sepsis

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Macrophages, lymphocytes and granulocytes are the main components of the cellular defense system. The extent of activation of inflammatory cell system under septic conditions may be measured for macropheges by neopterin and for granulocytes by PMN-elastase plasma levels. Neopterin is a product of the tetrahydrobiopterin pathway, liberated by macrophages after an interferon gamma stimulus. The physiological task of PMN-Elastase is degradation of phagocytosed material. The aim of this study was to investigate if neopterin and PMN-elastase help differentiate between nonseptic and septic patients, and evaluate them with regard to the severity of septicemia. A total of 63 patients (aged 32-56) were tested: 16 without symptoms of septicemia (control group), 33 with septicemia, and 14 with severe septicemia. Plasma levels were measured on admission to the hospital and then after 24 and 72 hours. PMN-elastase was measured in EDTA- plasma samples by turbidimetric immunoassay system from Merck on an Cobas Mira Plus autoanalyzer. Neopterin level was determined by competitive enzyme immunoassay on microtiter strips from Brahms. Absorbance at 405 nm was read using a Bio-Rad Microplate Reader 550. The results for all groups during 72 hours of hospital admission were as follows: for PMN-Elastase 188±32 μg/L, 226±26 μg/L and 276±120 μg/L; and for neopterin 43±18 nmol/L, 66±13 nmol/L and 120±99 nmol/L, respectively. Circulating PMN-elastase is not directly related to WBC counts and could differentiate between the control group and severe septicemia in the observation period of 72 hours after admission. Neopterin showed significant differences between groups in each study period. Highest levels are associated with poorer prognosis in patients with septicemia. We conclude that the two markers are useful parameters for the early diagnosis of septicemia and monitoring of the clinical course in septic patients.

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P9-2

Utjecaj HDL-razgradnih proizvoda na gensku ekspresiju u endotelnim stanicama

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Smatra se kako je ateroskleroza kronični upalni odgovor na upalne medijatore podrijetlom iz lipida koji se nakupljaju na određenim mjestima u arterijama. Endotelna lipaza (EL) je fosfolipaza s HDL česticama kao preferiranim supstratom. Cilj rada bio je istražiti promjene u genskoj ekspresiji u uzgojenim endotelnim stanicama nakon tretmana HDL-razgradnim proizvodima dobivenim djelovanjem endotelne lipaze (smjesa EL-HDL). Pokusi su izvedeni na stanicama ECA – primarnoj staničnoj kulturi dobivenoj iz humane placentne arterije, stanicama EAHY – humanoj staničnoj liniji iz umbilikalne vene i stanicama HAEC – humanoj staničnoj liniji iz aorte. HDL-razgradni proizvodi dobiveni su inkubacijom frakcije HDL3 s EL-kondicioniranim medijem te kontrolnim medijem bez EL kroz 3 sata na 37 °C. Endotelne stanice potom su inkubirane 3 sata na 37 °C s EL-HDL ili kontrolnom smjesom. Ispitana je relativna genska ekspresija 16 gena pomoću reverzne transkripcije-PCR; pritom je beta-aktin služio za normalizaciju. Rezultati su pokazali povećanu ekspresiju proupalnih kemokina i njihovih receptora (interleukin-6, RANTES, TNFalfa receptor, folistatin), molekula upletenih u signalnu transdukciju (fosfolipaza A2 IV A, ciklooksigenaza-2), adhezijskih molekula (ICAM-1, VCAM-1, E-selektin), koagulacijskih faktora (tromboplastin) i izvanstaničnih proteaza (hepsin, ADAMTS1). Porast ekspresije ICAM-1, COX-2 i IL-6 vremenski je i koncentracijski ovisan. Stanice su potom istodobno tretirane različitim inhibitorima kako bi se istražila uključenost pojedinih signalni putova. U stanicama tretiranima sa SN50 (snažnim NF-kapaB inhibitorom) uočeno je blago smanjenje ekspresije COX-2 i VCAM-1 u usporedbi sa stanicama koje su tretirane samo smjesom EL-HDL. Stanice tretirane smjesom EL-HDL i kvercetinom (protu-upalnim flavonoidom) neočekivano su pokazale koncentracijski ovisnu povećanu ekspresiju COX-2, ICAM-1, IL-6 i E-selektina (za koncentracije kvercetina od 1, 10, 25 i 50 μM). Tretman N-acetil-cisteinom (hvatač slobodnih radikala) inhibirao je učinak HDL-razgradnih proizvoda na ekspresiju VCAM-1, E-selektina, IL-6, COX-2 i PLA2 IV A, što ukazuje na upletenost redoks-osjetljivog signalnog puta. Nadalje, SB202190 (selektivni inhibitor p38 MAP kinaze)

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Effect of HDL-degradation products on gene expression in endothelial cells

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It has been suggested that atherosclerosis is a chronic inflammatory response to lipid-derived inflammatory mediators accumulating at selected arterial sites. Endothelial lipase (EL) is a phospholipase with HDL as the preferred substrate. The aim of the study was to evaluate gene expression changes in cultured endothelial cells after treatment with EL-derived HDL-degradation products (EL-HDL mixture). Experiments were performed on ECA cells – primary cell culture obtained from human placental artery endothelial cells; EAHY cells – human umbilical vein endothelial cell line, and HAEC cells – human aortic endothelial cell line. To obtain HDL-degradation products, HDL3 fraction was incubated with EL-conditioned medium and control medium without EL for 3 hours at 37 °C. Cells were then incubated for 3 hours at 37 °C with EL-HDL or control mixture. Relative gene expression of 16 genes was assessed by Reverse Transcription-PCR with beta-actin serving as a normalization gene. Results showed an increased expression of proinflammatory chemokines and their receptors (interleukin-6, RANTES, TNFalpha receptor, follistatin), molecules involved in signal transduction (phospholipase A2 IV A, cyclooxygenase-2), adhesion molecules (ICAM-1, VCAM-1, E-selectin), coagulation factors (thromboplastin) and extracellular proteases (hepsin, ADAMTS1). The increase in ICAM-1, COX-2 and IL-6 was shown to be time- and concentration-dependent. To gain an insight into the possible signaling pathways involved, cells were cotreated with various inhibitors. Cells treated with SN50 (a potent NF-kappaB inhibitor) showed a slight decrease in gene expression of COX-2 and VCAM-1 as compared to cells treated only with EL-HDL mixture. Cells treated with EL-HDL mixture and quercetin (an anti-inflammatory flavonoid) unexpectedly revealed that quercetin (at concentrations of 1, 10, 25 and 50 μM) actually increased the expression of COX-2, ICAM-1, IL-6 and E-selectin in a dose-dependent manner. Treatment with N-acetyl-cysteine (a free radical scavenger) inhibited the upregulation of VCAM-1, E-selectin, IL-6, COX-2 and PLA2 IV A, indicating involvement of a redox sensitive pathway. Furthermore, SB202190 (a selective p38 MAP kinase

spriječio je povećanje ekspresije VCAM-1 i COX-2, ukazujući na aktivaciju i upletenost barem dva različita signalna puta. Endotelna lipaza oslobađa upalne lipidne medijatore, čemu svjedoči povećana razina ekspresije upalnih gena u endotelnim stanicama koje su bile izložene HDL-razgradnim proizvodima dobivenim djelovanjem endotelne lipaze.

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inhibitor) abolished the upregulation of VCAM-1 and COX-2, indicating activation and involvement of at least two different signaling pathways. Endothelial lipase liberates inflammatory lipid mediators as evidenced by the upregulation of inflammatory genes in endothelial cells exposed to EL-derived HDL-degradation products.

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P9-3

IL-1 beta, IL-6, IL-8, IL-10 i TNF-alfa u serumu shizofrenih bolesnika u akutnoj fazi poremećaja

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Shizofreni bolesnici u cerebrospinalnoj tekućini imaju izmijenjene omjere imunokompetentnih stanica i varirajuće koncentracije citokina, poglavito proupalnih IL-6, IL-1beta i TNF-alfa. Promjene citokina jednostavno mogu biti posljedica mentalnog stresa ili nesanice udružene s napadom ili pogoršanjem bolesti. Cilj rada bio je istražiti aktiviranje odgovora upalnog sustava i koncentracije citokina u serumu shizofrenih bolesnika bez terapije u akutnom pogoršanju bolesti. Za ovaj rad odabrana su 22 muškarca u akutnoj fazi shizofrenije bez terapije i 26 po godinama i spolu izabranih zdravih kontrolnih ispitanika koji nemaju psihijatrijski poremećaj i ne uzimaju lijekove. IL-1 beta, IL-6, IL-8, IL-10 i TNF-alfa određeni su u serumu metodom ELISA (R&D Systems). Nije nađena statistički značajna razlika između skupine bolesnika (P) i kontrolne skupine (C) za IL-1 beta (2,53±0,53 pg/mL) (P), (2,29±0,08 pg/mL) (C); IL-6 (2,90±0,53 pg/mL) (P), (1,95±0,18 pg/mL) (C); IL-8 (28,19±9,7 pg/mL) (P), (21,55±6,26 pg/mL) (C); IL-10 (5,40±0,26 pg/mL) (P), (5,45±0,84 pg/mL) (C), kao ni za TNF-alfa (13,43±0,46 pg/mL) (P), (9,47±0,58 pg/mL) (C) na razini statističke značajnosti $p<0,05$. Prema našim nalazima, koncentracije citokina u serumu shizofrenih bolesnika bez terapije u akutnom pogoršanju poremećaja nisu izmijenjene. Pretpostavljamo da, ako je stvaranje citokina u shizofreniji promijenjeno, te promjene ne mogu biti uočljive u sistemskoj cirkulaciji.

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P9-3

Serum IL-1 beta, IL-6, IL-8, IL-10 and TNF-alpha in acute schizophrenic patients

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Schizophrenic patients have deranged proportions of immunocompetent cells and varying levels of cytokines, especially proinflammatory IL-6, IL-1beta and TNF-alpha in cerebrospinal fluid. Alterations in cytokine levels can simply be the consequence of mental stress or sleep deprivation associated with the episode or exacerbation of the disease. The aim of this study was to investigate the activation of the inflammatory response system and serum cytokine levels in drug-free schizophrenic patients in acute exacerbation of the disorder. Twenty-two drug-free schizophrenic males in exacerbation of the disorder and 26 age- and sex-matched healthy controls without psychiatric disorders and medication were recruited for the study. Serum IL-1 beta, IL-6, IL-8, IL-10 and TNF-alpha were determined by ELISA method (R&D Systems). No statistically significant differences were found betwen the patient (P) and control group (C) according to serum levels of IL-1 beta (2.53±0.53 pg/mL) (P), (2.29±0.08 pg/mL) (C); IL-6 (2.90±0.53 pg/mL) (P), (1.95±0.18 pg/mL) (C); IL-8 (28.19±9.7 pg/mL) (P), (21.55±6.26 pg/mL) (C); IL-10 (5.40±0.26 pg/mL) (P), (5.45±0.84 pg/mL) (C) and TNF-alpha (13.43±0.46 pg/mL) (P), (9.47±0.58 pg/mL) (C) at $p<0.05$. According to our results, serum initial cytokine concentrations are not altered in drug-free schizophrenic patients in exacerbation of the disorder. We suggest that, if cytokine production is altered in schizophrenic patients, these alterations may not be detectable in systemic circulation.

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P9-4

Glikozilacija u akutnom upalnom odgovoru

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Akutni upalni odgovor je niz staničnih i molekularnih događaja koji se javljaju kao reakcija na različite podražaje. Iako je akutni upalni odgovor jedinstven homeostatski mehanizam, razlike u proizvodnji medijatora i tijeku samog odgovora postoje u različitim patofiziološkim stanjima ovisno o naravi i mjestu upale. U našem radu ispitali smo promjene glikana otpuštenih sa serumskih glikoproteina bolesnika sa sepsom i bolesnika s akutnim pankreatitisom i usporedili ih sa zdravim pojedincem.
Serumi bolesnika uzimani su prilikom javljanja u bolnicu i zatim još tri puta tijekom prvih osam dana hospitalizacije. Kontrolni serum odgovarajuće dobi i spola uzet je samo jednom. Glikani su sa serumskih proteina otpušteni N glikozidazom F, enzimom koji specifično otpušta N vezane glikane i analizirani tekućinskom kromatografijom visoke djelotvornosti u kombinaciji s digestijom egzoglikozidazama. Prisutne glikanske strukture su identificirane, praćena je njihova promjena tijekom bolesti te su uspoređene s onima kontrolnog seruma. Naši rezultati pokazuju da se promjene serumskih glikana javljaju vrlo rano u akutnoj upali. Razine pojedinih struktura mijenjaju se na dnevnoj osnovi, neke u istom smjeru, dok neke variraju kroz dane. Ove promjene su složene i primjećujemo ih u tri- i tetrasijaliniziranim strukturama, manoznim strukturama, razini fukozilacije i stupnju razgranatosti. Promjene su prisutne već u prvom danu bolesti uspoređujući s kontrolnim serumom.
Složene promjene glikana tijekom akutnog upalnog odgovora nisu iznenađujuće ako uzmemo u obzir složenost akutnog upalnog odgovora te važnu ulogu glikana u mnogim procesima. Ove promjene mogu biti dio regulatornog mehanizma tijekom upale, jer postoje indikacije da manoza i fukoza sudjeluju u imunomodulaciji uz potencijalno dobrohotne učinke. Razlike pokazane u bolesnika sa sepsom u odnosu na onog s akutnim pankretitisom vjerojatno proizlaze iz činjenice da je akutni upalni odgovor izazvan različitim stimulusom i stoga ima različit obrazac proizvodnje citokina.

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P9-4

Glycosylation in acute inflammatory response

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Acute inflammatory response is a sequence of cellular and molecular events as a reaction to different stimuli. Although acute phase response is a unique homeostatic mechanism, differences in the patterns of mediator production and in acute phase response are present in different pathophysiological conditions depending on the nature and site of inflammation. In this work we observed changes in the pattern of glycans released from serum glycoproteins of a patient with sepsis and patient with acute pancreatitis during the first eight days of disease, and compared it with the glycans released from normal serum. Sera from the septic patient and patient with acute pancreatitis were obtained at the time of admission and then on three occasions during the first eight days of hospital stay. Patients claimed to have presented to the hospital on the first day of feeling ill, so we assumed it to be the first day of disease. Blood sampling from a healthy age- and sex-matched individual was performed on a single occasion. Serum glycans were released using N glycosidase F, an enzyme that specifically removes N linked glycans, and subjected to normal phase high performance liquid chromatography combined with exoglycosidase digestion. The glycan structures present in the sera were identified and their levels followed during the course of disease and compared to that released from healthy control. Our results show that changes of serum glycans occur very early in acute inflammation. The proportions of different glycans are changing daily, some in the same direction while others vary through days. These changes are complex and can be observed in tri- and tetrasialylated structures, mannose structures, level of fucosylation, and degree of branching. The changes are present as early as the first day of disease as compared to control. The complex changes in glycans during acute inflammation are not surprising, knowing how complex and diverse acute phase response is and in how many different processes glycans play important roles.
These changes can be part of regulatory processes during inflammation since it has been shown that mannose and fucose participate in immunomodulation, presumably having beneficial effects. The differences found between sepsis and acute pancreatitis were probably due to the fact that the acute phase response is triggered by a different stimulus, thus having different patterns of specific cytokine production.

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Identifikacija antinuklearnih antitijela pomoću imuno testa

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Antinuklearna antitijela (ANA) su klinički važni indikatori kolagenske bolesti. Pretraga na antinuklearna antitijela indicirana je kao pomoć u dijagnostici sustavne autoimune bolesti u području bolesti vezivnog tkiva poput SLE, MCTD, skleroderme, sindroma CREST i sindroma preklapanja. Hep2ANA EIA služi kao test probira prvoga izbora za isključivanje prisutnosti aktivne sustavne autoimune bolesti s visokom vjerojatnošću. Cobas Core Hep2ANA EIA je indirektni enzimski imuno test koji se provodi u dvije faze za kvalitativno određivanje antinuklearnih antitijela. Kad je rezultat ove pretrage pozitivan, bolesnika treba podvrgnuti diferencijacijskoj obradi kako bi se rasvijetlila specifičnost autoantitijela. Klinička osjetljivost testa Cobas Core Hep2ANA EIA bila je 79,8%, a klinička specifičnost 91,7%. Rezultate treba uvijek tumačiti u kontekstu kliničke slike dotičnoga bolesnika. Rezultati dobiveni ovom pretragom služe kao pomoć u dijagnostici sustavne autoimune bolesti.

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P9-5

Identification of antinuclear antibodies using an immunoassay system

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Antinuclear antibodies (ANA) are clinically important indicators of collagen diseases. A test for antinuclear antibodies is indicated as an aid in the diagnosis of systemic autoimmune disease in the area of connective tissue diseases like SLE, MCTD, scleroderma, CREST syndrome and overlap syndrome. The HEp2ANA EIA serves as a first line screening test to exclude with high probability the presence of an active systemic autoimmune disease. The Cobas Core HEp2ANA EIA is an indirect two-step enzyme immunoassay for qualitative detection of antinuclear antibodies. When the test result is positive, the patient should be submitted to differentiation testing to elucidate the specificity of the autoantibodies. The clinical sensitivity of the Cobas Core HEp2ANA EIA was 79.8%, and the clinical specificity 91.7%. The results should always be interpreted in the context of the patient's clinical picture. Results obtained with this assay are used as an aid in the diagnosis of systemic autoimmune diseases.

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P9-6

Enzim konverzije angiotenzina u sarkoidozi

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Određivanje aktivnosti enzima za konverziju angiotenzina (ACE) u serumu veoma je važno u diferencijalnoj dijagnostici i praćenju progresije sarkoidoze, kao i za praćenje i prilagodbu terapije. Cilj studije bio je ispitati ulogu ACE i utjecaj spola na aktivnost ovoga enzima kod bolesnika sa sarkoidozom. U studiju smo uključili 40 bolesnika sa sarkoidozom liječenih na Odjelu za pulmoalergologiju Sveučilišnog centra u Skopju. Kontrolnu skupinu činilo je 40 zdravih davatelja krvi. Aktivnost ACE određena je spektrofotometrijskom metodom Trinity (Biotech) na biokemij-

P9-6

Angiotensin converting enzyme of sarcoidosis

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Determination of angiotensin converting enzyme (ACE) activity in serum is of great significance for the differential diagnosis and monitoring of progression of sarcoidosis as well as to follow the course and adjustment of therapy. The aim of the study was to investigated the role of ACE and the impact of sex on this enzyme activity in sarcoidosis patients. The study included 40 patients with sarcoidosis, treated at Department of Pulmoallergology, Skopje University Clinical Center. Control group consisted of 40 healthy blood donors. ACE activity was detected by

skom analizatoru Cobas Mira. Statistička analiza dobivenih rezultata za aktivnost ACE u odnosu na spol (p<0,05) nije dovela do značajnih i čvrstih zaključaka. Razine aktivnosti ACE bile su značajno više u bolesnika sa sarkoidozom negoli u kontrolnoj skupini davatelja krvi (51,3±23,25 U/L prema 19,0±7,42 U/L; p<0,005). Dakle, aktivnost ACE pokazala je pozitivnu korelaciju sa sarkoidozom i njezinom progresijom, pa predstavlja dobar prognostički biljeg za ovu bolest. Korelacija aktivnosti ACE sa spolom nije bila statistički značajna.

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Trinity (Biotech) spectrophotometric method on a Cobas Mira biochemical analyzer. Statistical analysis of the results obtained on ACE activity in relation to sex (p>0.05) did not lead to any significant and firm conclusion. Significantly higher levels of ACE activity were obtained in sarcoidosis patiens (51.3±23.25 U/L) as compared to the control group of blood donors (51.3±23.25 U/L *vs.* 19.0±7.42 U/L; p<0.005). Accordingly, elevated ACE activity showed positive correlation with sarcoidosis and its progression, and is a good prognostic marker for this disease. The correlation of ACE activity and sex was not statistically significant.

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P9-7

Biljezi upale u prijevremenom porodu

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Smatra se da intrauterina infekcija ima ključnu ulogu u patogenezi jedne trećine prijevremenih poroda. Ako je prisutna, prati ju niz prijenatalnih i neonatalnih komplikacija od kojih je najteža periventrikularna leukomalacija (PVL). Subklinička infekcija dokazana je u 25% trudnoća s prijevremenim porodom. Dijagnozu je često teško postaviti, jer nema odgovarajućih točnih dijagnostičkih testova. Svrha ovoga rada bila je utvrditi mogu li vrijednosti interleukina-6 (IL-6), interleukina-1 beta (IL-1 beta) i C-reaktivnog proteina (CRP) poslužiti kao prognostički biljezi konatalne infekcije i oštećenja mozga novorođenčeta. U 47 trudnica s prijevremenim porodom uzorci krvi uzeti su kod prijma u Kliniku i tijekom prijevremenog poroda. Kontrolnu skupinu činilo je 20 zdravih trudnica bez komplikacija. IL-6 i IL-1 beta određivani su enzimskom imunokemijskom metodom, a CRP visokoosjetljivom imunoturbidimetrijskom metodom. Uobičajenim statističkim metodama analizirane su razlike u vrijednostima triju navedenih biljega između trudnoća s prijevremenim porodom i kontrolne skupine te između trudnica koje su rodile zdravu novorođenčad i onih kod čije je novorođenčadi utvrđena konatalna infekcija i PVL. Pomoću krivulja ROC utvrđene su granične vrijednosti predviđanja konatalne infekcije i PVL

P9-7

Inflammation markers in preterm labor

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Intrauterine infection is supposed to play a key role in the pathogenesis of about one third of preterm labors. If present, it may be followed by serious neonatal complications, with periventricular leukomalacia (PVL) as the most deleterious one. Subclinical infection has been demonstrated in about 25% of patients with preterm labor. The diagnosis is sometimes very difficult and often hampered by the lack of an accurate diagnostic test. The purpose of the study was to estimate whether maternal serum interleukin-6 (IL-6), interleukin-1 beta (IL-1beta) and C-reactive protein (CRP) could be used as markers of perinatal infection and possible neonatal brain damage. Forty-seven maternal blood samples were taken at admission for preterm labor and during the preterm delivery. Control group consisted of 20 gravidas with normal pregnancy. IL-6 and IL-1beta were determined by enzymatic immuno assay, and CRP by highly sensitive immunoturbidimetric method. Differences in maternal blood parameters between preterm labor and control group, and between those delivering healthy newborns and those delivering newborns that developed perinatal infection or PVL were analyzed. Receiver operating characteristic (ROC) curves were constructed to determine cut-off values for reli-

za ispitivane parametre. Sva tri parametra bila su značajno povišena u trudnica koje su prijevremeno rodile u odnosu na kontrolnu skupinu s još izraženijim razlikama koncentracija u prisutnosti konatalne infekcije i PVL. Naši rezultati pokazuju kako IL-6, IL-1 beta i CRP daju vrijednu informaciju o riziku od infekcijom uzrokovanog prijevremenog poroda, fetalne infekcije i nastanka PVL još prije poroda.

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able prediction of perinatal infection and PVL. All three parameters were significantly higher in patients with premature delivery than in control patients with term delivery, with even more pronounced differences in the rate of perinatal infection and PVL. Our results indicate that maternal IL-6, IL-1 beta and CRP levels provide information on the risk of infection-complicated preterm labor, fetal infection and PVL before birth.

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P9-8

Koncentracija TNF-alfa, CXCL8, velikog ET-1 i hsCRP kod zdravih nepušača, pušača i bolesnika s KOPB

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Kronična opstrukcijska plućna bolest (KOPB) je progresivna kronična upalna bolest obilježena smanjenjem protoka zraka kroz dišne putove koje nije potpuno reverzibilno. Premda patološki mehanizmi razvoja KOPB još nisu potpuno jasni, poznato je da različite stanice u plućima oslobađaju brojne upalne medijatore. TNF-alfa (čimbenik tumorske nekroze-alfa), oslobađanje kojega izaziva duhanski dim, ključni je proupalni citokin u patogenezi KOPB. TNF-alfa aktivira transkripcijske čimbenike i potiče transkripciju gena za citokine, kemokine, proteaze i endoteline. CXCL8 (interleukin-8) je kemokin koji privlači neutrofile u upalno područje. ET-1 (endotelin-1) i veliki ET-1 doprinose plućnoj vazokonstrikciji i plućnoj hipertenziji u KOPB. hsCRP (visoko osjetljivi C-reaktivni protein) je protein akutne faze povišen u upalnim bolestima. Cilj istraživanja bio je ispitati koncentracije i međusobnu korelaciju TNF-alfa, CXCL8, velikog ET-1 i hsCRP kod zdravih nepušača, zdravih pušača i bolesnika s KOPB. Koncentracije TNF-alfa, CXCL8 i velikog ET-1 određene su u plazmi kod zdravih nepušača (n=23, kontrolna skupina), zdravih pušača (n=30) i bolesnika s KOPB (n=30) gotovim ELISA test paketom, a koncentracija hsCRP u serumu nefelometrijskom metodom. Koncentracija hsCRP kod bolesnika s KOPB bila je značajno povećana u odnosu na vrijednosti kontrolne skupine (p=0,0004), dok koncentracije TNF-alfa (p=0,0788), CXCL8 (p=0,4839) i velikog ET-1 (p=0,3641)

P9-8

Concentrations of TNF-alpha, CXCL8, big ET-1 and hsCRP in healthy non-smokers, smokers and COPD patients

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COPD (chronic obstructive pulmonary disease) is a chronic progressive inflammatory disease characterized by limitations in lung airflow that is not fully reversible. Although the pathophysiology of COPD is not understood completely, it is well known that various cells in the lungs are releasing inflammatory mediators. Tobacco smoke induces the release of a proinflammatory cytokine TNF-alpha (tumor necrosis factor-alpha) that might play a key role in COPD. TNF-alpha activates certain transcription factors and switches on the transcription of genes for cytokines, chemokines, proteases and endothelins. CXCL8 (interleukin-8) is a chemokine that attracts neutrophils to the inflammatory site. ET-1 (endothelin-1) and big ET-1 contribute to pulmonary vasoconstriction and pulmonary hypertension in COPD patients. hsCRP (highly sensitivity C-reactive protein) is an acute phase protein elevated in inflammatory diseases. The aim of the study was to investigate the concentrations and correlation of TNF-alpha, CXCL8, big ET-1 and hsCRP in healthy non-smokers, healthy smokers and patients with COPD. The concentration of TNF-alpha and CXCL8 in serum and big ET-1 in plasma were measured with an ELISA test in healthy non-smokers (n=23, control group), healthy smokers (n=30) and COPD patients (n=30). hsCRP was measured in serum nephelometrically.
The concentration of hsCRP was significantly higher in

nisu bile značajno promijenjene. Koncentracije svih analita u uzorcima pušača također se nisu značajno razlikovale od vrijednosti u kontrolnoj skupini. Spearmanov koeficijent korelacije za koncentracije TNF-alfa i CXCL8 iznosio je r= 0,638 (p<0,0001). Značajno povećanje hsCRP ustanovljeno je u skupini bolesnika s KOPB, što govori u prilog upalnom značaju bolesti. Na temelju naših rezultata zaključujemo da je hsCRP osjetljiviji dijagnostički pokazatelj od TNF-alfa, CXCL8 i velikog ET-1 u sistemskoj cirkulaciji bolesnika s KOPB.

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patients with COPD than in the control group (p=0.0004) but the concentrations of TNF-alpha (p=0.0788), CXCL8 (p=0.4839) and big ET-1 (p=0.3641) were not statistically different. There was no significant difference in the measured analytes between smokers and control group. Spearman coefficient of correlation between the concentrations of TNF-alpha and CXCL8 was r=0.638 (p<0.0001). hsCRP was higher in COPD patients, which is in agreement with the fact that COPD is an inflammatory disease. hsCRP proved to be a more sensitive diagnostic parameter than TNF-alpha, CXCL8 and big ET-1 in the systemic circulation in patients with COPD.

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P9-9

Poremećaji unutarstaničnih signalnih putova kod bolesnika s kroničnom opstrukcijskom bolesti pluća

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Kronična opstrukcijska plućna bolest (KOPB) predstavlja svjetski zdravstveni problem i predviđa se da će postati trećim uzrokom smrti do 2020. godine. Kroničan i progresivan tijek KOPB povezuje se s razvojem lokalne i sistemske upale i s oksidacijskim stresom. Smatra se da je pušenje jedan od glavnih uzročnika KOPB. Oksidansi iz dima cigareta mogu izravno potaknuti upalni odgovor djelujući na nekoliko na redoks osjetljivih signalnih molekula poput proteinskih kinaza aktiviranih mitogenima (MAPK), proteina toplinskoga šoka (Hsp) i Bcl-2 proteina. Cilj ovog istraživanja bio je ispitati razinu ekspresije i aktivaciju MAPK (ERK, JNK, p38) te razinu ekspresije Hsp (Hsp70, Hsp27), Bcl-2 i Bax proteina u leukocitima bolesnika s KOPB (n=26) i zdravih dobrovoljaca (n=43). Bolesnici i kontrolne osobe su podijeljeni u 3 skupine: pušači, bivši pušači i nepušači. Svi su ispitanici bili muškarci prosječne životne dobi između 45 i 72 godine. Razine ekspresije MAPK nisu se promijenile ovisno o zdravstvenom stanju i o tome je li dotična osoba pušač ili ne. Ipak, razine ekspresije Hsp, Bcl-2 i Bax proteina, kao i aktivacija MAPK bili su ovisni o tim parametrima. Za razliku od zdravih nepušača, kinaza koja potiče preživljavanje stanica (ERK) nije se aktivirala kod pušača niti kod bivših pušača (s KOPB ili zdravih). S druge strane,

P9-9

Deregulation of intracellular signaling pathways in patients with chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a major global health problem and has been anticipated to become the third leading cause of death by the year 2020. Teh chronic and progressive course of COPD is associated with development of local and systemic inflammation, and with oxidative stress. Cigarette smoking is the crucial factor responsible for COPD. Oxidants found in cigarette smoke can act as direct messengers to propagate the inflammatory response through several redox-sensitive signaling molecules such as mitogen-activated protein kinases (MAPKs), heat shock proteins (Hsps) and Bcl-2 proteins. The aim of the study was to assess the expression and activation of MAPKs (ERK, JNK, p38), and the expression of Hsps (Hsp70, Hsp27), Bcl-2 and Bax in the leukocytes of COPD patients (n=26) and healthy volunteers (n=43). Both patients and controls were subdivided into 3 groups: smokers, ex-smokers and non-smokers. They all were men aged between 45 and 72 years. MAPK expression was unchanged regardless of health or smoking status. However, the expression of Hsps, Bcl-2 and Bax proteins as well as the activation of MAPKs were dependent on these parameters. Survival-enhancing ERK was not activated in COPD or healthy smokers and ex-smok-

kod pušača s KOPB snažno je aktivirana fosforilacija stresnih kinaza (JNK i p38), dok je intenzitet signala bio nešto slabiji kod bivših pušača s KOPB i zdravih pušača. Razine ekspresije Hsp i Bcl-2 snažno su potisnute kod pušača s KOPB, a nešto manje kod bivših pušača s KOPB i zdravih pušača. Suprotno tomu, razina ekspresije Bax inducirana je kod svih osoba s KOPB (osobito kod pušača s KOPB) i kod zdravih pušača. Rezultati ukazuju na to da KOPB djeluje na unutarstanične signalne putove. Ipak, najznačajnije su promjene primijećene kod pušača, neovisno o njihovom zdravstvenom stanju. Potrebna su daljnja istraživanja molekularnih mehanizama KOPB kako bi se razvili novi terapijski pristupi u liječenju ove bolesti.

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ers, as compared with healthy non-smokers. On the other hand, phosphorylation of stress kinases (JNK and p38) was strongly induced in COPD smokers, while the signal intensity slightly decreased in COPD ex-smokers and healthy smokers. Expression of Hsps and Bcl-2 was significantly reduced in COPD smokers and to a lesser extent in COPD ex-smokers and healthy smokers. In contrast, Bax expression was up-regulated in all COPD patients (especially in COPD smokers) and in healthy smokers. These results show that COPD affects intracellular signaling pathways. However, the most distinguished changes were observed in smokers regardless of their health status. Additional research into the basic cellular and molecular mechanisms of COPD is needed for development of new therapies for the disease.

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P9-10

Visoko osjetljivi C-reaktivni protein i serumski amiloid A u bolesnika s meningitisom

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Brza i točna dijagnoza infektivnih bolesti središnjega živčanog sustava (SŽS) je važna za pravodobnu terapiju. Obim oštećenja krvno-moždane barijere u bolestima SŽS određuje sadržaj proteina u cerebrospinalnoj tekućini (CSF). Visoko osjetljivi C-reaktivni protein (hsCRP) i serumski amiloid A (SAA) su proteini akutne faze jako povišeni u bakterijskim, a umjereno u virusnim infektivnim bolestima. Veličina i struktura ovih proteina ukazuje na to da bi ovi proteini mogli proći kroz krvno-moždanu barijeru u bolestima SŽS. Cilj studije bio je ispitati koncentracije hsCRP i SAA u serumu i CSF kao potencijalno korisnih pokazatelja oštećenja krvno-moždane barijere te u diferencijalnoj dijagnostici bakterijskih i virusnih meningitisa. Izmjerene su koncentracije SAA i hsCRP u serumu i CSF bolesnika s bakterijskim (n=11) i virusnim (n=13) meningitisom. Koncentracije albumina, IgG, IgA i IgM su izmjerene u serumu i CSF za procjenu funkcije krvno-moždane barijere. Svi analiti su izmjereni nefelometrijski. Koncentracije hsCRP (p<0,001) i SAA (p<0,01) u serumu bile su značajno veće u bolesnika s bakterijskim meningitisom. Koeficijent korelacije za hsCRP i SAA u serumu iznosio je r=0,944 (p<0,0001). U CSF bolesnika s bakterijskim menin-

P9-10

High sensitive C-reactive protein and serum amyloid A protein in patients with meningitis

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Rapid and accurate diagnosis of infectious diseases of the central nervous system (CNS) is important for proper treatment. The level of blood-brain-barrier (BBB) impairment in CNS diseases determines protein constituents in cerebrospinal fluid (CSF). High sensitivity C-reactive protein (hsCRP) and serum amyloid A protein (SAA) are acute phase proteins that are increased in bacterial and moderate in viral infectious diseases. The molecular size and structure of these proteins indicate that they could pass through the BBB and help in the differential diagnosis of meningitis. hsCRP and SAA were determined as potentially useful indicators of damaged BBB and also for the differential diagnosis of bacterial and viral meningitis. We measured the concentration of hsCRP and SAA in serum and CSF of patients with bacterial (n=11) and viral (n=13) meningitis. Albumin, IgG, IgA and IgM were determined to assess the BBB dysfunction. All analytes were determined nephelometrically. Serum concentrations of hsCRP (p<0.001) and SAA (p<0.01) were significantlly higher in patients with bacterial than in those with viral meningitis. The correlation coefficient for hsCRP and SAA in serum was r=0.944 (p<0.0001). CSF hsCRP (10/13) and

gitisom nađene su povišene koncentracije hsCRP (10/13) i SAA (5/13), dok je u bolesnika s virusnim meningitisom to povećanje bilo manje učestalo, hsCRP (3/13) i SAA (1/13). Disfunkcija krvno-moždane barijere bila je prisutna u bolesnika s virusnim (11/13) i u onih s bakterijskim meningitisom (11/11), s intratekalne sintezom imunoglobulina ili bez nje. Koncentracija hsCRP i SAA u CSF ovisi o koncentraciji tih proteina u serumu te o strukturi i veličini molekule. Disfunkcija krvno-moždane barijere bila je prisutna u svih bolesnika koji su imali povećane koncentracije hsCRP i SAA u CST. hsCRP i SAA imaju potencijalnu ulogu u procjeni disfunkcije krvno-moždane barijere, kao i u diferencijalnoj dijagnostici bakterijskog i virusnog meningitisa.

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SAA (5/13) were increased in patients with bacterial meningitis. In patients with viral meningitis this increase was less frequently recorded: hsCRP (3/13) and SAA (1/13). BBB dysfunction was present in patients with viral (11/13) and bacterial (11/11) meningitis, with or without intrathecal synthesis of immunoglobulin class. The concentrations of hsCRP and SAA in CSF depend on the concentration in the blood, their molecular size and structure. BBB dysfunction was present in all patients with increased hsCRP and SAA in CSF. With the severity of BBB damage larger proteins pass to CSF, so hsCRP and SAA seem to be useful in the assessment of BBB dysfunction as well as in the differential diagnosis of bacterial and viral meningitis.

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P10 – Cerebrovaskularne bolesti, P10-1

Utjecaj Holeste na serumske razine lipoproteina(a) i homocisteina u bolesnika s primarnom hiperlipidemijom

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Povišene serumske razine lipoproteina(a) (Lpa)) i hiperhomocisteinemija smatraju se novisnim čimbenicima rizika za kardiovaskularne bolesti. Nema više podataka o učincima nedavno uvedenog statina holesta na Lp(a) i homocistein. Cilj studije bio je ispitati učinke statina Holeste na serumske razine lipida, Lp(a) i ukupnog homocisteina (tHcy) u bolesnika s primarnom hiperlipidemijom. Skupina od 32 bolesnika (20 žena i 12 muškaraca) s primarnom hiperlipidemijom praćeno je bazalno i 6 puta kroz 6 mjeseci mjerenjem Lp(a) (prijelomna vrijednost <30 mg/dL) i tHcy (prijelomna vrijednost <10 mmol/L). Serumske razine Lp(a) mjerene su imunoturbidimetrijskom metodom, a tHcy testom Abbott AxSYM. Doze terapije holestom iznosile su 40 mg, 20 mg i 10 mg, ovisno o razinama kolesterola. Dobiveni rezultati nisu pokazali statistički značajnih razlika ni bazalno ni nakon terapije: Lp(a) mg/dL 108,6±72,4 prema 99,8±76,5 i tHCYmmol/L 17,8±3,3 prema 15,7±3,1 (p>0,05 oba). Utvrđena je statistički visoka korelacija između koncentracije Lp(a) bazalno i nakon 6 mjeseci: r=0,936, p<0,0001 i tHCy r=0,896, p<0,001. Liječenje holestom dovelo je do značajnog sniženja ukupnog kolesterola mmol/L (8,64±4,22 prema 4,11±1,03), LDL-kolesterola mmol/L (5,62±4,32 prema 2,89±1,32) i apolipoproteina B

P10 – Cerebrovascular diseases, P10-1

Effect of Hollesta on serum lipoprotein(a) and homocysteine levels in patients with primary hyperlipidemia

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Elevated levels of serum lipoprotein(a) (Lp(a)) and hyperhomocysteinemia are regarded as independent risk factors for cardiovascular disease. There is no more information on the effects of the recently introduced Hollesta (a statin) on Lp(a) and homocysteine. The aim of the study was to investigate the effects of hollesta on serum levels of lipids, Lp(a) and total homocysteine (tHcy) in patients with primary hyperlipidemia. A group of 32 patients (20 women and 12 men) with primary hiperlipidemia were monitored at baseline and 6 times within 6 months, with measurement of: Lp(a) cut-off <30 mg/dL and tHcy cut-off <10 mmol/L Serum levels of Lp(a) were measured by immunoturbidimetric method and tHcy by Abbott AxSYM assay. The doses of hollesta therapy were 40 mg, 20 mg and 10 mg, depending of cholesterol levels. According to our results there were no statistically significant differences at baseline and after therapy: Lp(a) mg/dL 108.6±72.4 *vs*. 99.8±76.5 and tHcy mmol/L 17.8±3.3 *vs*. 15.7±3.1 (p>0.05 both). There was a statistically high correlation between Lp(a) concentration at baseline and 6 months later: r=0.936, p<0.0001 and tHcy r=0.896, p<0.001. Hollesta treatment produced significant reduction in total cholesterol mmol/L (8.64±4.22 *vs*. 4.11±1.03),

mg/dL (2,32±0,8 prema 1,36±0,32), dok se HDL-kolesterol i apolipoprotein A-I nisu značajno promijenili od bazalnih razina. Osim učinkovitog snižavanja lipida holesta nema učinka na serumske razine Lp(a) i tHcy. Hiperhomocisteinemija se može lako liječiti dodatcima vitamina, kojima se daje prednost zbog njihovih kardioprotektivnih svojstava, dok se razine Lp(a) s vremenom stabiliziraju.

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LDL-cholesterol mmol/L (5.62±4.32 *vs.* 2.89±1.32) and apolipoprotein B mg/dL (2.32±0.8 *vs.* 1.36±0.32), whereas HDL-cholesterol and apolipoprotein A-I did not significantly change from baseline. Besides its lipid lowering efficacy, hollesta has not effect on serum Lp(a) and tHcy levels. Hyperhomocysteinemia can easily be treated with vitamin supplements, which are favored for their cardioprotective properties, while Lp(a) levels are stable over time.

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P10-2

Učestalost genotipova ljudskih trombocitnih antigena u djece s arterijskim ishemijskim moždanim udarom

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Arterijski ishemijski moždani udar u djece je relativno rijetka bolest s višestrukom etiologijom i incidencijom od 2,7 /100 000 djece godišnje, koja još nije u potpunosti razjašnjena. Uz dobro utvrđene rizične čimbenike za arterijski ishemijski moždani udar, sve više podataka ukazuje na važnost protrombotičnih čimbenika koji nastaju zbog poremećaja u koagulacijskom i fibrinolitičkom sustavu, endotelu i trombocitima, s tim što uloga trombocita još nije dovoljno istražena. Cilj našega istraživanja bio je odrediti učestalost genotipova ljudskih trombocitnih antigena (HPA) u skupini djece s arterijskim ishemijskim moždanim udarom potvrđenim slikovnim pretragama mozga, te ih usporediti s kontrolnom skupinom. Skupina s arterijskim ishemijskim moždanim udarom (skupina AIS) je uključivala 22 djece (15 dječaka, 7 djevojčica kod kojih je moždani udar dijagnosticiran u dobi od 11 mjeseci do 16 godina), dok je 26 zdrave djece (20 dječaka, 6 djevojčica starosne dobi od 2 do 18 godina) predstavljalo kontrolnu skupinu. Genotipizacija HPA-1, HPA-2, HPA-3 i HPA-5 je izvršena lančanom reakcijom polimerazom pomoću početnica sa specifičnim slijedom. Dobivene su slijedeće učestalosti alela:

P10-2

Frequency of human platelet antigen genotypes in children with arterial ischemic stroke

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Arterial ischemic stroke (AIS) in children is a relatively rare disease (with an incidence of 2.7 *per* 100,000 children/year) that is not yet clearly understood and with multifactorial etiology. Besides well-established risk factors for AIS, there are accumulating data indicating the importance of prothrombotic abnormalities due to defects in coagulation and fibrinolytic system, endothelial cells and platelets. Among these, the role of platelets has not been well studied. The aim of our study was to determine the frequency of human platelet antigen (HPA) genotypes in the group of children with AIS (confirmed by brain imaging), and to compare the data with those obtained in a control group. The AIS group consisted of 22 children (15 male and 7 female; age at first onset 11 months to 16 years), whereas 26 children (20 male and 6 female, aged from 2 to 18 years) were included in the control group. The genotypes of HPA-1, HPA-2, HPA-3 and HPA-5 were determined by the sequence-specific primer polymerase chain reactions. The calculated allele frequencies were as follows: for AIS patients, HPA-1a/b 0.75/0.25, HPA-2a/b 0.86/0.14, HPA-3a/b 0.77/0.23, HPA-5a/b 0.89/0.11; and for

u skupini AIS (HPA-1a/b 0,75/0,25; HPA-2a/b 0,86/0,14; HPA-3a/b 0,77/0,23 i HPA-5a/b 0,89/0,11), a u kontrolnoj skupini (HPA-1a/b 0,94/0,06; HPA-2a/b 0,92/0,08; HPA-3a/b 0,50/0,50 i HPA-5a/b 0,89/0,11). Nije utvrđena statistički značajna razlika u učestalosti HPA-2 (p=0,5429) i HPA-5 (p=0,7729) između skupine AIS i kontrolne skupine. Statistički značajna razlika je utvrđena za učestalost alela HPA-1 (p=0,0195) i HPA-3 (p=0,0118). Dobiveni rezultati ukazuju na moguću ulogu glikoproteina II b/IIIa te polimorfizama HPA-1 i HPA-3 u aktivaciji trombocita, kao i na mogući utjecaj na trombotički događaj.

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control subjects HPA-1a/b 0.94/0.06, HPA-2a/b 0.92/0.08, HPA-3a/b 0.50/0.50, HPA-5a/b 0.89/0.11. No statistically significant differences in the frequencies of HPA-2 (p=0.5429) and HPA-5 (p=0.7729) between AIS patients and control subjects could be detected, while statistically significant differences were obtained for HPA-1 (p=0.0195) and HPA-3 (p=0.0118) allele frequencies. These results indicate the potential role of glycoprotein IIb/IIIa and HPA-1 and HPA-3 polymorphisms in platelet activation, and possible involvement in the thrombotic event.

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P10-3

C-reaktivni protein kao biomarker visokog stupnja stenoze moždanih arterija

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Nedavne spoznaje ukazuju na to da C-reaktivni protein (CRP) kao nespecifični upalni biomarker aktivno doprinosi svim stupnjevima aterogeneze. Mjerenje serumske razine CRP visoko-osjetljivim metodama može ukazati na subklinička upalna stanja krvožilnog sustava. Cilj ove studije bio je ispitati odnos serumske koncentracije CRP i stupnja razvoja aterosklerotskih promjena objektivno utvrđenog prema angiografskim kriterijima hemodinamske značajnosti stenoze metodom digitalne suptrakcijske angiografije u odnosu na kontrolnu skupinu s normalnim moždanim arterijama prema ultrazvučnom nalazu. Lipidni status i koncentracija CRP određeni su u serumu 119 bolesnika u dobi od 40-83 (medijan 66) godina sa stenozom ekstrakranijskih moždanih arterija utvrđenom angiografski i uspoređeni s kontrolnom skupinom ispitanika u dobi od 44-82 (medijan 61) godine u kojih su ultrazvučnim pregledom nađene normalne moždane arterije. Kod 73 bolesnika utvrđen je stupanj stenoze manji od 70% širine lumena, a kod 46 bolesnika veći od 70% širine lumena ili obliteracija. Koncentracije CRP određene su lateks imunoturbidimetrijskom meodom visoke osjetljivosti na analizatoru Olympus AU 600 i reagensima Olympus. Nepreciznost u seriji iskazana kao koeficijent varijacije (KV) bila je 1,98%

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C-reactive protein as a biomarker for severe stenosis of cerebral arteries

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C-reactive protein (CRP) as a non-specific inflammatory biomarker has been demonstrated to actively contribute to all stages of atherogenesis. Measurement of serum concentrations of CRP using a high sensitivity assay (hs-CRP) can demonstrate subclinical inflammatory states, which may reflect vascular inflammation. The aim of this study was to investigate the relation between CRP concentrations and severity of stenosis of cerebral arteries scored by digital subtraction angiography *versus* group with normal cerebral arteries on ultrasound examination. Lipid parameters and CRP concentrations were measured in the sera of 119 patients, median age 66 (range 40-83) years, with stenosis of extracranial cerebral arteries established by angiography and compared with age- and sex-matched controls, median age 61 (range 44-82) years, with normal cerebral arteries on ultrasound examination. Patient group included 73 patients with stenosis less than 70% and 46 patients with stenosis of 70% or more including obliteration. CRP concentrations were determined by high-sensitivity latex-enhanced immunoturbidimetric assay (Olympus) on an Olympus AU 600 analyzer. The intra-assay coefficient of variation (CV) was 1.98% at a concentration of 2.37 mg/L. The inter-assay CV for hs-CRP assay

u koncentracijskom području od 2,37 mg/L. Nepreciznost iz dana u dan za koncentracijsko područje od 1,30 mg/L i 16,00 mg/L izražena kao KV bila je 4,1% odnosno 2,7%. U skupini bolesnika sa stenozom moždanih arterija CRP pokazuje porast u odnosu na kontrolnu skupinu (medijan 1,8 mg/L u skupini sa stupnjem stenoze manjim od 70% i 3,4 mg/L u skupini sa stupnjem stenoze većim od 70% u odnosu na 1,5 mg/L u kontrolnoj skupini, p<0,05). Utvrđena je statistički značajna povezanost između koncentracije CRP i omjera ukupnog kolesterola i HDL-kolesterola (r=0,25; p<0,05) u skupini bolesnika sa stenozom manjom od 70%, a u skupini bolesnika sa stenozom većom od 70% s ukupnim kolesterolom, omjerom ukupnog kolesterola i HDL-kolesterola i indeksom ateroskleroze (r=0,304; 0,423; 0,341). Logistička regresijska analiza pokazala je značajnu povezanost CRP sa stupnjem stenoze većim od 70%. Dobiveni rezultati ukazuju na to da se povišene koncentracije CRP koje su još unutar granica referentnog intervala mogu smatrati dodatnim diskriminirajućim biokemijskim pokazateljem visokog stupnja stenoze moždanih arterija.

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at concentrations of 1.30 mg/L and 16.0 mg/L was 4.1% and 2.7%, respectively. CRP concentrations showed an increasing tendency in the groups of patients with cerebrovascular stenosis compared to the control group (median 1.8 mg/L in the group with less than 70% stenosis and 3.4 mg/L in the group with more than 70% stenosis *versus* 1.5 mg/L in the control group; p<0.05). A statistically significant association was found between CRP concentrations and the total cholesterol/HDL-cholesterol ratio in the group of patients with stenosis less than 70% (r=0.25, p<0.05), and total cholesterol, total cholesterol/HDL-cholesterol ratio and index of atherosclerosis (r=0.304, 0.423 and 0.341, respectively) in the group with stenosis of more than 70%. On logistic regression analysis, CRP was significantly associated with stenosis of more than 70%. The findings obtained indicate that elevated CRP concentrations, which are still within the reference interval, are significantly associated with cerebrovascular stenosis of more than 70% and appear to be an additional discriminating indicator of severe stenosis of cerebral arteries.

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P11 – Bubrežne bolesti, P11-1

Endotelin-1, veliki endotelin-1 i dušikov oksid u bolesnika s kroničnim bubrežnim bolestima

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Čimbenici endotelinskog sustava (endotelini, endotelinski receptori) i dušikov oksid (NO) igraju značajnu ulogu u složenoj patogenezi kroničnih bubrežnih bolesti (KBB), ne samo zbog njihovih vazoaktivnih svojstava, nego i zbog uloge u općoj modulaciji vaskularne homeostaze. Različite bubrežne stanice sintetiziraju NO i endoteline (ET) koji djeluju na bubrežnu hemodinamku te na izlučivanje soli i vode putem mokraće. Endotelin-1 (ET-1) uza snažno vazoaktivno djelovanje modulira mitozu i apoptozu pojedinih staničnih vrsta. Cilj istraživanja bio je ispitivanje uloge i međusobne interakcije ET-1, velikog-ET-1 i NO kod kroničnih bubrežnih bolesti. Koncentracije navedenih vazoaktivnih molekula određene su u plazmi/serumu i/ili mokraći bolesnika (n=57) s dijabetičnom nefropatijom (I. skupina), arterijskom hipertenzijom (II. skupina), KBB dru-

P11 – Nephrological diseases, P11-1

Endothelin-1, big endothelin-1 and nitric oxide in patients with chronic renal diseases

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The complex pathogenesis of chronic renal disease (CRD) depends on endothelin axis (endothelins and endothelin receptors) as well as nitric oxide (NO) because of their vasoactive effects and role in the general modulation of vascular homeostasis. Distinct renal cells synthesize endothelins (ET) and NO that play a significant role in renal hemodynamics as well as in water and salt excretion *via* urine. Endothelin-1 (ET-1) is a strong vasoconstrictor. Besides its vasoactive effects, ET-1 modulates mitosis and apoptosis in a cell type dependent manner, and may play an important role in CRD pathogenesis. The aims of this study were to determine the role and interactions of ET-1, big ET-1 and NO in CRD. Concentrations of these vasoactive molecules were measured in patients with diabetic nephropathy (group I), arterial hypertension (group II) and

ge etiologije (III. skupina) te kod zdravih ispitanika (n=18). Koncentracija velikog ET-1 u mokraći bolesnika s KBB bila je značajno povećana (13,13 pmol/L; p<0,001) u odnosu na kontrolnu skupinu (11,34 pmol/L), dok se koncentracije ET-1 u plazmi i mokraći nisu značajno razlikovale od kontrolne skupine. Koncentracija NO u serumu bila je značajno povećana u skupini bolesnika (72.55 µmol/L; p<0,001), kao i u mokraći bolesnika (141,74 µmol/L; p<0,05) u odnosu na kontrolnu skupinu. Određivanjem koncentracije velikog ET-1 u mokraći (dijagnostička osjetljivost 56,1%, dijagnostička specifičnost 88,9%) i NO u plazmi (dijagnostička osjetljivost 66,7%, dijagnostička specifičnost 83,3%) mogu se razlikovati bolesnici s KBB različitih etiologija u odnosu na zdrave ispitanike. Dijagnostička osjetljivost velikog ET-1 za dijagnostiku dijabetične nefropatije je 78,6%, a specifičnost 88,9%.

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CRD of other etiology (group III). The concentrations of ET-1 (plasma and urine), big ET-1 (urine) and NO (serum and urine) were measured in CRD patients (n=57) and healthy controls (n=18). In CRD patients, the concentration of big ET-1 in urine was significantly increased (13.13 pmol/L; p<0.001) as compared to the control group (11.34 pmol/L). However, ET-1 values in plasma and urine showed no significant changes. NO concentrations were also significantly increased in CRD patients (in serum: 72.55 µmol/L, p<0.001; and in urine 141.74 µmol/L, p<0.05) as compared to the control group. Study results indicated that big ET-1 and NO could be useful as diagnostic parameters for CRD because of their diagnostic sensitivity and diagnostic specificity (for big ET-1 in urine: 56.1% and 88.9%; and for NO in serum: 66.7% and 83.3%, respectively). In addition, big ET-1 may prove useful in the differential diagnosis of diabetic nephropathy (diagnostic sensitivity of 78.6% and diagnostic specificity of 88.9%).

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P11-2

Praćenje učinka konvencionalne dijalize, hemodijafiltracije i parne hemodijafiltracije na razinu beta-2-mikroglobulina u serumu

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Beta-2-mikroglobulin protein je protein male molekularne mase (11,8 kDa). Nalazi se gotovo u svim tjelesnim tekućinama u niskoj koncentraciji, a serumska koncentracija znatno je povećana kod bolesnika na hemodijalizi. Jedan je od biljega tubularne lezije. Najvažnija mu je primjena u dijagnosticiranju nefropatije, praćenju bolesnika s limfomom, multiplim mijelomom i leukemijom. Jedna od niza komplikacija u bolesnika na dugotrajnoj hemodijalizi je taloženje beta-2-mikroglobulina u tkivu sinovija, što izaziva niz komplikacija poput oštećenja kostiju, frakture kostiju i atrofije kostiju s razvojem amiloidoze. Cilj ispitivanja bio je praćenje uklanjanja beta-2-mikroglobulina kod bolesnika na konvencionalnoj dijalizi (HD), hemodijafiltraciji (HDF) i parnoj hemodijafiltraciji (PHF). Ukupno je ispitano 68 bolesnika, od toga 20 bolesnika na konvencionalnoj dijalizi na *high-flux* dijalizatorima (HD), skupina N1; 14 bo-

P11-2

Monitoring of the effect of conventional hemodialysis, hemodiafiltration and paired hemodiafiltration on-line on serum beta-2-microglobulin levels

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Beta-2-microglobulin is a low molecular weight protein (11.8 kDa). It is found in almost all body fluids and its serum level is significantly increased in patients on hemodialysis. Beta-2-microglobulin is one of the markers of tubular damage. It has a great significance in the diagnosis of nephropathy as well as in monitoring of patients with lymphoma, multiple myelomas and leukemias. One of the numerous complications in patients on long-term hemodialysis is deposition of beta-2-microglobulin, which causes complications such as bone lesions, fractures and atrophy with the development of amyloidosis. The aim of the study was to monitor the elimination of beta-2-microglobulin in patients on conventional hemodialysis (HD), hemodiafiltration (HDF) and paired hemodiafiltration on-line (PHF). The study included 48 patients, 20 of them dialysed with conventional dialysis on a high-flux dialysis

lesnika dijaliziranih na Polyflax 21S Gambro (HDF), skupina N2; 14 bolesnika dijaliziranih na dijalizatorima Lympha Belko (PHF), skupina N3. Srednja dob bolesnika bila je 43 ±2,8 godine. Kt/v u svim skupinama bio je veći od 1,2. Svim bolesnicima na početku i na kraju dijalize određen je beta$_2$-mikroglobulin metodom imunoturbidimetrije na Olympusu AU 600. U statističkoj obradi rabio se je Mann-Whitney test. Razlika u koncentraciji beta$_2$-mikroglobulina kod bolesnika prije i poslije hemodijalize pokazala se statistički značajnom u skupinama N2 ($p<0,05$) i N3 ($p<0,01$), dok u skupini N1 nije bila statistički značajna ($p>0,08$). Ispitivanje je pokazalo značajno uklanjanje serumskog beta-2-mikroglobulina kod bolesnika na HDF i PHF dijalizi u odnosu na bolesnike na HD dijalizi, što smanjuje brojne komplikacije, a sve to dovodi do smanjenja pobola i smrtnosti u bolesnika na hemodijalizi.

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machine (HD), group N1; 14 patients dialysed on Polyflax 21S Gambro (HDF), group N2; and 14 patients dialysed on Lympha Belko (PHF), group N3. Median age of the patients was 43±2.8 years. Kt/v was higher than 1.2 in all three groups. Beta-2-microglobulin was determined at the beginning and at the end of hemodialysis by immunoturbidimetric method on an Olympus AU 600 analyzer. On statistical analysis Mann-Whitney test was used. The difference in beta-2-microglobulin levels before and after hemodialysis was statistically significant in groups N2 ($p<0.05$) and N3 ($p<0.01$), while in group N1 the difference did not reach statistical significance ($p>0.08$). Our study showed a significant elimination of beta-2-microglobulin in patients on HDF and PHFas compared to patients on HD, which reduces the rate of complications, morbidity and mortality among hemodialysis patients.

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P11-3

Određivanje slobodnih masnih kiselina u tkivu leđne moždine u uvjetima liječenja indometacinom

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Ishemija i hipoksija leđne moždine tipična su patološka stanja koja prate traumu kičmenog stupa. U uvjetima traume pokreću se mehanizmi lipidne peroksidacije membrana živčanih stanica i oslobađaju masne kiseline (*free fatty acids*, FFA), a njihovom razgradnjom stvaraju se raznoliki metaboliti. Patofiziološka pozadina poremećaja koji nastaju nakon traume leđne moždine vrlo je složena i samo djelomice poznata. U namjeri da doprinesemo spoznavanju etiologije ovakvoga stanja, istražili smo učinkovitost različitih terapijskih pristupa u eksperimentalnom modelu traume kičmene moždine kod kunića. Cilj ovoga istraživanja bio je odrediti razinu slobodnih masnih kiselina u uzorcima tkiva kičmene moždine u uvjetima liječenja indometacinom, nesteroidnim protuupalnim lijekom (NSAID) koji suzbija ciklooksigenazu i blokira sintezu prostaglandina. Iz uzoraka tkiva leđne moždine izolirani su ukupni lipidi metodom po Folchu. Preparativnom tankoslojnom kromatografijom odvojene su slobodne masne kiseline koje su analizirane kao metilni esteri plinskom kromatografijom. Razine ispitivanih slobodnih masnih kiselina porasle su u uvjetima traume, a indometacin u četiri

P11-3

Determination of free fatty acids in spinal cord tissue in the conditions of indomethacin therapy

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Traumatic injury of the spinal cord leads to a series of pathological events that result in tissue necrosis and paralysis. Among the earliest biochemical reactions are hydrolysis of membrane phospholipids, release of fatty acids, production of biologically active eicosanoids, and peroxidation of lipids. The aim of this study was to investigate the influence of indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), a potent inhibitor of arachidonate cyclooxygenase (COX) and thus inhibitor of the production of prostaglandins and thromboxanes, on the spinal cord tissue concentration of free fatty acids (FFA) in rabbits with spinal cord injury (SCI). Spinal cord samples were taken from the impact injury site. Total lipids were isolated and purified by a modification of the method of Folch. FFA were separated from total lipid extract by preparative thin-layer chromatography, converted to the corresponding methyl esters and identified using gas chromatography. The concentrations of all FFA analyzed were increased in the spinal cord after neurotrauma, in comparison to control tissues. Treatment of injured rabbits with indomethacin resulted in a significant decrease

različite doze značajno je snizio njihovu razinu, te popravio motorički deficit kod promatranih eksperimentalnih životinja. Slobodne masne kiseline, osobito arahidonska, koristan su pokazatelj nekroze tkiva. Liječenje specifičnim protuupalnim lijekovima može biti korisno u liječenju oštećenog tkiva.

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in spinal cord FFA and exerted a positive effect on neurotrauma-induced motor impairment. The accumulation of FFA is a possible indicator of nervous cell damage. Treatment with specific anti-inflammatory agents modulates various components of the pathological process thereby selectively attenuating neurologic damage and encouraging wound healing.

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P11-4

Značenje nalaza atipičnih stanica u svježoj mokraći

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Kad se u mokraćnom sedimentu spontane mokraće zapaze atipične stanice, treba saznati uvjete njihove pojave, kao i njihovo podrijetlo i značenje. Ova je studija bila usredotočena na prisutnost atipičnih stanica i drugih stanica koje ukazuju na različite upalne bolesti (bubrežne ili nebubrežne) u mokraćnom sedimentu. U ispitivanje je bilo uključeno 62 bolesnika, većina s različitih odjela Županijske bolnice za hitna stanja u Timisoari. Odabrane su fotografije nekih najzanimljivijih slučajeva. Kako su ovi slučajevi vrlo rijetki, studija je trajala gotovo dvije godine (od veljače 2003. do siječnja 2005.). Mokraćni sediment je najprije promatran pod manjim povećanjem (x100), a zatim su promijenjene strukture ispitane pod većim povećanjem (x400, x1000). Ispitivanje je provedeno različitim mikroskopskim tehnikama (jasno polje, fazni kontrast te imunofluorescentna mikroskopija), te upotrebom obojenih uzoraka (bojenje prema May-Grünwald Giemsa i Sternheimer-Malbin). Fokusiranje atipičnih stanica između stakalca i pokrova stakalca (obojeni i neobojeni), kao i u razmazima predstavlja element citoprevencije, jer sumnja na prisutnost atipičnih stanica dovodi do preporuke za patološku pretragu. Dokazivanje atipičnih stanica u mokraćnom sedimentu pri rutinskoj mikroskopskoj pretrazi dovodi do ranog dokazivanja malignih stanica. Ova činjenica ima veliku kliničku važnost. To ovdje naglašavamo, jer je ovaj odjel jedini koji može sa sigurnošću potvrditi ili isključiti prisutnost tumorskih stanica. Prisutnost atipičnih stanica u mokraćnom sedimentu ne mora uvijek ukaziva-

P11-4

Significance of atypical cell finding in fresh urine

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When atypical cells are observed in urinary sediment from spontaneous urine, it is necessary to know the conditions of their occurrence as well as their origin and significance. The aim of the study was focused on the presence of atypical cells and other cells indicative of various inflammatory diseases (renal or nonrenal) in urinary sediment. The study included 62 patients, most of them from different department of the Timisoara County Emergency Hospital. Some photos representing the most interesting cases were selected. Due to the rarity of cases, the study lasted for almost two years (February 2003 – January 2005). Urinary sediment was first screened at a low power (x100) and thereafter modified structures were examined at a higher power (x400, x1000). The investigation was performed by different microscopic techniques (bright field, phase contrast, and immunofluorescence microscopy), and also using stained samples (May-Grünwald Giemsa stain and Sternheimer-Malbin stain). Focusing on atypical cells between the slide and the cover slip (stained and unstained) as well as in smears is a cytoprevention element because suspicion of the presence of atypical cells leads to recommendation of pathologic examination. The detection of atypical cells in urinary sediment on routine microscopic examination will lead to an early detection of malignant cells. This fact has clinical implications of great importance. We emphasize it because this department is the only one that can certainly confirm or exclude the presence of tumor cells. The presence of atypical cells

ti na malignitet. Benigne promjene uzrokovane upalom mogu također biti uzrokom pojave atipičnih stanica u mokraći. Kako bi se izbjegli mogući nesporazumi, primijenili smo paralelnost mikroskopsih tehnika (poglavito imunofluorescentne mikroskopije) te obojene i neobojene uzorke, prema potrebi. Kao laboratorij prvoga kontakta možemo bolesnika upozoriti i preporučiti patološku pretragu. Predložene tehnike nisu niti skupe niti zahtijevaju puno vremena; jedino je potreban stručnjak s iskustvom u mikroskopiranju.

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in urinary sediment need not necessarily indicate malignancy. Benign changes due to inflammation may be the cause of atypical cells in urine. To avoid any possible confusion, we used the parallelism of microscopic techniques (especially immunofluorescence microscopy) and both stained and unstained samples as necessary. As the first contact laboratory, we can warn the patient and recommend pathologic examination. The techniques proposed are neither expensive nor time-consuming, only an experienced microscopist is required.

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P12 – Bolesti jetre i gastrointestinalnog trakta, P12-1

Mutacija gena za kationski tripsinogen (PRSS1) hereditarnog pankreatitisa: prikaz obitelji

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Akutni i kronični pankreatitisi su relativno češće upalne bolesti gušterače, katkada s ozbljnim komplikacijama. U većini slučajeva pankreatitisi su uzrokovani bilijarnim kamencima ili alkoholom, no danas je poznato da postoji genetička komponenta u vidu mutacija u genima za kationski tripsinogen (PRSS1), sekretorni inhibitor tripsina (SPINK) ili u genu za cističnu fibrozu (CFTR). Današnji je stav da se dijagnostički preporuča testirati jedino za mutaciju PRSS1 gena. U dvije trećine slučajeva s PRSS1 mutacijom prisutna je R122H, a u jednoj trećini mutacija N29I. Ove mutacije prekomjerno aktiviraju tripsinogen. U obiteljima s mutacijama između 60% i 80% osoba razvije klinički pankreatitis. Ovdje opisujemo šestoročlanu obitelj u kojoj je kćer u dobi od 14 godina razvila recidivirajući pankreatitis. Danas, u dobi od 18 godina ona je klinički stabilna. U ove bolesnice smo primijenili test RFLP-PCR za dokazivanje mutacije R122H, koji se temelji na činjenici da u mutaciji tranzicija G u A nukleotid u DNA sekvenci stvara rezno mjesto za AflIII restrikcijsku endonukleazu (Bell *et al*. J Clin Pathol Mol Pathol 1998;51:115). Primijenjeni molekularni test potvrdio je postojanje mutacije R122H kod ove bolesnice. Testirani su i ostali članovi obitelji, tj. roditelji, dvojica braće i sestra, no oni nisu nosili mutaciju. Iako su mutacije PRSS1 autosomno dominantne, moguće

P12 – Liver and gastrointestinal diseases, P12-1

Hereditary pancreatitis with cationic trypsinogen gene (PRSS1) mutation: a family study

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Acute and chronic pancreatitis are relatively common but potentially serious clinical conditions. In most cases acute pancreatitis is caused by gallstones or alcohol, but unexplained recurrent acute pancreatitis may be associated with a known genetic mutation in the cationic trypsinogen gene (PRSS1), SPINK1 gene or CFTR gene. Currently, the only gene for which genetic testing is recommended is trypsinogen. About two thirds of families with hereditary pancreatitis have R122H and one third N29I mutation. These are function mutations that lead to premature trypsinogen activation. In families with R122H or N29I, 60%-80% of individuals who inherit the mutation will develop pancreatitis. We describe a family with six members where one daughter developed recurrent acute pancreatitis at age 14. Now clinically stable, at age 18, she was tested for PRSS1 R122H mutation by RFLP-PCR analysis. Molecular test is based on the fact that the mutation with transition of G to A in the DNA sequence creates a cutting site for the restriction endonuclease AflIII (Bell *et al*. J Clin Pathol Mol Pathol 1998;51:115). The R122H mutation of cationic trypsinogen gene was confirmed in the patient. Testing of parents and three siblings, all free from clinical history of pancreatitis, revealed normal PRSS1 R122H allele. Although autosomal dominant in inheritance, tryp-

su i pojave spontanih mutacija, o čemu ćemo raspraviti detaljnije.

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sinogen mutation can occur as a spontaneous mutation, which will be discussed in more detail.

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P12-2

Dokazivanje prisutnosti *Helicobacter pylori* pomoću ureja izdisajnog testa

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Ureja izdisajni test se primjenjuje za dokazivanje prisutnosti i aktivnosti bakterije *Helicobacter pylori* na stijenci želuca. Izdisajnim testovima se analizira CO_2 iz izdahnutog zraka. Svojstvo bakterije *Helicobacter pylori* je sinteza ureaze. Kad ispitanik konzumira ureju obilježenu ugljikovim izotopom, a bakterija je prisutna na sluznici želuca, tada će ureaza koju sintetizira bakterija razgraditi testnu otopinu na amonijak koji se izlučuje urogenitalnim traktom i CO_2 s izotopom koji u određenom vremenskom razdoblju krvlju dospijeva u pluća i konačno u izdahnuti zrak. Tehnike za određivanje količine izotopa su razne: masena spektrometrija, plinska kromatografija-masena spektrometrija, optička spektroskopija i infracrvena spektroskopija. Cilj studije je bio utvrditi prisutnost bakterije *Helicobacter pylori* na stijenci želuca ispitanika. Primijenili smo metodu ureja izdisajnog testa ugljikovim izotopom C13. Tehnika određivanja omjera koncentracije ugljikovog izotopa C12 i C13 je infracrvena spektroskopija. Izvor infracrvenog zračenja emitira konstantno svjetlo. Ova radijacija prolazi kroz mjerne komore ispunjene zrakom koji se analizira, a zatim kroz detektorske komore od kojih je svaka ispunjena jednim od izotopski čistog ugljičnog dioksida. Plin u svakoj detektorskoj komori apsorbira energiju u spektru dotičnog izotopa i zagrijava se do stupnja proporcionalno valnoj radijaciji na ulasku u sustav. Intenzitet ovisi o količini apsorbirane energije u mjernoj komori, dakle, o koncentraciji i odgovarajućem parcijalnom tlaku komponente u mjernoj komori. Veličina koja se određuje jest omjer izotopa C12 i C13 u uzorku, a izražava se kao Delta. Rezultat se izražava kao DOS=Delta *over baseline*. U ovom testu se tako uspoređuju Delta C13 i C12 u nultom uzorku prije konzumacije testne otopine i Delta C13 i C12 u uzorku nakon određenog vremena za koje je utvrđeno da je potrebno za razgradnju supstrata i pojavu razgradnog proizvoda u izdahnutom zraku. Ureja izdisajni test je neinvazivna metoda dokazivanja prisutnosti i aktivnosti bakterije *Helicobacter pylori* na stijenci želuca ispitanika obilježena

P12-2

Urea breath test to detect the presence and activity of Helicobacter pylori

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Urea breath test is used to determine the presence and activity of *Helicobacter pylori* on gastric mucosa. Breath tests analyze CO_2 from breath. *Helicobacter pylori* synthesizes urease. The patient takes urea labelled with carbon isotope and if the bacteria is present, then the urease that is synthesized breaks the test solution up to produce ammonia which is eliminated by urogenital pathway and CO_2 with isotope which will reach the lungs by blood within a certain period of time and eventually be detectable in breath. The techniques used to determine isotope concentration are: mass spectrometry, gas chromatography-mass spectrometry, optical spectroscopy and infrared spectroscopy. The aim of the study was to determine the presence of *Helicobacter pylori* on gastric mucosa. Urea breath test with C13 carbon isotope was used. Infrared spectroscopy was used for determination of C12 and C13 isotope concentration. In the measuring system, the infrared sources emit constant infrared light. This radiation passes measuring cells filled with measuring gas and then detector cells loaded with the isotopically pure gaseous component to be measured. The gas in each of detector cells absorbs energy in the spectral range of the particular component and is warmed up to the level proportional to the intensity of this radiation at its entrance window. This intensity depends on the degree of radiation absorption in the measuring cells and therefore also on the concentration or partial pressure of the particular component in these measuring cells. Diagnostic criterion of C13-urea breath test is Delta Over Baseline (DOB) value. Delta values are deviations from a certain standard expressed *per* million. For carbon isotope analysis, standard PDB is used as internal standard and its Delta value is 0 *per* million. Delta values are determined at zero time, Delta baseline, and 30 minutes after test solution intake, Delta after tracer intake. Urea breath test is a noninvasive method to determine the presence and activity of *Helicobacter pylori* on gastric mucosa, characterized by high sensitivity and

visokom osjetljivošću i specifičnošću. Preporučljiva je za postavljanje dijagnoze u kombinaciji s gastroskopijom, pričem se eliminira uzimanje bioptičkog materijala i za procjenu uspješnosti eradikacijske terapije.

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specificity. It is recommended for diagnostic use in combination with gastroscopy, thus eliminating the need of biopsy, and for evaluation of eradication therapy efficacy.

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P13 – Endokrinologija, P13-1

Laboratorijska potpora dijagnostici i praćenju bolesti štitnjače

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Laboratorijske pretrage neophodna su potpora liječnicima za racionalno i ekonomski opravdano liječenje bolesnika. Pritom je vrlo važno odabrati visoko kvalitetne i isplative dijagnostičke pretrage. U tom duhu MBL Opće bolnice Pula primjenjuje smjernice za dijagnostiku i praćenje bolesti štitnjače prema preporuci svjetskih organizacija za liječenje bolesti štitnjače. U MBL Opće bolnice Pula svakodnevno se zaprima oko 70 zahtjeva za ispitivanje funkcije štitnjače (TSH, fT4, T3). Status štitnjače ispitivali smo u skupini od 498 bolesnika koje smo svrstali u 3 skupine: I. bolesnici s 5 ili više simptoma (n=71), II. s 3 ili 4 simptoma (n=156) i III. s 2 ili manje simptoma poremećaja štitnjače (n=271). Od ukupnog broja bolesnika bolest štitnjače dijagnosticirana je u 15,8% slučajeva. U I. skupini bolest štitnjače utvrđena je u 32 (45%), u II. skupini u 26 (16,6%) i u III. skupini u 21 (7,7%) ispitanika. Nizak udio bolesti štitnjače u odnosu na ispitivane bolesnike navodi nas na razmišljanje o opravdanosti velikog broja zahtjeva za ispitivanje funkcije štitnjače. Racionalizacija pretraga znači i racionalizaciju sredstava za nabavu reagensa i potrošnog materijala za izvođenje pretraga. U 2004. godini bi za primljene zahtjeve (n=37.409) neselektivnim određivanjem svih parametara za ispitivanje funkcije štitnjače bilo potrebno izdvojiti 274.661,00 kn za troškove nabave reagensa. Međutim, primjenom smjernica broj potrebnih pretraga je značajno smanjen (n=25.754), što preračunato u cijenu reagensa iznosi 184.390,00 kn, a to predstavlja uštedu od 90.271 kn ili 32,9%. Kako svaka ušteda omogućuje ispravniju preraspodjelu ukupnih sredstava, treba ustrajati na oblikovanju kriterija struke i provedbi novih znanstvenih spoznaja u laboratorijskoj dijagnostici. Respektabilni iznos postignute uštede navodi nas na razmišljanje o opravdanosti velikog broja zahtjeva za pojedine skupine analiza, jer racionalnu je dijagnostičku obradu moguće postići uz manje materijalne troškove.

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P13 – Endocrinology, P13-1

Laboratory support to thyroid disease diagnosis and follow up

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Laboratory tests are indispensable support to doctors in reaching an accurate diagnosis and economical processing of patients with thyroid diseases. It is extremely important to choose high quality and cost-effective thyroid function analyses for a certain group of patients. The Laboratory of Medical Biochemistry at Pula General Hospital has implemented guidelines for thyroid disease diagnosis and follow up recommended by world organizations for treatment of thyroid diseases. The Laboratory receives about 70 requests for thyroid function tests (TSH, fT4, T3) daily. Thyroid status was tested in 498 patients divided into three groups: I, patients with 5 or more symptoms (n=71), II, patients with 3-4 symptoms (n=156), and III, patients with 2 or less symptoms of thyroid malfunction (n=271). From the total number of patients, thyroid disease was diagnosed in 15.8% of cases: 32 (45%) in group I, 26 (16.6%) in group II, and 21 (7.7%) in group III. The low rate of thyroid disease in the total number of study patients suggested that justification for the large number of thyroid function test requests should have been reconsidered. Rationalization of the number of tests implies rationalization of the reagent and expendable material procurement. According to the number of requests (n=37,409) received in 2004, the overall cost of non-selective measurement of all thyroid function parameters would be 274,661 HRK in reagent procurement prices. However, the implementation of the guidelines reduced significantly the number of necessary tests (n=25,754). Calculated in reagent prices, the total cost was 184,390 HRK , yielding a saving of 90,271 HRK (32.9%). As every saving allows for better allotment of total funds, it is necessary to be consistent in efforts to implement professional criteria and new scientific concepts in laboratory diagnosis. The respectable saving has prompted us to consider the justification for the large number of requests for specific test groups. Optimal diagnostic processing is possible with lower material expenses.

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P13-2

TSH u praćenju supresijske terapije pomoću L-T4 u bolesnika s karcinomom štitnjače

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Klinički i eksperimentalni podaci u literaturi potvrđuju da TSH može stimulirati rast tumora. Primjena L-T4 suprimira TSH i stoga smanjuje rast tumora štitnjače. Supresija serumskog TSH se općenito primjenjuje u dugovremenom medicinskom liječenju bolesnika s karcinomom štitnjače. Današnje metode mjerenja TSH velike osjetljivosti i visoke specifičnosti omogućuju dokazivanje niskih vrijednosti TSH i stoga se TSH rabi kao terapijska završna točka u prilagođavanju supresijske doze tiroksinom. Osobito je važno individualno prilagoditi supresiju TSH za svakog bolesnika. Za određivanje TSH u serumu kod 160 bolesnika s operiranim karcinomom štitnjače rabio se je pribor treće generacije DELFIA hTSH Ultra kit. Terapijska završna točka u prilagođavanju supresijske doze tiroksinom za svakog je bolesnika određena vaganjem između čimbenika kao što su dob, klinički status uključujući srčane čimbenike i povrat tumora nasuprot potencijalnim štetnim učincima na srce i kosti zbog jatrogene (subkliničke) hipertireoze. U skupini od 88 bolesnika na fiziološkoj dozi terapije pomoću L-T4, 51 (57%) bolesnik je imao vrijednost TSH u subnormalnom području mjerenja (0,05-0,40 mIJ/L), a 34 (38%) bolesnika u ovoj skupini imalo je normalne vrijednosti (0,40-4,2 mIJ/L). Nemjerljive vrijednosti TSH imalo je troje bolesnika. U skupini od 72 bolesnika na suprafiziološkoj dozi terapije pomoću L-T4 61 (84,7%) bolesnik je imao nemjerljive vrijednosti TSH (<0,05 mIJ/L), a 7 (9,7%) bolesnika je imalo vrijednost TSH u subnormalnom području mjerenja. Četvoro bolesnika u ovoj skupini je imalo normalne vrijednosti TSH. U zaključku, učinak supresijske ili supstitucijske terapije pomoću L-T4 u dugovremenom medicinskom liječenju bolesnika s karcinomom štitnjače može se motriti mjerenjem serumskog TSH.

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P13-2

TSH monitoring in patients with thyroid carcinoma on L-T4 suppressive therapy

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Clinical and experimental literature has documented that TSH can stimulate the growth of thyroid tumors. The administration of L-T4 will suppress TSH, thus reducing the growth of thyroid cancer. Thyroid suppression of serum TSH is currently employed for long-term medical management of patients with thyroid cancer. Current TSH methods with their enhanced sensitivity and specificity are capable of detecting low TSH values, thus the measurement of TSH is used as a therapeutic endpoint for adjusting the thyroxine suppression dose. We employed a third-generation assay, DELFIA hTSH Ultra kit, for determination of serum TSH in 160 patients with thyroid cancer. The therapeutic endpoint for adjusting the thyroxine suppression dose for each patient was judged by weighing the patient's factors such as age, clinical status including cardiac factors and cancer recurrence risk, against the potentially deleterious effects of iatrogenic mild (subclinical) hyperthyroidism on the heart and bone.
In the group of 88 patients on physiological dose of L-T4 therapy, 51 (57%) patients had TSH value in the subnormal range (0.05-0.42 mIU/L) and 34 (38.6%) patients had TSH value in the normal range (0.4-4.2 mIU/L). Three patients had undetectable TSH. Out of 72 patients on suphraphysiological dose of L-T4 therapy, 61 (84.7%) patients had undetectable TSH (<0.05 mIU/L) and 7 (9.7%) patients had TSH value in the subnormal range. Four patients had normal TSH value. In conclusion, the efficacy of L-T4 suppression or substitution long-term medical therapy in patients with thyroid cancer can be monitored by measuring serum TSH.

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P13-3

Usporedba triju potpuno automatiziranih imunokemijskih metoda za mjerenje ukupnog HCG u serumu

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Mjerenje ukupnog HCG rabi se u ranom otkrivanju i praćenju normalne i patološke trudnoće te specifičnih malignih bolesti. Danas na tržištu postoje brojne potpuno automatizirane imunokemijske metode za mjerenje ovoga analita. U ovoj studiji uspoređene su tri homogene imunokemijske metode s dva protutijela i to: A. metoda s elektrokemiluminiscentnom detekcijom (Roche, Elecsys 2010), B. metoda s fluorescentnom detekcijom (BioMerieux, Vidas) te C. biotin-streptavidinska metoda s luminiscentnom detekcijom (Johnson&Johnson, Vitros). Nepreciznost (CV%) unutar i između serija bila je prihvatljiva za sve tri ispitane metode i to: A: 2,1 i 4,1; B: 4,5 i 5,7 i C: 3,2 i 6,1. Funkcionalna osjetljivost izražena kao najniža koncentracija s CV 20% bila je: A 0,7 U/L, B: 2,5 U/L i C: 1,4 U/L. Poznato je da se beta-HCG u serumu, s obzirom na stadij trudnoće ili prisutnost maligne bolesti, može nalaziti u vrlo širokom rasponu mjernih koncentracija. U tom smislu, veći raspon linearnosti metode omogućuje brži i jednostavniji rad uz manji utrošak reagenasa. Linearnost ispitanih metoda bila je kako slijedi: A: 0,1-10.000 U/L, B: 2-1500 U/L i C: 0,5-1000 U/L. Usporedba rezultata dobivenih svim trima ispitanim metodama (N=50) ukazala je na visoku razinu korelacije (r>0.96 za sva tri para vrijednosti), s obzirom na to da se radi o imunokemijskim metodama. Međutim, zapaženo je izrazito pozitivno odstupanje rezultata dobivenih metodom B, i to samo u području visokih vrijednosti koje prelaze linearnost te zahtijevaju prethodnu diluciju za sve tri metode. Ovaj podatak još jednom ukazuje na nemogućnost bilo kakvog praćenja bolesnika primjenom različitih imunokemijskih metoda.

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P13-3

Comparison of three fully automated immunassays for serum total HCG determination

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HCG determination in serum is used for detection and follow up of early pregnancy and some specific malignancies. There are numerous fully automated beta-HCG immunoassays available. In this study, we compared three methods, all of them homogeneous sandwich immunassays: A) electrochemiluminescence detection method (Roche, Elecsys 2010), B) fluorescence detection method (BioMerieux, Vidas), and C) biotin-streptavidin method with luminescent signal detection (Johnson&Johnson, Vitros). Imprecision (expressed as CV%) both within- and between-run was within acceptable limits: A) 2.1 and 4.1, B) 4.5 and 5.7, and C) 3.2 and 6.1, respectively. Functional sensitivity, expressed as the lowest beta-HCG concentration that could be measured with a CV 20%, was as follows: A) 0.7 U/L, B) 2.5 U/L and C) 1.4 U/L. As it is commonly known, serum HCG concentrations, depending on the stage of pregnancy or the presence of malignant growth, can vary within several orders of magnitude. Thus, methods with higher linearity are highly preferable, since they allow for ease of operation and cost reduction. Linearities of the three methods studied were as follows: A) 0.1-10,000 U/L, B) 2-1500 U/L and C) 0.5-1000 U/L. Comparison of results obtained by all three methods (N=50) revealed a high degree of correlation, taking into account the different antibodies and signal detection used (r>0.96 for all three pairs of results). However, we noticed a substantial positive bias in the results obtained by method B, not throughout the concentration range but only in the results that required prior dilution. This finding confirms the known fact that patient follow-up should always be performed by using the same immunochemistry method.

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P13-4

Poremećaji funkcije štitnjače u bolesnika na terapiji amjodaronom

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Amjodaron je derivat benzofurana, lijek iz III. skupine antiaritmika, koji se upotrebljava za liječenje i prevenciju aritmija. Maseni udio joda u molekuli amjodarona iznosi oko 37%. Uzimanjem amjodarona dnevni unos joda se povećava 50 do 100 puta. Amjodaron može uzrokovati neke poremećaje funkcijskih testova, kao i manifestnu bolest štitnjače. Amjodaron u perifernim tkivima inhibira aktivnost tipa I 5' dejodinaze i inhibira ulazak hormona štitnjače u periferna tkiva, što doprinosi povećanju serumske vrijednosti tiroksina (T4) i sniženju vrijednosti trijodtironina (T3). Podaci iz različitih dijelova svijeta pokazuju incidenciju amjodaronom uzrokovane tireotoksikoze (AIT) od 1% do 23% i amjodaronom uzrokovane hipotireoze (AIH) od 1% do 32%. Cilj studije bio je odrediti učestalost AIT i AIH u našoj populaciji bolesnika na terapiji amjodaronom i usporediti dobivene podatke s podacima iz literature. U razdoblju od 2002. do 2006. godine u bazi podataka Odsjeka za laboratorijsku dijagnostiku bolesti štitnjače ukupno je evidentirano 447 bolesnika na terapiji amjodaronom. U istraživanje je uključeno 97 bolesnika s medijanom praćenja od 12 (raspon 1-47) mjeseci. Serumske koncentracije TSH i hormona štitnjače određivane su imunometrijskim metodama: a) TSH (Immulite Third Generation TSH (DPC), referentne vrijednosti: 0,36-4,20 mU/L), b) T3 i T4 (DELFIA T3: 1,1-2,8 nmol/L, T4:65-160 nmol/L), c) FT3 i FT4 (BRAHMS (FT3: 4,9-7,9 pmol/L FT4: 12,6-20,9 pmol/L) i Immulite (DPC) (FT3: 2,3-6,3 pmol/L FT4: 10,3-24,5 pmol/L). AIH je definirana nalazom povišenih vrijednosti TSH uza snižene vrijednosti T4/FT4, a subklinička hipotireoza povišenim vrijednostima TSH uz uredne vrijednosti FT4/T4. AIT je definirana sniženim vrijednostima TSH uz povišene razine T3/FT3 ili izrazito povišene vrijednosti FT4/T4, a subklinička tireotoksikoza sniženim vrijednostima TSH uz uredne vrijednosti T3/FT3. U 59 od 97 (61%) bolesnika utvđena je eutireoza. AIH je utvrđena u 14 (14%), subklinička hipotireoza u 9 (9%), AIT u 8 (8%), a subklinička tireotoksikoza u 7 (7%) bolesnika. U promatranoj populaciji zabilježen je značajan broj bolesnika s poremećajem funkcije štitnjače, od kojih je najveći broj bio s AIH, a manji dio s AIT, što je u skladu s podacima za područja s dostatnim unosom joda.

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P13-4

Thyroid function dysfunction in patients on amiodarone therapy

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Amiodarone is a benzofurane derivative, a class III antiarrhythmic drug used for the prevention and management of tachyarrhythmias. Quantitive proportion of iodine in the amiodarone molecule is 37%. Amiodarone treatment increases daily iodine intake approximately 50-100 times. Amiodarone has an effect on thyroid function in the spectrum from abnormal thyroid tests to overt thyroid dysfunction. It inhibits the activity of type I '5 deiodinase as well as thyroid hormone entry in peripheral tissues, which contributes to serum thyroxine (T4) level increase and triiodothyronine (T3) level decrease. Data from different parts of the world indicate the incidence of amiodarone induced thyrotoxicosis (AIT) to be 1% to 23%, and of amiodarone induced hypothyroidism (AIH) 1% to 32%. The aim of the study was to assess the prevalence of AIT and AIH on the basis of thyroid tests in our group of patients on amiodarone therapy, and to compare these findings with literature data. According to the Division of Thyroid Diseases database, in the 2002-2006 period there were 447 patients on amiodarone therapy. Only 97 patients with a follow up median of 12 (range 1-47) months were included in the study. Serum concentrations of TSH and thyroid hormones were measured by immunometric assay methods: (a) TSH (Immulite Third Generation TSH (DPC, reference range: 0.36-4.20 mU/L); (b) T3 and T4 (DELFIA T3:1.1-2.8 nmol/L, T4: 65-160 nmol/L); (c) FT3 and FT4 (BRAHMS (FT3: 4.9-7.9 pmol/L FT4: 12.6-20.9 pmol/L) and Immulite (DPC) (FT3: 2.3-6.3 pmol/L FT4: 10.3-24.5 pmol/L). AIH was defined by increased serium TSH level followed by lower value of T4/FT4. Subclinical hypothyroidism was defined by increased TSH level followed by normal FT4 and T4 values. AIT was defined by lower TSH level followed by increased T3/FT3 or extremely elevated FT4/T4 levels, and subclincal thyrotoxicosis was defined by lower TSH level followed by normal T3/FT3 value. Euthyroidism was recorded in 59 of 97 (61%), AIH in 14 (14%), subclinical hypothyroidism in 9 (9%), AIT in 8 (8%), and subclincal thyrotoxicosis in 7 (7%) patients. Accordingly, thyroid dysfunction was found in a significant number of patients. The higher incidence of AIH and lower incidence of AIT were consistent with data reported from areas with adequate iodine intake.

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P13-5

Biokemijske promjene u središnjem živčanom sustavu miševa s inaktivnim genom za receptor folitropina ovisne su o starosti i spolu

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Tijekom starenja dolazi do povećanja gonadotropina u plazmi i do disbalansa spolnih hormona, što može povećati rizik za razvoj neurodegenerativnih poremećaja. Ispitivali smo izraženost proteina toplinskoga šoka (Hsp) te izraženost i aktivaciju proteinskih kinaza aktiviranih mitogenima (MAPK) u hipokampusu i korteksu miševa s inaktivnim genom za receptor folitropina (FORKO), što uzrokuje povišenje LH i FSH te disbalans estradiola i testosterona. Razine izraženosti MAPK u ispitivanim strukturama mozga nisu se promijenile bez obzira na genotip, starost i spol miševa, ali su razine izraženosti Hsp te aktivacija MAPK bile ovisne i o starosti i o spolu. U hipokampusu je izraženost Hsp70 smanjena kod FORKO mužjaka starih 20 mjeseci, dok su kod ženka iste dobi bile potisnute razine i Hsp70 i Hsp25. Fosforilacija ERK opažena je već kod 3 mjeseca starih FORKO ženka, dok se kod mužjaka ERK značajno aktivirala tek kod 20 mjeseci starih miševa. U korteksu su najznačajnije promjene opažene samo kod starijih miševa (20 mjeseci). Izraženost Hsp25 umjereno je smanjena kod mužjaka, dok je izraženost Hsp70 i Hsp25 bila značajno smanjena kod ženka. ERK je snažno aktivirana kod oba spola. Funkcionalne značajke MAPK ovise o njihovoj lokalizaciji unutar stanice. U hipokampusu 12 mjeseci starih FORKO ženka smanjena je translokacija P-ERK u jezgru u odnosu na zdrave životinje iste dobi, uz istodobno povećanje P-ERK u citosolu. U korteksu FORKO mužjaka starih 20 mjeseci značajno je smanjena P-JNK u jezgri uza snažno povećanje količine aktiviranih kinaza u mitohondrijima. Dakle, povećanje gonadotropina uz disbalans spolnih hormona ili bez njega tijekom starenja može dovesti do biokemijskih promjena u središnjem živčanom sustavu, koje se očituju kao poremećaji u izraženosti Hsp te u aktivaciji i razdiobi MAPK unutar stanice. Ove promjene mogu predstavljati čimbenik rizika za razvoj neurodegenerativnih poremećaja u menopauzi i kasnoj andropauzi.

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P13-5

Age and gender dependent biochemical changes in central nervous system of follitropin receptor knockout mice

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Aging is accompanied by enhanced plasma concentrations of gonadotropins and sex hormone imbalance, which could increase the risk of neurodegenerative disorders. We assessed the status of heat shock proteins (Hsps) and mitogen-activated protein kinases (MAPKs) in the hippocampus and cortex of follitropin receptor knockout (FORKO) mice with enhanced LH and FSH together with estradiol and testosterone imbalance as a consequence of FSH-R deletion. MAPK expression was unchanged in the brain structures examined, regardless of the genotype, age or sex. However, Hsp expression and MAPK activation were age- and sex dependent. In the hippocampus, the expression of Hsp70 was reduced in 20-month-old FORKO males, while both Hsp70 and Hsp25 were down-regulated in age-matched females. In addition, female FORKO mice exhibited enhanced P-ERK already at 3 months of age, while these changes became significant only in 20-month-old male knockouts. In the cortex, the most distinguished changes were observed in older animals (20 months of age). Hsp25 was slightly decreased in males, while the expression of Hsp70 and Hsp25 was significantly lower in females. The signal intensity of P-ERK was strong in both sexes. Functional characteristics of MAPKs are dependent on their subcellular localization. In the hippocampus of 12-month-old FORKO females, translocation of P-ERK to the nucleus decreased and more cytosolic P-ERK was found than in age-matched wild-type animals. On the other hand, nuclear P-JNK was significantly reduced, whereas it was markedly enhanced in the mitochondria of the cortex of 20-month-old FORKO males. In summary, enhanced gonadotropins alone or in combination with sex hormone imbalance in aging lead to biochemical changes in the central nervous system in terms of disturbed expression of Hsps, activation and subcellular distribution of MAPKs, and those changes could present a risk factor for neurodegeneration in menopause and late andropause.

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P14 – Onkologija i tumorski biljezi, P14-1 (UP5-1)

Je li Ca 19-9 XR bolji?

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Ca 19-9 je glikoprotein velike molekularne mase poznat kao mucin. To je antigen koji je povišen u serumu osoba s raznim gastrointestinalnim karcinomima. Može biti povišen u stanjima kao što su hepatitis, ciroza, pankreatitis, kao i kod cistične fibroze. Rabi se kao pomoć u praćenju bolesnika s karcinomom gušterače ili drugim gastrointestinalnim karcinomima. Prije nekoliko mjeseci počeli smo rabiti novi test Ca 19-9 XR. Proizvođač je uz novi test poslao obavijest da je Ca 19-9 XR poboljšan u smislu povećane kliničke specifičnosti, poglavito kod nemalignih bolesti, ali mu je zadržana ista osjetljivost. Izveli smo usporedno određivanje s prethodnim testom Ca 19-9 kako bismo ustanovili postoje li razlike u rezultatima. Određivanje je izvršeno testovima Ca 19-9 i Ca 19-9 XR tehnologijom CMIA na analizatoru Architect i2000 SR tvrtke Abbott. Dobiveni rezultati su razvrstani u skupine: zdravi, bolesni – nemaligne bolesti, početno ispitivanje bolesnika, maligne bolesti i maligne bolesti – poslijeoperacijski. Analiza rezultata navodi na zaključak kako postoje značajne razlike u dobivenim rezultatima kod nemalignih bolesti, dok su se kod malignih bolesti rezultati dobro slagali. S obzirom na različitosti u samom testu prethodni rezultati dobiveni pomoću Ca 19-9 i novi pomoću Ca 19-9 XR ne mogu se uspoređivati.

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P14 – Oncology and tumor markers, P14-1 (UP5-1)

Is Ca 19-9 XR better?

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Ca 19-9 is a glycoprotein of high molecular weight, known as mucin. It is an antigen which is elevated in serum of patients with various gastrointestinal carcinomas, hepatitis, cirrhosis, pancreatitis, and in cystic fibrosis. It is used as an aid in monitoring patients with pancreatic carcinoma or other gastrointestinal carcinomas. The manufacturer has declared the new Ca 19-9 XR test to have better clinical specificity, especially in nonmalignant diseases, while retaining the same sensitivity. We tested the samples with both Ca 19-9 and Ca 19-9 XR to see if there were some diferences. Testing was performed with Ca 19-9 and Ca 19-9 XR tests using CMIA technology on an Architect i2000 SR analyzer (Abbott). Results were divided into groups: healthy, disease – nonmalignant, initial testing, malignant diseases, and malignant diseases – postoperative. The analysis revealed significant differences in the results in nonmalignant diseases, whereas in malignant diseases the results showed good concordance. Thus, the results obtained by Ca 19-9 and Ca 19-9 XR cannot be compared due to test variation.

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P14-2

MTHFR u karcinomu prostate i benignoj hiperplaziji prostate

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Metilentetrahidrofolat-reduktaza (MTHFR) je najvažniji enzim uključen u procese metilacije DNA i katalizira pretvorbu 5,10-metilentetrahidrofolata u 5-metiltetrahidrofolat. Polimorfizam C677T povezan je sa 70%-tnim smanjenjem aktivnosti enzima i hipometilacijom. Učestalost

P14-2

MTHFR in prostatic cancer and benign prostatic hyperplasia

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Methylenetetrahydrofolate reductase (MTHFR) is the most important enzyme involved in DNA methylation process and converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The C677T polymorphism of the MTHFR gene is associated with 70% lower enzyme

mutiranog T alela ispitivana je kod bolesnika s karcinomima i većina autora navodi smanjenu učestalost, no dosad objavljeni rezultati su oprečni. Prema hipotezi ove studije, u naših bolesnika s karcinomom prostate očekuje se manji udio T alela nego kod bolesnika s benignom hiperplazijom prostate (BPH). Također se očekuje da će taj udio biti manji kod malignijih tumora. Ovo je istraživanje obuhvatilo 45 bolesnika s BPH i 95 bolesnika s karcinomom prostate kojima je određen Gleason *score*, pokazatelj stupnja malignosti tumora. Polimorfizam MTHFR C677T određen je metodom PCR-RFLP. Genotipovi u obje skupine bili su u Hardy-Weinbergovoj ravnoteži. Udio genotipova u skupini bolesnika s karcinomom bio je C/C=0,40, C/T=0,51 i T/T=0,09, a u skupini s BPH C/C=0,47, C/T=0,42 i T/T=0,11. Nije pronađena statistički značajna razlika u raspodjeli genotipova (p=0,6558), OR i 95%-tni CI za C/T genotip iznosi 1,3961(0,658-2,963), a za T/T genotip 0,9947(0,295-3,357). Kod bolesnika s karcinomom prostate manje malignosti (Gleason *score* 4-6) udjeli su bili C/C=0,39, C/T=0,47 i T/T=0,14, dok su kod skupine s malignijim tumorima (Gleason *score* 7-9) udjeli iznosili C/C=0,40, C/T=0,53 i T/T=0,07. Udjeli genotipova C677T MTHFR u tumorima veće i manje malignosti nisu se razlikovali (p=0,8693). Rezultati našega istraživanja nisu potvrdili navedenu hipotezu. Na temelju dobivenih rezultata zaključujemo da T alel polimorfizma MTHFR C677T nije povezan s nastankom karcinoma prostate.

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activity and hypomethylation. Many studies investigated C677T in patients with carcinoma and although most authors report a lower frequency of mutated T allele in cancer patients, results are inconclusive.
According to the study hypothesis, a lower frequency of mutated T allele would be expected in patients with prostatic cancer (PC) than in patients with benign prostatic hyperplasia (BPH). A lower frequency would also be expected in tumors of higher malignancy. The study included 45 patients with BPH and 95 patients with prostatic cancer. For carcinoma patients, Gleason score was determined as an index of the degree of malignancy. PCR-RFLP method was used to determine MTHFR C677T polymorphism. Genotypes in both groups were in the Hardy-Weinberg equilibrium. The proportions of genotypes in PC group were C/C=0.40, C/T=0.51 and T/T=0.09, and in BPH group C/C=0.47, C/T=0.42 and T/T=0.11. There was no statistically significant difference between groups in genotype distribution (p=0.6558). Odds ratio and 95% confidence interval for the C/T genotype was 1.3961(0.658-2.963) and for the T/T genotype 0.9947(0.295-3.357). The proportions in the group of patients with prostatic cancer of lower malignancy (Gleason score 4-6) were C/C=0.39, C/T=0.47 and T/T=0.14; and in the group with more malignant prostatic cancer (Gleason score 7-9) C/C=0.40, C/T=0.53 and T/T=0.07. The proportions did not differ between the groups with higher and lower malignancy (p=0.8693). Our study results did not confirm the above hypothesis. According to the findings we conclude that T allele of the MTHFR C677T polymorphism is not associated with prostatic cancer.

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P14-3

Tumorska povezanost proteolitičnih faktora uPA i PAI-1 kod karcinoma štitnjače

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P14-3

Tumor-associated proteolytic factors uPA i PAI-1 in thyroid carcinoma

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U velikom broju tumora sastavnice sustava aktivatora plazminogena, serinska proteaza uPA (urokinaza aktivator plazminogena), inhibitor PAI-1 (inhibitor aktivatora

There is abundant experimental evidence that the plasminogen activator (PA) system with the key components, the serine protease uPA (urokinase-type plasminogen ac-

plazminogena tip 1) i uPA-R CD87 (receptor na površini stanice), imaju važnu ulogu u invaziji i metastaziranju malignih tumora. Mnoga klinička istraživanja pokazala su da se u solidnim tumorima s lošijom prognozom nalaze visoke koncentracije uPA i PAI-1. U karcinomima štitnjače imunohistokemijski je pokazana izraženost uPA i PAI-1, ali nije nađena povezanost s kliničko patološkim parametrima. Mi smo pokazali kako izraženost i aktivacija uPA i PAI-1 u karcinomima štitnjače mogu imati značajnu ulogu u definiranju prognostičkih čimbenika. Koncentracije uPA i PAI-1 izmjerene su metodom ELISA u citosolu tkiva karcinoma štitnjače i u citosolu tkiva zdrave štitnjače u 23 bolesnika (18 žena i 5 muškaraca u dobi od 3-76 godina). Vrijednosti uPA i PAI-1 usporedili smo s veličinom tumora, metastazama u limfnim čvorovima, udaljenim metastazama, ali i s drugim prognostičkim pokazateljima. Značajno visoke koncentracije uPA i PAI-1 nađene su u tkivima karcinoma štitnjače (uPA 1,11ng/mg; PAI-1 15,036 ng/mg) u usporedbi sa zdravim tkivom štitnjače, gdje su nađene niže koncentracije (uPA 0,004 ng/mg; PAI-1 2,349 ng/mg). uPA i PAI su pokazali značajnu različitost u različitim histološkim gradusima (p<0,024; p<0,017). Ovi rezultati su pokazali značajnu razliku između histološkog tipa karcinoma štitnjače i multicentričnosti. Nađena je korelacija između koncentracije uPA i PAI-1 u karcinomima štitnjače (p<0,001; koeficijent korelacije = 0,71). Pokazali smo korelaciju između uPA i PAI-1 i standardnih prognostičkih čimbenika. Buduća istraživanja pokazati će prognostičku ulogu uPA i PAI-1 u karcinomima štitnjače.

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tivator), its inhibitor PAI-1 (plasminogen activator inhibitor type 1), and its cell surface receptor (uPA-R, CD87), play a fundamental role in tumor invasion and metastasis. A large body of clinical data indicate that high levels of uPA and PAI-1 can be used to predict poor prognosis for multiple types of solid tumors. In thyroid cancer very intense and diffuse uPA i PAI-1 immunohistochemical expression has been described, but no relationship between the expression of these proteins and clinicopathologic parameters could be determined. Therefore, we evaluated wheter the expression and activation of uPA and PAI-1 might be of clinical value as a tumor/biologically defined risk faktor in patients with thyriod carcinoma. The levels of uPA and its inhibitor (PAI-1) were measured by use of ELISA in the cytosol of tissue homogenates obtained from thyroid cancer and tumor-free thyroid in 23 patients (18 female and 5 male, median age 56, range 3-76 yrs). All patients were staged according to tumor size, nodal involvement, distant metastasis, and other relevant predictors of prognosis. Significantly higher mean levels of uPA i PAI-1 were found in thyroid carcinoma (uPA 1.11ng/mg; PAI-1 15.036 ng/mg) as compared to tumor-free thyroid (uPA 0.004 ng/mg; PAI-12.349 ng/mg). Both uPA and PAI-1 were significantly different in various histologic grades (p<0.024 and p<0.017, respectively). The uPA and PAI-1 results showed significant differences among thyroid histologic type and multicentricity. There was a correlation between the levels of uPA and PAI-1 expression in cancerous tissue (p<0.001; correlation coefficient = 0.72). We found significant correlation between uPA (urokinase-type plasminogen activator), its inhibitor PAI-1 (plasminogen activator inhibitor type 1) and the standard prognostic parameters in thyroid cancer. Additional investigations have to clarify whether uPA and PAI-1 could be used as independent prognostic factors.

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P14-4

Tumorski biljezi S100 i MIA u praćenju bolesnika s malignim melanomom

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Posljednjih godina provedena su brojna istraživanja vezana uz tumorske biljege za maligni melanom (MM) radi

P14-4

Tumor markers S100 and MIA proteins in the follow up of patients with malignant melanoma

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Much effort has been made to establish a reliable tumor marker for malignant melanoma (MM) in order to select

što ranijeg prepoznavanja bolesnika s lošijom prognozom i mogućnošću praćenja tijeka bolesti te odgovora na terapiju. Nove spoznaje potvrđuju da su proteini S100 i MIA korisni tumorski biljezi za bolesnike s MM. I naše ranije istraživanje potvrdilo je korisnost određivanja serumskih razina proteina S100 i MIA u bolesnika s MM. Cilj ovoga rada bio je usporediti rezultate određivanja S100 i MIA u odnosu na stadij bolesnika s MM tijekom petgodišnjeg praćenja. Razine proteina S100 i MIA u serumu određene su imunoradiometrijskim (IRMA) i imunoenzimskim (EIA) kompletima: Sangtec 100 IRMA (Sangtec Medical, Švedska), CanAg S100 EIA (CanAg Diagnostics, Švedska), Sangtec100 ELISA (DiaSorin, SAD), MIA ELISA (Roche Diagnostics, Njemačka). U statističkoj analizi rabili smo χ^2-test. U istraživanje je bilo uključeno 154 bolesnika s MM (96 žena u dobi od 21-81 godine i 58 muškaraca u dobi od 16-85 godina), kojima je koncentracija proteina S100 i MIA određena najmanje tri ili više puta tijekom razdoblja od 2001. do 2006. godine. Razvrstani su prema stadiju bolesti: I. (n=76), II. (n=50), III. (n=15) i IV. (n=13). Od ukupno 601 učinjene dijagnostičke pretrage (stadij I.-IV.: 268, 222, 65, 46) zabilježeno je 110 (18,3%) povišenih rezultata za S100, te 137 (22,8%) povišenih rezultata za MIA. Postotci povišenih vrijednosti S100 u stadijima I. do IV. bili su: 19%, 17,8%, 15,4% i 21,7%, a postotci povišenih vrijednosti MIA 21,3%, 22,5%, 23,1% i 32,8%. Razina obaju biljega bila je u fiziološkim granicama u 47 (30,5%) bolesnika (stadij I.=14,3%, II.=10,4%, III.=3,2%, IV.=2,6%). Metastaze su dijagnosticirane u 25 (16%) bolesnika, od kojih je 13 bilo u III. stadiju i 12 u IV. stadiju. U skupini bolesnika s metastazama oba su biljega bila povišena u 8 (32%) bolesnika, a oba biljega unutar fizioloških vrijednosti također u 8 (32%) bolesnika. Povišena razina samo S100 nađena je u 2 (8%) bolesnika, a samo povišena razina MIA u 7 (28%) bolesnika. Izvedeni su slijedeći zaključci: 1. Zabilježene su statistički značajne razlike u vrijednosti tumorskih biljega u krvi bolesnika s MM između stadija I. i stadija III. i IV., kao i između stadija II. i stadija III. i IV.; 2. Nije utvrđena statistički značajna razlika u koncentraciji biljega između stadija I. i II. niti između stadija III. i IV.; 3. Postavlja se pitanje zašto je u skupini bolesnika s metastatskim melanomom zabilježen jednak broj bolesnika s oba povišena biljega i s oba biljega u fiziološkim granicama?

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patients with poor prognosis who might benefit from an adjuvant therapy. Moreover, MM markers could be useful in monitoring the clinical course of disease during the treatment. So far, studies have described S100 and MIA (melanoma inhibiting activity) proteins as new suitable tumor markers for MM. Our earlier work confirmed the usefulness of determination of the melanoma markers S100 and MIA serum proteins in patients with melanoma. The aim of this study was to evaluate S100 and MIA results of MM patients according to the stage of disease during the five-year follow up period. Serum MIA and S100 protein levels were measured three or more times during the 5-year follow up period (2001-2006) in patients with MM stage I-IV (AJCC 2002 classification). Serum concentrations of S100 and MIA proteins were determined by immunoradiometric (IRMA) and immunoenzymatic (EIA) kits: Sangtec 100 IRMA (Sangtec Medical, Sweden), CanAg S100 EIA (CanAg Diagnostics, Sweden), Sangtec 100 ELISA (DiaSorin, USA) and MIA ELISA (Roche Diagnostics, Germany). χ^2-test was used on statistical analysis. A total of 154 MM patients (96 women and 58 men, age range 21-81 and 16-85 yrs, respectively) were classified by stages: I (n=76), II (n=50), III (n=15) and IV (n=13).
Of 601 diagnostic tests performed (stage I-IV: 268, 222, 65 and 46, respectively), there were 110 (18.3%) elevated S100 levels and 137 (22.8%) elevated MIA levels. The percentage of elevated levels according to stages I-IV for S100 were 19.0%, 17.8%, 15.4% and 21.7%, and for MIA 21.3%, 22.5%, 23.1% and 32.8%, respectively. Both markers were within the normal range in 47 (30.5%) patients (stage I=14.3%, stage II=10.4%, stage III=3.2% and stage IV=2.6%). Metastases were diagnosed in 25 (16%) patients: 13 stage III and 12 stage IV. In patients with metastases, both markers were increased and within the normal range in 8 (32%) patients each. Only 2 (8%) patients showed an increased level of S100, and only 7 (28%) patients showed an increased level of MIA. Study results pointed to the following conclusions: 1) there were significant differences in serum marker levels between stage I and stages III and IV, as well as between stage II and stages III and IV; 2) there was no significant difference in the level of serum markers between stages I and II, and between stages III and IV; and 3) it remains unclear why there were an equal number of patients with both markers increased or both markers within the normal range among the patients with metastases?

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P15 – Toksikologija i TDM, P15-1 (UP10-1)

Određivanje lamotrigina u plazmi metodom tekućinske kromatografije visokog razlučivanja uz primjenu ultraljubičastog detektora

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Lamotrigin (LTG) je antiepileptik novije generacije djelovanje kojega se očituje u blokiranju potencijala ulaznih vrata natrijskih kanala. Lamotrigin suzbija patološko otpuštanje glutamata i glutamatom izazvano izbijanje akcijskog potencijala. Terapeutsko praćenje LTG je važno kako bi se izbjeglo njegovo toksično djelovanje, kao i utjecaj na ostale antiepileptike. Cilj rada bio je uvesti metodu HPLC za određivanje LTG te istražiti kakav je odnos između primijenjene doze LTG i koncentracije u plazmi. Uzorku plazme (100 μL) dodaje se unutarnji standard (kloramfenikol) te se LTG ekstrahira dodatkom smjese kloroform:izopropanol u omjeru 95:5. Nakon odvajanja organski sloj se odpari do suhog, a LTG otopi u metanolu. Dobiveni ekstrakt (5 μL) injicira se na sustav HPLC. Odvajanje se provodi na koloni C18 uz mobilnu fazu sastava: fosfatni pufer, pH 6,0, acetonitril i metanol u omjeru 70:20:10, pri protoku od 1,0 mL/min. LTG se određuje UV detekcijom pri 214 nm. Vrijeme zadržavanja LTG na koloni je 4,7 minuta, a unutarnjeg standarda 7,8 minuta. Preciznost metode ispitana je određivanjem kontrolnog uzorka plazme kojem su dodavane poznate koncentracije LTG. Koeficijenti varijacije kretali su se između 3,1% i 4,5% za uzastopne analize unutar dana, te između 2,3% i 6,7% među opetovanim analizama tijekom nekoliko dana. Iskorištenje ekstrahiranog LTG kao i unutarnjeg standarda bilo je između 88,1% i 91,7%. Metoda je primijenjena određivanjem koncentracije LTG u 39 djece oboljele od epilepsije, od čega ih e 17 bilo na monoterapiji lamotriginom (4,66 mg/kg), a ostali na kombiniranoj terapiji LTG (3,92-5,66 mg/kg) i ostalim antiepilepticima (VPA, CLB, CZP). Prekoračena dozvoljena koncentracija LTG u plazmi (5 mg/L) pronađena je u 5 bolesnika na monoterapiji i 11 bolesnika na kombiniranim terapijama. Najviše vrijednosti LTG nađene su u bolesnika na kombiniranoj terapiji LTG i VPA. Također je zaključeno kako nema međusobnog odnosa između primijenjene doze LTG i koncentracije u plazmi. Metoda HPLC uz UV detektor može se preporučiti kao metoda izbora za određivanje LTG u plazmi zbog osjetljivosti, preciznosti i jednostavnosti.

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P15 – Toxicology and TDM, P15-1 (UP10-1)

Determination of lamotrigine in plasma by high performance liquid chromatography with UV detection

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Lamotrigine (LTG) is a novel antiepileptic, which has a phenytoin-like membrane stabilizing mechanism *via* blockade of voltage-dependent sodium channels and inhibition of glutamate release. Therapeutic monitoring of lamotrigine is useful for patient management and avoidance of toxicity. The aim of the study was to develop HPLC method for determination of plasma LTG and to investigate the relationship between oral dose and plasma concentration of LTG. To 100 μL of plasma sample the internal standard (chloramphenicol) was added. The extraction was performed by a mixture of chloroform and isopropanol (95:5% v/v) in the presence of phosphate buffer. After evaporation the residue was reconstituted with methanol and injected to HPLC system. The separation was performed on a C18 stainless steel column. Mobile phase consisted of phosphate buffer pH 6.0, acetonitrile and methanol (70:20:10 v/v/v), with a flow rate of 1 mL/min. The detection was obtained by UV detector at 214 nm.The retention times for LTG and IS were 4,7 and 7,8 minutes, respectively. Within- and between-day precision was examined by analysis of control plasma sample with coefficients of variation of 3.1%-4.5% and 2.3%-6.7%, respectively. The absolute recovery of LTG and of IS ranged from 88.1% to 91.7%. The method was used to analyze plasma LTG concentrations from 39 children with epilepsy; 17 samples were obtained from patients receiving LTG monotherapy (4.66 mg/kg), and the others received combined therapy with LTG (3.92-5.66 mg/kg) and other antiepileptics (VPA, CLB, CZP). In 16 plasma samples LTG level exceeded the reference range of 5 mg/L (5 on monotherapy and 11 on combined therapy). The highest plasma LTG levels were found in 4 patients receiving VPA. There was no relationship between oral dose and plasma concentration. The HPLC method with UV detection is the method of choice for the measurement of lamotrigine in plasma because of its sensitivity, selectivity and simplicity.

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P15-2

Serumske koncentracije oligoelemenata u shizofrenih bolesnika i ovisnika o alkoholu

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Literaturni podatci pokazuju kako je u raznim psihijatrijskim poremećajima snižena koncentracija Cu, a povišena koncentracija Zn i Mg. Cilj rada bio je utvrditi serumske koncentracije oligoelemenata (Cu, Zn, Fe i Mg) u bolesnika koji boluju od shizofrenije (n=35), ovisnika o alkoholu (n=38) i kontrolnoj skupini (n= 20) koju su činili zdravi ispitanici. U istraživanje su uključeni svi bolesnici s navedenim dijagnozama koji su se liječili na Klinici za psihijatriju KB Sestre milosrdnice. Dijagnoza shizofrenije ili ovisnosti o alkoholu postavljena je pomoću kriterija MKB 10 te potvrđena pomocu kriterija PANSS (za shizofreniju) i upitnika CAGE (za ovisnost o alkoholu). Koncentracije Cu i Zn određene su metodom atomske apsorpcije na atomskom apsorpcijskom spektrofotometru Zeeman 3030 (Perkin Elmer), dok su Fe i Mg određeni spektrofotometrijskom metodom na analizatoru Olympus AU 2700. Rezultati su pokazali slijedeće srednje vrijednosti (i SD) koncentracije u kontrolnoj skupini, skupini bolesnika sa shizofrenijom i skupini alkoholičara za Cu 19,25±2,36, 16,17±2,93 i 17,44±2,32 µmol/L; Zn 16,19±1,54, 15,52±1,55 i 14,85±1,54 µmol/L; Fe 16,73±5,61, 16,77±6,94 i 18,29±10,09 µmol/L; i Mg 0,91±0,027, 0,86±0,055 i 0,86±0,096 µmol/L. Statistička obrada rezultata pokazala je da je distribucija normalna (Smirnoff test). Kako su sve distribucije bile normalne, primijenjen je parametrijski test, a s obzirom na više od dvije skupine ispitanika rabili smo test Anova. Anova je pokazala kako postoji statistički značajna razlika između testiranih skupina (p=0,035), a Student-Newman-Keulsov test pokazuje da postoje statistički značajne međusobne razlike između skupina, p<0,05 za Cu, Zn i Mg, ali ne i za Fe. Rezultati potvrđuju kako je koncentracija Cu niža u psihijatrijskih bolesnika u odnosu na kontrolnu skupinu, tako da je u bolesnika sa shizofrenijom niža negoli u alkoholičara, što je u skladu s literaturnim podatcima. Koncentracija Zn je niža u shizofrenih bolesnika, nešto viša u alkoholičara i još viša u kontrolnoj skupini, što ne odgovara literaturnim podatcima. Koncentracija Mg je viša u kontrolnoj skupini u odnosu na shizofrene bolesnike i alkoholičare, što također ne odgovara literaturnim podatcima. Koncentracije Fe ne pokazuju značajne razlike između skupina.

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P15-2

Serum oligoelement concentrations in schizophrenia patients and alcohol addicts

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Literature data indicate the concentration of Cu to be decreased, and the concentrations of Zn and Mg to be increased in various psychiatric disorders. The aim of the study was to determine serum concentrations of oligoelements (Cu, Zn, Fe and Mg) in schizophrenic patients (n=35), alcoholics (n=38) and control group of healthy subjects (n=20). The study included all patients with the above diagnoses treated at University Department of Psychiatry, Sestre milosrdnice University Hospital. The diagnosis of schizophrenia or alcohol dependence was made on the basis of ICD 10 criteria and confirmed by use of PANSS criteria (for schizophrenia) and CAGE questionnaire (for alcohol dependence). Cu and Zn concentrations were determined by the method of atomic absorption on a Zeeman 3030 absorption spectrophotometer (Perkin Elmer), while Fe and Mg concentrations were determined by spectrophotometric method on an Olympus AU 2700 analyzer. Results showed the following mean (±SD) concentrations in the control group, group of schizophrenia patients and group of alcoholics: Cu 19.25±2.36, 16.17±2.93 and 17.44±2.32 µmol/L; Zn 16.19±1.54, 15.52±1.55 and 14.85±1.54 µmol/L; Fe 16.73±5.61, 16.77±6.94 and 18.29±10.09 µmol/L; and Mg 0.91±0.027, 0.86±0.055 and 0.86±0.096 µmol/L, respectively. Statistical analysis of the results showed normal distribution (Smirnoff test). As all distributions were normal, the parametric test was used, while Anova test was employed considering more than two subject groups. Anova yielded a statistically significant difference between study groups (p=0.035), whereas Student-Newman-Keuls test revealed statistically significant between-group differences: p<0.05 for Cu, Zn and Mg but not for Fe. Study results confirmed the concentration of Cu to be lower in psychiatric patients as compared with control group; it was lower in schizophrenic patients than in alcoholics, which is consistent with literature data. The concentration of Zn was decreased in schizophrenic patients, slightly higher in alcoholics, and even higher in the control group, which is inconsistent with literature data. The concentration of Mg was higher in the control group as compared with schizophrenic patients and alcoholics, which is also in disagreement with literature data. The concentration of Fe showed no significant between-group differences.

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P15-3 (UP10-2)

Naša iskustva u primjeni imunokemijskih testova u toksikološkom prosijavanju na lijekove i sredstva ovisnosti

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Imunokemijsko testiranje mokraće na lijekove i sredstva ovisnosti općenito se rabi za brzo prosijavanje u jedinicama hitne medicinske pomoći i bolnicama kada bolesnici pokazuju znakove intoksikacije ili predoziranja. U svrhu toksikološkog prosijavanja u Općoj bolnici Sveti Duh rabi se imunokemijski kompetitivni test za toksikološko prosijavanje s 10 parametara (lijekova i sredstava ovisnosti). Cilj je prikazati naša iskustva u primjeni imunokemijskih testova s obzirom na poznata ograničenja u njihovoj upotrebi. Retrospektivno su obrađeni rezultati analiza izvršenih tijekom 2004. i 2005. godine. Sve kvalitativne analize mokraće izvršene su primjenom imunotesta MultiDrug control (kat. Br. 008A410, UltiMed, Njemačka), koji sadrži 10 parametara: amfetamini, barbiturati, benzodiazepini, kokain, metamfetamin, morfin, metadon, ecstasy, triciklični antidepresivi i tetrahidrokanabinol. Semikvantitativna analiza benzodiazepina u serumu i opijata u mokraći učinjena je metodom EMIT na analizatoru Dimension RxL, Dade Behring. Ukupno su analizirana 204 uzorka u 2004. godini i 228 uzoraka u 2005. godini, od čega ih je bilo 31 odnosno 75 negativnih (nije ustanovljena prisutnost lijeka ili opojnih sredstava). Kod pozitivnih rezultata prevladavali su sami benzodiazepini (48% odnosno 40%) ili u kombinaciji s tricikličnim antidepresivima (15% odnosno 8,5%), te u kombinaciji s opojnim sredstvima (26% odnosno 25%). Ostalo su bili samo triciklični antidepresivi, odnosno samo opojna sredstva (morfin/heroin, metadon, kokain, marihuana). Usporedna semikvantitativna određivanja benzodiazepina u serumu i opijata u mokraći pokazala su kod benzodiazepina 31% odnosno 11% negativnih rezultata usprkos pozitivnom nalazu u mokraći. Pozitivni kvalitativni i kvantitativni nalazi nađeni su u 69% odnosno 89% slučajeva. Kod kvantitativnog određivanja opijata u mokraći bilo je 20% odnosno 7,7% negativnih rezultata. Unatoč jednostavnosti izvedbe i brzini dobivanja rezultata, imunokemijske kvalitativne testove u toksikološkom prosijavanju treba rabiti pažljivo uzimajući u obzir sve moguće uzroke lažno pozitivnih i negativnih rezultata.

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P15-3 (UP10-2)

Our experience with immunochemistry testing on screening for drugs and abuse substances

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Immunochemical testing of urine for drug and substance of abuse screening is generally used in emergency units and hospitals for the treatment of intoxicated or overdosed patients. At our Department immunochemical competitive test for toxicologic screening with 10 parameters is applied.

The aim of this report is to present our experience in the use of immunochemical tests, considering their known limitations of application. Data on testing performed during 2004 and 2005 were retrospectively analyzed. All qualitative urine analyses were done by use of the Multi-Drug Control immunoassay (No. 008A410, UltiMed, Germany), consisting of 10 parameters: amphetamines, barbiturates, benzodiazepines, cocaine, metamphetamine, morphine, methadone, ecstasy, tricyclic antidepressants, and tetrahydrocannabinol. Semiquantitative determination of benzodiazepines in serum and opiates in urine was done by EMIT method on a Dimension RxL analyzer (Dade Boehringer). A total of 204 and 228 samples were analyzed in 2004 and 2005 (31 and 75 negative, i.e. the presence of drugs or substances abuse was not proved), respectively. Positive results mostly referred to benzodiazepines (48% and 40%) or their combination with tricyclic antidepressants (15% and 8.5%) or opiates (26% and 25%), respectively; the rest were tricyclic antidepressants or opiates alone (morphine/heroin, methadone, cocaine, marijuana). Parallel semiquantitative determination of benzodiazepines in serum and opiates in urine yielded 31% and 11% of negative findings for benzodiazepines in spite of positive urine finding. Positive qualitative and quantitative findings were recorded in 69% and 89% of cases. Quantitative determination of opiates in urine showed 20% and 7.7% of negative results. In spite of the simple procedure and rapid result production, the immunochemical qualitative tests in toxicologic screening should be very carefully used, with due consideration of the possible causes for false-positive and negative results.

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P15-4

Koncentracija beta-endorfina u serumu i mozgu štakora izloženih trazodonu

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Desetak supstanca sličnih opijatima u koje spadaju endorfini, met- i leu-enkefalini i dinorfin nađeno je u različitim dijelovima živčanoga sustava. Beta-endorfini su otkriveni u hipotalamusu i u hipofizi, ali imaju, u manjoj mjeri, lokalizaciju i u nekim drugim organima. Cilj našega istraživanja bio je ustanoviti u kojoj mjeri psihoaktivni lijekovi utiču na koncentraciju beta-endorfina u serumu i mozgu eksperimentalnih životinja. Istraživanja su provedena na albino štakorima soja Wistar, uz primjenu pripravka trazodon iz skupine antidepresiva. Za određivanje koncentracije beta-endorfina u serumu i mozgu štakora primijenjena je tehnika RIA. Dobiveni rezultati pokazuju značajne razlike u koncentraciji beta-endorfina u serumu i mozgu životinja kojima se je davao trazodon u odnosu na kontrolnu skupinu životinja koje su dobivale fiziološku otopinu. Ovo istraživanje ukazuje na to da uzimanje psihoaktivnih lijekovia utiče na koncentraciju beta-endorfina u serumu i mozgu, te da bi beta-endorfini mogli korisno poslužiti u procjeni učinaka psihoaktivnih lijekova. Ujedno bi praćenje koncentracije beta-endorfina moglo biti vrijednim pokazateljem u terapiji ovim farmakološkim pripravcima.

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P15-4

Brain and serum beta-endorphin concentration in trazodone treated rats

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A dozen of opioid-like substances that include endorphins, met- and leu-enkephalins and dinorphin have been found in different parts of the nervous system. Beta-endorphins have been detected in the hypothalamus and hypophysis, and in a small amount in other organs. The aim of our study was to establish the extent to which psychotropic drugs influence serum and brain beta-endorphins in experimental animals. The study was performed on albino Wistar rats, using the antidepressant trazodone. RIA technique was employed for quantification of serum and brain beta-endorphins. Our study showed a significant difference in serum and brain beta-endorphin concentrations between trazodone treated animals and control group of animals administered 0.95% NaCl. The study showed the use of psychoactive drugs to influence serum and brain beta-endorphin concentrations. Beta-endorphins could be used as valuable markers to evaluate effects of psychoactive drugs, and a useful parameter in therapy with these psychopharmaceuticals.

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P15-5

Koncentracije alfa-1-mikroglobulina u mokraći stanovnika istočne Hrvatske izloženih arsenu pitkom vodom

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Određene su koncentracije arsena i alfa-1-mikroglobulina u mokraći stanovnika Osijeka i Andrijaševaca koji piju vodu s različitim koncentracijama arsena, 37,9 μg/L (Osijek) i 612 μg/L (Andrijaševci). Ukupna koncentracija arsena

P15-5

Urine levels of alpha-1-microglobulin among residents of eastern Croatia exposed to high concentrations of arsenic in drinking water

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We determined urine levels of arsenic and alfa-1-microglobulin in the groups of Osijek and Andrijaševci residents at chronic exposure to high concentratinos of arsenic in drinking water (37.9 μg/L and 612 μg/L, respectively). Total

određena je hidridnom atomskom apsorpcijskom spektrometrijom (HGAAS), kreatinina kinetičkom metodom po Jaffeu, a alfa-1-mikroglobulina imunonefelometrijski. Srednje koncentracije arsena u prvoj jutarnjoj mokraći bile su 28,2 mg/g kreatinina (Osijek n=16) i 653,7 mg/g kreatinina (Andrijaševci n=34). Skupine su se statistički značajno razlikovale (p<0,001). Srednja koncentracija alfa-1-mikroglobulina u mokraći izložene skupine bila je 7,6 mg/g kreatinina, a kontrolne skupine 7,2 mg/g kreatinina. Nije bilo statistički značajne razlike u koncentraciji alfa-1-mikroglobulina. Iz rezultata zaključujemo da je kod ispitanika iz Andrijaševaca prisutna vrlo visoka koncentracija arsena u mokraći i zbog toga postoji visok rizik toksičnosti arsena. Budući da nije bilo razlike u koncentracijama alfa-1-mikroglobulina kao biljega oštećenja bubrega u odnosu na skupinu iz Osijeka s niskom koncentracijom arsena u mokraći, potrebno je ispitati druge biljege oštećenja tubula bubrega.

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arsenic was determined using hydride generation atomic absorption spectrometry (HGAAS). Creatinine was determined using Jaffe rate method, and alfa-1-microglobulin using nephelometric immunoassay. The mean value of total arsenic in first void urine of the control group (Osijek, n=16) and exposed group (Andrijaševci, n=34) was 28.2 mg/g creatinine and 653.7 mg/g creatinine, respectively. There was a statistically significant difference between the two groups (p<0.001). The respective mean concentrations of alfa-1-microglobulin were 7.2 mg/g creatinine and 7.6 mg/g creatinine. There was no statistically significant difference between the two groups. According to the results, urine levels of arsenic were seriously high and probably toxic in the group of Andrijaševci residents. Since alfa-1-microglobulin as a marker of tubular damage showed no difference between the two groups, it is necessary to examine other markers of tubular damage.

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P15-6

Katalitička koncentracija ukupne glutation S-transferaze (GSTs) u serumu zdravih ispitanika i bolesnika sa stabilnom KOPB

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Kronična opstrukcijska plućna bolest (KOPB) je stanje obilježeno smanjenjem protoka zraka kroz dišne putove te progresivnim i ireverzibilnim smanjenjem forsiranog ekspiracijskog volumena u 1. sekundi (FEV1). Više od 90% bolesnika s KOPB su pušači, ali se KOPB ne razvije kod svih pušača. Dim cigarete sadrži više od 1016-1017 oksidacijsih molekula po oblačiću dima i to je važan uzrok oksidativnog stresa u KOPB. Glutation S-transferaze (GSTs) štite stanicu protiv brojnih oksidacijskih molekula nastalih iz dima cigarete. Cilj ovoga rada bio je procijeniti postoji li u serumu skupine bolesnika sa stabilnom KOPB (pušači i nepušači) promjena u katalitičkoj koncentraciji GSTs, alfa 1-antitripsina (AAT) i laktat dehidrogenaze (LDH) u usporedbi sa zdravim isptanicima (pušači i nepušači) koji su činili kontrolnu skupinu. Ispitivanja su izvršena kod ukupno 90 ispitanika (muškarci, starija dobna skupina). Prvu skupinu činilo je 60 zdravih ispitanika (kontrolna skupina,

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Total glutathione S-transferase (GSTs) activity in serum of healthy subjects and patients with stable COPD

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Chronic obstructive pulmonary disease (COPD) is an obstructive airway disorder characterized by a slowly progressive and irreversible decrease in FEV1. More than 90% of patients with COPD are smokers, but not all smokers develop COPD. Cigarette smoke containing more than 1016-1017 oxidant molecules *per* puff is an important cause of oxidative stress in COPD. Glutathione S-transferases (GSTs) protect cells against numerous oxidant generating compounds from cigarette smoke. The aim of this study was to assess the possible changes in the catalytic concentrations of GSTs, alpha 1-antitrypsin (AAT) and lactate dehydrogenase (LDH) in serum of elderly men with stable COPD (smokers and non-smokers) in comparison to age-matched healthy control men (smokers and non-smokers). A total of 90 subjects were included in the study. Group 1 included 60 healthy men (control group, mean age 63±5): 19 non-smokers, 16 ex-smokers and 25

srednje dobi 63±5 godina): 19 nepušača, 16 bivših pušača i 25 pušača. Drugu skupinu činilo je 30 bolesnika sa stabilnom KOPB (srednje dobi 65±7 godina): 12 bivših pušača, 14 pušača i 4 nepušača. Venska krv je uzorkovana u jutarnjim satima prije medikacije. Katalitička koncentracija GSTs izmjerena je novom, brzom spektrofotometrijskom metodom koju su opisali Habdous i sur. Koncentracija AAT određena je metodom RID. Aktivnost LDH mjerena je standardnom metodom upotrebom testa za analizator Olympus AU 400. Rezultati pokazuju da je ukupna aktivnost GSTs u serumu bolesnika sa stabilnom KOPB niža (medijan: 67 U/L (21-104 U/L) u usporedbi sa zdravom kontrolnom skupinom (medijan: 68,5 U/L (38-111 U/L), ali ta razlika nije bila statistički značajna (p>0,05). Ovaj trend pada aktivnosti GSTs u bolesnika s KOPB može se objasniti padom koncentracije reduciranog glutationa (GSH) u KOPB, jer je GSH kofaktor raznih enzima koji smanjuju oksidacijski stres. Dobiveni rezultati koncentracije ukupnog AAT (protein akutne faze) za skupinu bolesnika s KOPB bili su značajno viši u usporedbi s kontrolnom skupinom (p<0,05). Kao biljeg povećanog anaerobnog metabolizma mjerena je LDH aktivnost. Dobivene vrijednosti za ukupnu LDH aktivnost u bolesnika s KOPB bile su statistički značajno više u usporedbi s kontrolom (p<0,05). Nakon provedene analize ROC zaključujemo da GSTs, AAT i LDH imaju malu učinkovitost za razlikovanje bolesnika sa stabilnom KOPB od zdravih kontrolnih ispitanika.

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current life-long smokers. Group 2 included 30 patients with stable COPD (mean age 65±7): 12 ex-smokers, 14 current life-long smokers and 4 that had never smoked. Blood was collected by venipuncture during morning hours and prior to administration of any medication. GSTs activity was determined by a new, rapid spectrophotometric method described by Habdous *et al*. AAT concentration was determined by RID technique. LDH activity was measured by standard method using available tests for the Olympus AU 400 analyzer. Analysis of GSTs activity in serum of all COPD patient subgroups showed lower values (median: 67 U/L (21-104 U/L) as compared with the respective control group (median: 68.5 U/L (38-111 U/L) but the differences were not statistically significant (p>0.05). This decreasing trend of all GSTs activity in COPD patients could probably be explained by the fall of GSH level in COPD, since GSH is a cofactor for various enzymes that decrease oxidative stress. The results on total AAT (acute phase protein) concentrations in COPD patients were significantly higher as compared with control group (p<0.05). LDH activity was measured as a marker of enhanced anaerobic metabolism. The value of total LDH activity in COPD patients was statistically significantly higher as compared with control total (p<0.05). From ROC analysis we concluded that GSTs, AAT and LDH had low accuracy in differentiating patients with stable COPD and healthy controls.

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P15-7

Farmakogenetička analiza i individualizacija terapije u liječenju epilepsije – prikaz slučaja

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Među važne genske kandidate u farmakogenetičkoj analizi bolesnika od epilepsije ubrajamo gene koji kodiraju proteine izravno uključene u biotransformaciju i prijenos lijeka kroz membrane. Reakcije 1. faze metabolizma uključuju skupinu citokroma P450 (CYP), među kojima su za antiepilepsijske i psihotropne lijekove najvažniji CYP2C9, CYP2C19, CYP3A4, CYP2D6. Postoji nekoliko vrsta proteina koji prenose lijek u organizmu, a koji su relevantni s

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Pharmacogenetic analysis and therapy individualization in epilepsy treatment – a case report

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Genes coding the proteins directly included in biotransformation and membrane drug transport belong to important candidate genes in pharmacogenetic analysis of epileptic patients. Phase I metabolic reactions involve a group of cytochromes P450 (CYP), among which CYP2C9, CYP2C19, CYP3A4 and CYP2D6 are most important for antiepileptic and psychotropic drugs. There are several types of drug-transporting proteins in the body, which

farmakogenetičkoga stajališta. Jedna takva skupina su proteini ABC, koji su izraženi u mnogim tkivima od probavnog sustava, jetre, bubrega do krvno-moždane brane, te stoga mogu utjecati na bioraspoloživost, odnosno učinkovitost/toksičnost lijeka od periferije do središnjega živčanog sustava. Proteini ABC su proizvodi gena MDR (*multidrug resistance genes*). Prototipna molekula je P-glikoprotein (P-gp) koji je prepoznat kao ključni element u reguliranju pristupa niza terapijskih agenasa u mozak i druga tkiva. Učestalost psihičkih poremećaja je znatno viša među bolesnicima s epilepsijom nego u općoj populaciji. Kako se u tih bolesnika antiepilepsijski i psihotropni lijekovi moraju primjenjivati u kombinaciji, rizik od farmakokinetskih interakcija na razini metaboličkih enzima i transportnih proteina je relativno visok. U ovom radu prikazan je 10-godišnji bolesnik s refraktornom epilepsijom i psihičkim poremećajem. U kliničkom tijeku bolesti su uz nekontrolirane epilepsijske napade značajni i različiti oblici nuspojava koji su se mogli pripisati primjenjivanim lijekovima. Farmakogenetički nalaz (PCR-RFLP i *real time* PCR): (CYP2C9-*1/*1, CYP2C19-1/*2-, CYP3A4-*1/*1, CYP2D6-*4/*6, MDR1-2677T/T, 3435T/T i SERTPR-S/S, SERTin2-s/l). Prema genotipu, bolesnik pripada intermedijarnom metaboličkom fenotipu za CYP2C19, sporom metaboličkom fenotipu za CYP2D6 i ima značajno sniženu ekspresiju P-glikoproteina. Fenotipizacija CYP2D6 s dekstrometorfanom (GC-MS) ukazivala je također na spori metabolički fenotip (MR>0,3). Terapijsko praćenje koncentracija lijekova pokazivalo je vrijednosti za lamotrigin u rasponu od 20-60 µmol/L (HPLC), a za valproate od 350-650 µmol/L (imunokemijska metoda). Prema farmakogenetičkom nalazu možemo zaključiti da su se u bolesnika pri standardnim dozama mogle nakupiti značajno povišene koncentracije lijekova supstrata CYP2D6, CYP2C19 i P-gp, što je moglo dovesti do različitih oblika neželjenih reakcija na lijekove. U ovom je slučaju farmakoterapija bila prilagođena na osnovi farmakogenetičkog nalaza.

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are of pharmacogenetic relevance. One such group are ABC proteins, which are expressed in many tissues, from gastrointestinal tract, liver, kidneys to blood brain barrier, and may therefore affect the availability or efficacy/toxicity of a drug from peripheral to central nervous system. ABC proteins are products of multidrug resistance genes (MDR). The prototype molecule is P-glycoprotein (P-gp), which has been recognized as a key element in regulating access of a series of therapeutic agents to brain and other tissues. The frequency of mental disturbances is considerably higher among epileptic patients than in general population. As a combination of antiepileptic and psychotropic medications must be used in these patients, the risk of pharmacokinetic interactions at the level of metabolic enzymes and transport proteins is relatively high. In this study, a 10-year-old patient with refractory epilepsy and mental disturbance is presented. Besides uncontrollable epileptic seizures, various types of side effects were pronounced in the clinical course of the disease, and they could be ascribed to the drugs administered. Pharmacogenetic results (PCR-RFLP and real time PCR): (CYP2C9-*1/*1, CYP2C19-1/*2-, CYP3A4-*1/*1, CYP2D6-*4/*6, MDR1-2677T/T, 3435T/T i SERTPR-S/S, SERTin2-s/l). According to genotype, the patient had intermediary metabolic phenotype for CYP2C19 and slow metabolic phenotype for CYP2D6, and a significantly decreased P-gp expression. CYP2D6 phenotyping with dextromethorphan (GC-MS) also suggested slow metabolic phenotype (MR>0.3). Therapeutic monitoring of drug concentrations showed lamotrigine values to range between 20-60 µmol/L (HPLC), and valproate values between 350-650 µmol/L (immunochemical method). Based on pharmacogenetic results, we may conclude that significantly increased CYP2D6, CYP2C19 and P-gp drug substrate concentrations could accumulate under standard drug doses and thus lead to various types of adverse drug reactions. In this case pharmacotherapy was adjusted on the basis of pharmacogenetic results.

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Fenotipizacija s dekstrometorfanom u procjeni metaboličkog kapaciteta enzima CYP2D6 i dometa interakcija lijekova

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Genetički utemeljene razlike u aktivnosti metaboličkih enzima koji metaboliziraju lijekove prepoznate su kao glavni izvor farmakokinetičkih varijabilnosti između bolesnika. Polimorfizam gena CYP2D6 određuje četiri kategorije fenotipova s obzirom na intenzivnost metabolizma: brzi, spori, intermedijarni i vrlo brzi. Metabolički fenotip može se odrediti fenotipizacijom ili genotipizacijom. Fenotipizacija se izvodi pomoću testnog lijeka metabolizam kojega isključivo ovisi o funkciji ispitivanog enzima. Prednost fenotipizacije je u mogućnosti praćenja interakcija lijekova koje bolesnik mora istodobno uzimati. Fenotipizacija za enzim CYP2D6 najčešće se provodi testnim lijekom dekstrometorfanom. Cilj ovoga rada bio je ispitati metabolički kapacitet enzima CYP2D6 u psihijatrijskih bolesnika (n=18) na terapiji paroksetinom (20 mg/dan) i maprotilinom (50 mg/dan). Paroksetin je supstrat i snažan inhibitor enzima CYP2D6, dok je maprotilin samo supstrat navedenog enzima. Fenotipizacija je provedena prije početka terapije i 14. dana terapije. U 8-satnom uzorku mokraće metodom GC-MS određen je dekstrometorfan (DM) i njegov metabolit dekstrorfan (DX). Iz omjera vrijednosti navedenih analita određen je metabolički omjer (MR) koji predviđa metabolički kapacitet enzima CYP2D6. Vrijednosti MR prije terapije bile su u rasponu od 0,001-0,034 (n=18; medijan 0,006), dok su nakon postizanja ravnoteže primijenjenih lijekova (14. dan) bile u rasponu od 0,084-1,239 (n=18; medijan 0,24). Dobiveni su rezultati ukazali na značajan inhibicijski učinak paroksetina. Ispitanici, vrlo brzi i brzi metabolizatori, postali su intermedijarni i spori metabolizatori (fenokopiranje). Pri politerapiji lijekovima koji se metaboliziraju putem enzima CYP2D6, osobito ako su neki od njih inhibitori ili induktori enzima, mogu nastati značajne interakcije lijekova koje mogu rezultirati promijenjenim terapijskim učincima i razvojem štetnih nuspojava. Metoda fenotipizacije CYP2D6 dekstrometorfanom je brza, jednostavna i pouzdan je pokazatelj metaboličkog kapaciteta enzima.

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Dextromethorphan phenotyping in assessing CYP2D6 enzyme metabolic capacity and drug interaction

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Genetically based differences in the activity of drug-metabolizing enzymes have been recognized as a principal source of pharmacokinetic variabilities among patients. CYP2D6 gene polymorphism determines four phenotype categories depending on the metabolism intensity: extensive (EM), poor (PM), intermediary (IM) and ultraextensive (UEM) metabolizing capacity. Metabolic phenotype can be determined by phenotyping or genotyping. Phenotyping is performed by a test drug the metabolism of which depends solely on the function of the enzyme examined. The advantage of phenotyping is the possibility of monitoring interactions of the drugs concomitantly administered to the patient. CYP2D6 enzyme phenotyping is most often performed by using dextromethorphan as a test drug. The aim of this study was to investigate the metabolic ratio of CYP2D6 enzyme in psychiatric patients (n=18) on paroxetine (20 mg/day) and maprotiline (50 mg/day) therapy. Paroxetine is a substrate and a potent inhibitor of CYP2D6 enzyme, while maprotiline is only a substrate of this enzyme. Phenotyping was performed prior to and on day 14 of therapy. Dextromethorphan (DM) and its metabolite dextrorphan (DX) were determined in 8-h urine sample by GC-MS method. Metabolic ratio (MR) was determined from the value of the mentioned analytes that predict metabolic capacity for CYP2D6 enzyme. Pretherapeutic MR values ranged from 0.001-0.034 (n=18; median 0.006), while having achieved balance between the drugs administered (day 14) they ranged from 0.084 to 1.239 (n=18; median 0.24). The results obtained indicated a significant inhibitory effect of paroxetine. The subjects who had been UEM and EM became IM and PM (phenocopying). Polytherapy with drugs that are metabolized by CYP2D6, particularly if some of them are enzyme inhibitors or inductors, may result in significant drug interactions with the possible altered therapeutic effects and development of adverse side effects. The method of CYP2D6 phenotyping with dextromethorphan is rapid and simple, and a reliable indicator of the enyzme metabolic capacity.

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P15-9

Analitička validacija testa Seradyn-Innfluor za određivanje koncentracije everolimusa

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Jedan od preduvjeta za čim bolji ishod transplantiranog bolesnika svakako je izabrati optimalnu individualnu kombinaciju imunosupresivne terapije. Everolimus je novi antiproliferativni imunosupresivni lijek koji se rabi u transplantaciji solidnih organa. On je dio imunosupresivnih protokola, obično u kombinaciji s inhibitorom kalcineurina ili s mikofenolatom, uz istodobnu primjenu steroida ili bez nje. Terapijsko praćenje everolimusa rezultira smanjenom incidencijom akutnog odbacivanja i manjom incidencijom nuspojava u primatelja solidnih organa liječenih everolimusom. Zbog kraćeg poluživota everolimusa lakše se postiže ciljni učinak i ujednačeniji terapijski raspon lijeka. Stoga smo za potrebe bolesnika u kojih je učinjena transplantacija bubrega u našoj ustanovi evaluirali test Seradyn-Innofluor za everolimus, koji je zasad jedini dostupan na tržištu. Test Seradyn-Innofluor za određivanje koncentracije everolimusa koncipiran je na načelu imunokemijskog određivanja uz ekscitaciju fluorofora polariziranim svjetlom (metoda FPIA). Sva određivanja učinjena su na analizatoru TDX tvrtke Abbott. Kratka analitička validacija testa obuhvaćala je: nepreciznost u seriji, nepreciznost iz dana u dan, netočnost, osjetljivost i linearnost. Kao terapijsko područje testa navodi se koncentracija od 3-8 μg/L. Nepreciznost u seriji za nisko, srednje i visoko koncentracijsko područje (n=10) iznosila je: 7,2%, 7,9% i 3,4%. Pripadajuća netočnost za nisko, srednje i visoko koncentracijsko područje, izražena na komercijalnim kontrolnim uzorcima, iznosila je 2,5%, 10,8% i 6,1%. Nepreciznost iz dana u dan u navedenim kontrolnim uzorcima testa (nisko, srednje, visoko, n=10) iznosila je 18,5%, 17,5% i 18,5%. Dilucijom uzoraka dokazali smo donju granicu osjetljivosti od 2 μg/L i linearnost od 40 μg/L. Dobivena je zadovoljavajuća analitička validacija testa. Ističemo nešto lošiju nepreciznost iz dana u dan, KVa=18,5%, što smatramo nedostatkom testa.

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Analytical validation of Seradyn-Innfluor assay for everolimus measurement

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Individualized optimal combination of immunosuppressive therapy is one of the necessary prerequisites for the best possible outcome of transplant patients. Everolimus is a novel antiproliferative immunosuppressant used in solid organ transplantation. It is a part of immunosuppressive protocols, usually in combination with calcineurine inhibitor, or with micophenolate, with or without simultaneous steroid administration. Therapeutic drug monitoring of everolimus results in a decreased incidence of acute rejection and lower incidence of side effects in solid organ recipients treated with this immunosuppressive drug. The shorter half-life of everolimus facilitates achieving the target effect and a more homogeneous therapeutic scope of the drug. Therefore, for the needs of patients who had kidney transplantation performed at our Hospital, we evaluated the everolimus Seradyn-Innofluor assay, which is currently the sole such assay available on the market. The Seradyn-Innofluor assay for measuring the everolimus concentration is based on the principle of immunochemical determination with fluorophore excitation with polarized light (FPIA method). All determinations were performed on an Abbott TDx analyzer. Short analytical validation of the assay included the following: imprecision in series, day to day imprecision, inaccuracy, sensitivity, and linearity. The concentration range between 3 and 8 μg/L was set as a therapeutic scope of the assay. Within-series imprecision for the low, medium and high concentration range (n=10) was 7.2%, 7.9% and 3.4%, respectively. Inaccuracy for the low, medium and high concentration range, expressed on the commercial control samples, was 2.5%, 10.8% and 6.1%, respectively. Day-to-day imprecision in the samples stated above (low, medium and high, n=10) was 18.5%, 17.5% and 18.5%, respectively. Using sample dilution we demonstrated minimum sensitivity level of 2 μg/L and linearity of 40 μg/L. Satisfactory analytical assay validation was obtained. We point out a somewhat poorer day-to-day imprecision rate, KVa=18.5%, which we consider to be a limitation of the assay.

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P15-10

Prikaz slučaja: otrovanje etilen-glikolom

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U jedinicu intenzivne skrbi zaprimljen je četrdesetgodišnji muškarac poslije pokušaja samoubojstva. Bolesnik je pronađen kod kuće, bez svijesti, a pokraj njega je bila otvorena boca antifriza. Glavni sastojak antifriza je etilen-glikol koji nije toksičan, ali su toksični njegovi metaboliti, glikolna i oksalna kiselina, jer uzrokuju oštećenja organa i smrt. Kod prijma je bolesnik bio bez svijesti, a kliničkom obradom isključena su neurološka oštećenja. Rezultati početnoga toksikološkog probiranja seruma i mokraće bili su negativni. Laboratorijski rezultati ukazivali su na metaboličku acidozu (pH 7,123; pCO_2 2,03 kPa) s leukocitozom (L 32,0 10^9/L). Analiza mokraće pokazala je prisutnost hematurije i amorfnih kristala. Osmolalnost i osmotski procijep nisu mjereni kod prijma bolesnika. Zbog metaboličke acidoze, anamnestičkih podataka i kliničkog stanja bolesnika započeto je liječenje etilnim alkoholom kao protulijekom te hemodijaliza, pa se bolesnikovo stanje postupno poboljšalo. Naknadna toksikološka analiza glikolne i oksalne kiseline u serumu i mokraći napravljena je ionskom kromatografijom metodom HPLC. Vrijednosti glikolne kiseline u slučajnom uzorku mokraće bile su kod prijma 28171,2, a poslije hemodijalize 3,01 mmol/mol kreatinina, a oksalne kiseline 370,5 kod prijma i 80,5 mmol/mol kreatinina nakon hemodijalize. U ultrafiltratu seruma dobivene vrijednosti za navedene kiseline bile su: glikolna kiselina 17661,0 mmol/L kod prijma, 35,2 mmol/L poslije hemodijalize; oksalna kiselina 353,5 mmol/L kod prijma, 19,5 mmol/L poslije hemodijalize. Otrovanje etilen-glikolom uzrokuje tešku metaboličku acidozu koja može izazvati smrt ako dijagnoza nije pravodobna i ako se specifično liječenje ne započne na vrijeme. Laboratorijski testovi značajno mogu pridonijeti bržem postavljanju dijagnoze, a uključuju: određivanje plinova u krvi s anionskim procijepom, povišenu osmolalnost s osmotskim procijepom, prisutnost kristala kalcijevog oksalata u mokraći i mjerenje etilen-glikola u serumu, ako je moguće. Prema preporukama NACB klinički laboratorij mora osigurati mjerenje etilen-glikola u serumu ili plazmi, a ako se rabi metoda GC, tada analiza mora uključivati i toksični metabolit glikolnu kiselinu.

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P15-10

Case report: ethylene glycol poisoning

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A 40-year-old man was admitted to the emergency department after a suicide attempt. The patient was found at home unconscious, with an open bottle of antifreeze near him. The main component of antifreeze is ethylene glycol that is not toxic but produces toxic metabolites that cause organ failure and death. The patient was in coma on admission, but was neurologically intact. Results of initial urine and serum toxicological screening tests were negative. Laboratory values indicated metabolic acidosis (pH 7.123; pCO_2 2.03 kPa) with leukocytosis (L 32.0 10^9/L). Urinalysis revealed hematuria and amorphous crystals. Osmolality and osmol gap were not determined at patient admission. Treatment with ethanol as an antidote and hemodialysis were started because of metabolic acidosis, history data and clinical status of the patient, and subsequently led to improvement of his condition.
Further toxicological analyses of glycolic and oxalic acids in serum and urine samples were performed by the ion-chromatography HPLC method. Results of glycolic and oxalic acid tests in spot urine were 28171.2 and 370.5 mmol/mol creatinine, and 3.01 and 80.5 at admision and post-hemodialysis, respectively. The values of glycolic and oxalic acids in serum ultrafiltrate were 17661.0 and 353.5 mmol/L, and 35.2 and 19.5 mmol/L at admission and post-hemodialysis. Intoxication by ethylene glycol causes severe metabolic acidosis which may lead to death if diagnosis is delayed and specific treatment is not initiated promptly. Laboratory tests for ethylene glycol poisoning include determination of blood gases with anion gap, elevated osmolality with osmol gap, calcium oxalate in urine, and serum ethylene glycol if possible. According to NACB guideline, clinical laboratory should provide direct measurements of ethylene glycol in serum or plasma, and the GC assay should target glycolic acid in addition to parent intoxicant, ethylene glycol.

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P16 – Molekularna dijagnostika, P16-1 (UP7-1)

Polimorfizam gena za citokine IL-6, IL-10, TNF-alfa, TGF-beta1 i IF-gama u bolesnika s presađenim bubregom

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Polimorfizam gena za citokine svakodnevno se istražuje te povezuje s nastankom mnogih bolesti. Proizvodnja citokina je pak povezana s imunom reakcijom na transplantirani organ. Dosad nisu objavljeni rezultati koji bi se odnosili na hrvatsku populaciju. Cilj ovoga rada bio je utvrditi raspodjelu genotipova za citokine IL-6, IL-10, TNF-alfa, TGF-beta1 i IF-gama u uzorku hrvatske populacije s transplantiranim bubregom te ispitati utjecaj spola na genetski polimorfizam istih. Istraživanje je provedeno na 56 neselektiranih bolesnika iz istočne Hrvatske (od toga 25 muškaraca, dob 43±13 godina) s funkcionalnim, prvi puta transplantiranim bubregom (od toga 53 kadaverična bubrega, a 3 od živih donora). DNA je izolirana iz leukocita periferne krvi uporabom JETquick blood DNA Spin Kit. Genotipovi za IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gama određeni su metodom PCR-SSP uz primjenu Cytokine Genotyping Tray. U skladu s utvrđenim genotipom bolesnici su svrstani u određenu razinu izraženosti citokina: IL-6 i TNF-alfa u slabo i jako izražene, a IL-10, TGF-beta1 i IFN-gama u slabo, srednje i jako izražene. Kod ispitanih 56 bolesnika utvrđena je slijedeća raspodjela izraženosti citokina: TNF-alfa slabo (40), jako (16); TGF-beta jako (40), srednje (12), slabo (4); IL-10 jako (9), srednje (29), slabo (18); IL-6 jako (49), slabo (7); IFN-gama jako (12), srednje (30), slabo (14). Raspodjela genotipova IL-10 značajno se razlikovala između muškaraca i žena (χ^2=13,884, p=0,008). Distribucija razine ekspresije IL-10 se također značajno razlikovala između muškaraca i žena (χ^2=12,931, p=0,002). Kod ostalih ispitanih gena za citokine nije nađena značajna razlika u raspodjeli genotipova s obzirom na spol. Raspodjela razine izraženosti za ostale ispitane citokine također se nije značajno razlikovala s obzirom na spol. Uz to, nema značajne razlike ni u raspodjeli genetskog polimorfizma s obzirom na primarnu bubrežnu bolest. Polimorfizam gena za citokine IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gama kod bolesnika s presađenim bubregom iz istočne Hrvatske podudara se s podacima dobivenim u različitim

P16 – Molecular diagnostics, P16-1 (UP7-1)

Determination of the IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gamma cytokine gene polymorphism in the population of kidney transplanted patients from eastern Croatia – a single center study

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Cytokine gene polymorphism is currently examined in association with many diseases. Production of the IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gamma cytokines is related to the immune reaction to the transplanted organ. Currently there are no published data on the respective Croatian population. The aim of the study was to determine the distribution of genotypes for IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gamma in the Croatian population of kidney transplanted patients and to examine sex difference in the gene polymorphism. The study included 56 nonselected patients (25 males, mean age 43±13 years) from eastern Croatia with functional first kidney transplant (53 cadaveric and 3 from living donors). DNA was extracted from peripheral blood leukocytes using JETquick blood DNA Spin Kit. Genotypes for IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gamma were determined by PCR-SSP method using Cytokine Genotyping Tray. The genotypes obtained were grouped according to the level of cytokine production as follows: for TNF-alfa and IL-6, patients with low and high expression; and for TGF-beta1, IL-10 and IFN-gamma, patients with high, intermediate and low expression. The distribution of cytokine production levels in 56 patients was as follows: TNF-alfa low (40), high (16); TGF-beta high (40), intermediate (12), low (4); IL-10 high (9), intermediate (29), low (18); IL-6 high (49), low (7); and IFN-gamma high (12), intermediate (30), low (14). IL-10 genotype distribution was significantly different between men and women (χ^2=13.884, p=0.008). The distribution of IL-10 expression levels also differed significantly between men and women (χ^2=12.931, p=0.002). Sex differences in genotype distribution were not significant for other cytokine genes under study. The distribution of cytokine expression levels according to sex and determined genotypes was not significantly different for the cytokines other than IL-10. There was no difference in the distribution of gene polymorphisms according to primary renal disease either. Data on the polymorphism of genes for the IL-6,

svjetskim populacijama. Spolna razlika je značajno izražena kod IL-10 genotipova te posljedično kod pretpostavljene razine ekspresije IL-10. Iz navedenog možemo zaključiti da imuna reakcija može biti i spolno uvjetovana. Dobiveni podaci mogu pomoći u istraživanju genetski uvjetovane imune reakcije na transplantirani bubreg.

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IL-10, TNF-alfa, TGF-beta1 and IF-gamma cytokines in the kidney transplanted patients from eastern Croatia were found to be consistent with the respective data on populations from other parts of the world. Sex difference was significant for IL-10 genotypes and the related IL-10 production predetermination. Therefore, the immune reaction could differ according to sex. These data could be used in the study of genetic determination of immune reaction to transplanted kidney.

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P16-2 (UP7-2)

Kvantitativna fluorescentna PCR – brza metoda za prenatalnu dijagnostiku najčešćih autosomnih aneuploidija

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Kvantitativna fluorescentna lančana reakcija polimerazom (QF-PCR) već se više od deset godina stalno razvija u području prenatalne dijagnostike u cilju dokazivanja najučestalijih aneuploidija. Autosomne trisomije čine više od 80% značajnih kromosomskih poremećaja, a rutinski se utvrđuju kariotipizacijom kultiviranih fetalnih stanica. Ipak, relativno dugo vrijeme potrebno za izradu kariograma ukazuje na potrebu alternativnog pristupa dijagnostici, poput metode QF-PCR koja značajno smanjuje kako vrijeme nesigurnosti za bolesnike, tako i financijska sredstva potrebna za provođenje analize. Svrha istraživanja bila je ispitati mogućnost primjene metode multipleks QF-PCR na nekoliko tipova uzoraka, te informativnost odabranih mikrosatelitskih lokusa u hrvatskoj populaciji i pouzdanost rezultata dobivenih na ovaj način. DNA je izolirana iz uzoraka krvi, plodovih voda, kulture fetalnih stanica i tkiva u parafinu na kolonicama komercijalnog kita za izolaciju. Mikrosatelitski (STR) lokusi na autosomima 13, 18 i 21, te amelogeninski lokus umnoženi su pomoću QF-PCR u multipleks reakciji, pričem su za svaki autosom umnožena po tri lokusa. Dobiveni proizvodi umnažanja razdvojeni su kapilarnom elektroforezom genetičkog analizatora ABI 3130 i analizirani pripadajućim računalnim programom. Izolati DNA svih početnih uzoraka, osim dva uzorka kultiviranih fetalnih stanica, pokazali su se pogodnima za umnažanje u reakciji multipleks QF-PCR. Rezultati analize za uzorke krvi, plodovih voda i kulture fetalnih stanica do-

P16-2 (UP7-2)

Quantitative-fluorescent PCR – a rapid approach to prenatal diagnosis of common autosomal aneuploidies

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Quantitative fluorescent polymerase chain reaction (QF-PCR) is constantly being improved as a diagnostic tool for the detection of aneuploidies in prenatal samples. Autosomal trisomies account for more than 80% of significant chromosomal disorders and are routinely being detected by karyotype analysis of cultivated fetal cells. However, this approach is time-consuming and requires a significant level of training and expertise. Therefore, there is the need of a faster method like QF-PCR, which significantly decreases both the anxiety period for the patients and the financial input required for the analysis. The aim of our study was to evaluate the suitability of different sample types for multiplex QF-PCR, the usefulness of the chosen marker set in the Croatian population, and the reliability and accuracy of the results obtained. DNA was extracted from different samples, including blood, amniotic fluid, cultivated fetal cells and paraffin-embedded tissue, using the column-based commercial DNA isolation kit. Three microsatellite (STR) loci on each of the autosomes 13, 18 and 21, and amelogenin locus were amplified together in a single assay QF-PCR. The reaction products were subsequently separated using capillary electrophoresis on an ABI 3130 genetic analyzer and analyzed with the appropriate software. STR loci of all but two DNA extracts from the cultivated fetal cells were successfully amplified in the single-assay QF-PCR. The results were obtained within 24 hours for the blood, amniotic fluid and culti-

biveni su unutar 24 sata od primitka uzoraka u laboratorij, a unutar 48 sati za tkivo u parafinu. Od ukupno 57 analiziranih uzoraka utvrđeno je 7 uzoraka s aneuploidijom, uključujući trisomiju 21 (Downov sindrom), trisomiju 18 (Edwardsov sindrom), te XYY sindrom. Utvrđena je potpuna podudarnost rezultata analize uzoraka plodovih voda i staničnih kultura pomoću QF-PCR i kariotipizacijom. Također, izabrani set primera predstavlja pogodan i robustan sustav koji uključuje lokuse visoke heterozigotnosti, a samim tim i informativnosti. S obzirom na brzinu i relativnu jednostavnost ovoga pristupa u usporedbi s klasičnom citogenetičkom metodologijom, QF-PCR predstavlja značajnu inovaciju i vrlo dobro rješenje za pouzdanu analizu većeg broja uzoraka u vrlo kratkom vremenu. Izabrani set biljega pokazao se vrlo dobrim u prenatalnoj dijagnostici aneuploidija u hrvatskoj populaciji.

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vated fetal cells samples, while 48 hours were needed to obtain results for the paraffin-embedded tissue sample. In the total of 57 different samples, 7 aneuploidies were detected, including trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) and XYY syndrome. All results of QF-PCR analysis were in full compliance with karyotype analysis of the amniotic fluid and cultivated fetal cells samples. Finally, the chosen marker set was found to be suitable for usage in Croatian population, since it is a robust system which includes loci with high heterozigosity and, therefore high information content. Considering the speed and simplicity of this approach compared with the classic cytogenetic procedure, QF-PCR represents a substantial innovation and a very good solution for reliable analysis of a large number of samples in a short period of time. The chosen marker set was found suitable for prenatal diagnosis of aneuploidies in the Croatian population.

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P16-3

Jednostavan i brz protokol za molekularno dokazivanje normalnih i produljenih alela miotonične distrofije tipa 2

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Miotonična distrofija tipa 2 (MD2) je autosomno dominantno nasljediva multisistemska bolest koju uzrokuje produljenje slijeda ponavljanja CCTG u intronu 1 gena ZNF9 na kromosomu 3q21. Raspon ponavljanja je iznimno varijabilan, počevši od 75 pa sve do 11000 ponavljanja. Takva se produljenja obično dokazuju metodom hibridizacije po Southernu nakon restrikcije genomske DNA. Međutim, ova je metoda dugotrajna, zahtijeva velike količine genomske DNA i ne uspijeva otkriti oko 20% zahvaćenih osoba. Stoga je cilj bio uspostaviti jednostavnu i neradioaktivnu metodu za molekularnu dijagnostiku miotonične distrofije tipa 2. Naš posebno dizajniran protokol koji se zasniva na reakciji PCR omogućava umnažanje normalnih i vrlo dugih sljedova ponavljanja koji se vizualiziraju nakon oligonukleotidne hibridizacije. Ova metoda rabi vrlo male količine genomske DNA (30 ng), jednostavna je i stoga smanjuje troškove laboratorijske dijagnostičke obrade bolesnika s MD2. Molekularnom dijagnostikom MD2 u Hr-

P16-3

A simple and rapid molecular protocol to detect normal and expanded alleles in myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is an autosomal dominant multisystemic disease caused by expansion of CCTG repeats in intron 1 of ZNF9 gene on chromosome 3q21. The range of expansion is extremely variable, starting from 75 to up to 11000 repeats. Such expansions are usually detected by Southern blot after restriction enzyme digestion of genomic DNA. However, this method is time consuming, requires large amounts of genomic DNA and fails to detect approximately 20% of affected individuals. Therefore, the aim was to establish a simple and non-radioactive method for molecular diagnosis of MD2. Our specially designed PCR-based protocol enables amplification of normal and very long repeat tracts that are visualized after oligonucleotide hybridization. The method uses very small amounts of genomic DNA (as little as 30 ng), is simple, relatively fast, and thus reduces the cost of diagnostic laboratory processing of DM2 patients. During one year of molecular diagnosis of DM2 in Croatia, we

vatskoj tijekom jedne godine ispitali smo 78 bolesnika koji su imali različite simptome povezane s miotoničnom distrofijom, a kod kojih je genetičkim testiranjem isključena miotonična distrofija tipa 1 (MD1). Analizom DNA potvrđena je mutacija gena ZNF9 kod 8 bolesnika, tako da ovi rezultati upućuju na potrebu za uspostavljanjem protokola u laboratorijima za molekularno genetičko ispitivanje bolesnika negativnih na MD1 za mutaciju koja uzrokuje MD2 zbog preklapanja njihovih kliničkih simptoma.

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tested 78 patients showing different symptoms related to myotonic dystrophy but with negative test results for myotonic dystrophy type 1 (DM1). DNA analysis confirmed mutation of ZNF9 gene in 8 patients and these results suggest the need for laboratories to establish the protocol for molecular genetic testing of DM1 negative patients for mutation causing DM2 due to their overlapping clinical symptoms.

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P16-4

Mutacije gena PMM2 i ALG6: postoje li u hrvatskoj populaciji

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Urođeni poremećaji glikozilacije (CDG, engl. *congenital disorders of glycosylation*) uzrokovani su točkastim mutacijama gena koji kodiraju enzime uključene u N-glikozilacijski put te se očituju kao poremećaji sinteze glikanskog dijela glikoproteina ili drugih glikokonjugata. Najučestaliji urođeni poremećaji glikozilacije su CDG-Ia i CDG-Ic. Sindrom CDG-Ia čini 80% svih urođenih poremećaja glikozilacije, a uzrokovan je mutacijama u genu PMM2 koji kodira enzim fosfomanomutazu. Najučestaliji genotip za sindrom CDG-Ia je R141H/F119L. CDG-Ic uzrokuje mutacije u genu ALG6, čiji je proteinski proizvod enzim alfa1,3-glukozil-transferaza. Mutacija A333V je najučestalija u genu ALG6 i dosad je nađena u bolesnika europskog podrijetla. Nedavno smo započeli opsežan projekt određivanja učestalosti različitih mutacija/polimorfizama u genima PMM2 i ALG6 u hrvatskoj populaciji. Dosad u Hrvatskoj nije potvrđen niti jedan bolesnik obolio od sindroma CDG. Prikazani su rezultati pretraživanja mutacija u eksonu 5 i dijelova introna IVS4 i IVS5 PMM2 gena te eksona 11 ALG6 gena. Dosad su analizirani uzorci dobiveni od 350 dobrovoljnih davatelja krvi. Za utvrđivanje prisutnosti mutacija R141H i F119L rabili smo umnažanje lančanom reakcijom polimeraze i ispitivanje analizom polimorfizma konformacije jednolančane DNA (PCR-SSCP analiza - 6% PAG-elektroforeza, 15 °C, 5 h, 3 W). Dvadesetčetiri fragmenta koji su pokazali različit elektroforetski obrazac od normalnoga dodatno su sekvencirani na genetskom analizatoru ABI-Prism 310. Mutacije R141H i F119L nisu pronađene u analiziranoj skupini. Međutim, otkriveno je šest heterozigota za IVS5+19T/T, tri homozigota za IVS5+19C/C i petnaest

P16-4

Are there any PMM2 and ALG6 gene mutations and polymorphisms in the Croatian population

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Congenital disorders of glycosylation (CDGs) are a growing group of genetic disorders caused by a deficient assembly or processing of glycoproteins. The most common types of CDGs are CDG Ia and CDG Ic. Type Ia is caused by reduced N-glycosylation due to phosphomannomutase 2 deficiency as a consequence of mutations in PMM2 gene. The most frequent single-base mutations are present in exon 5, 422G>A and 357C>A, resulting in R141H and F119L substitution, respectively. CDG Ic is caused by mutations in ALG6 gene, encoding Man(9)GlcNAc(2)-PP-Dol alpha1,3-glucosyltransferase. The most significant mutation found in this gene, C998T, resulting in an A333V substitution, has been detected in patients of European origin. We have recently undertaken a comprehensive project to determine the frequency of various mutations/polymorphisms in PMM2 and ALG6 genes in Croatian population. Until now no patient with CDG was detected in Croatia. Here we present results of screening for mutations in exon 5 and parts of intervening sequences IVS4 and IVS5 of PMM2 gene and exon 11 of ALG6 gene. To date, we analyzed samples obtained of 350 unrelated Croats. Screening for R141H and F119L was performed using PCR-SSCP analysis (6% PAG-electrophoresis, 15 °C, 5 h, 3 W). Twenty-four fragments that showed aberrant electrophoretic patterns were additionally sequenced on ABIPrism 310 Genetic Analyzer. R141H and F119L mutations were not found in the group analyzed. However, we detected six homozygotes for IVS5+19T/T, three homozygotes for IVS5+19C/C and fifteen heterozygotes (IVS5+19T/C) for intragenic single nucleotide polymorphism IVS5+19T/C, while all 24

heterozigota IVS5+19T/C za polimorfizam IVS5+19T/C, dok su sve 24 osobe bile homozigoti za IVS5+22T/T. Jedan od heterozigota za IVS5+19T/C je bio također i heterozigot za deleciju od 3 bp (ATG) na poziciji -58 u intronu 4. Kako bismo mogli odrediti učestalost mutacije A333V u genu ALG6 optimirali smo postupak PCR-SSCP postupak: elektroforeza je izvedena na 6% PAG, na 4 °C u trajanju od 5 sati uz 6 W. U dosad analiziranim uzorcima nismo identificirali mutaciju A333V.

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individuals were homozygous for IVS5+22T/T. One of the heterozygotes for IVS5+19T/C was also a heterozygote for deletion of 3bp (ATG) at position -58 in intron 4. To be able to analyze the frequency of A333V mutation in ALG6 gene we optimized PCR-SSCP procedure: electrophoresis was performed on 6% PAG, at 4 °C for 5 hours using 6 W. In the samples analyzed until now A333V mutation has not been identified.

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P16-5

Polimorfizam gena za TNF-alfa +489G/A kod bolesnika s KOPB

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Tumorski nekrotizirajući faktor (TNF)-alfa je proupalni citokin čiji je gen smješten na 6. kromosomu u području III. glavnog kompleksa tkivne podudarnosti. Poznato je nekoliko polimorfizama ovoga gena, a za neke od njih utvrđena je povezanost s kroničnim upalnim bolestima. Kod bolesnika s kroničnom opstrukcijskom plućnom bolesti (KOPB) nađene su povišene koncentracije TNF-alfa u sputumu i cirkulaciji, što ukazuje na važnost ovoga citokina u lokalnom i sistemskom upalnom procesu koji je djelatan u KOPB. Opisani polimorfizmi gena za TNF-alfa povezuju se stoga sa sklonošću obolijevanju od ove bolesti. Analiziran je polimorfizam na mjestu +489G/A u prvom intronu gena za TNF-alfa za koji postoje literaturni podatci o povezanosti s pojavom KOPB u nizozemskoj populaciji. Postupkom PCR-RFLP taj je polimorfizam određen kod 31 bolesnika s KOPB te kod 51 zdravog ispitanika. Utvrđene su slijedeće frekvencije genotipa kod bolesnika oboljelih od KOPB: AG genotip 0,39, GG genotip 0,61 i AA genotip 0,00, te u zdravoj populaciji: AG genotip 0,53, GG genotip 0,45 i AA genotip 0,02. Usporedba alelnih i genotipskih frekvencija za +489G/A u bolesnika s KOBP i zdravih ispitanika ne ukazuje na postojanje značajnih razlika između ovih dviju ispitivanih populacija (p=0,299). Temeljem dobivenih rezultata može se zaključiti kako nije utvrđena povezanost između polimorfizma u prvom intronu gena za TNF-alfa na mjestu +489G/A i KOPB u hrvatskoj populaciji.

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P16-5

TNF-alpha +489G/A polymorphism and COPD

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The proinflammatory cytokine tumor necrosis factor (TNF)-alpha plays an important role in inflammatory processes. The gene coding for this cytokine is located on chromosome 6 in the class III region of the major histocompatibility complex. Several biallelic polymorphisms of this gene are known. Some of them have been reported to be associated with chronic inflammatory diseases. Chronic obstructive pulmonary disease (COPD) is characterized by an increased level of TNF-alpha in sputum and in circulation, indicating that this cytokine is involved in both local and systemic inflammation present in COPD. Analysis of the possible TNF-alpha gene polymorphisms in COPD could be related to a higher susceptibility to developing this disabling pathological condition. We analyzed TNF-alpha gene polymorphism at position +489G/A located in the first intron of the gene which has been reported to be associated with COPD in Dutch population. Polymorphism at position +489G/A of TNF-alpha gene was examined in 31 COPD patients and 51 healthy volunteers by PCR-RFLP procedure. We found the following genotype frequencies in the group of COPD patients: AG 0.39, GG 0.61 and AA 0.00; and in the control group: AG 0.53, GG 0.45 and AA 0.02. There were no differences in the allele and genotype frequency between the two groups (p=0.299). We could not find any significant link between the +489G/A polymorphism of TNF-alpha gene and COPD in Croatian population.

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P16-6

Analitička procjena sustava za umnažanje DNA u stvarnom vremenu Rotor-Gene 3000

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Rotor-Gene 3000 je otvoreni sustav koji omogućava uporabu različitih reagensa za umnažanje DNA u stvarnom vremenu. Programska potpora omogućava analizu podataka te apsolutnu i relativnu kvantifikaciju. Cilj studije bio je analitički procijeniti Rotor-Gene 3000 kvantifikacijom fuzijskog transkripta BCR-ABL u bolesnika s kroničnom mijeloičnom leukemijom u stvarnom vremenu i dobivene rezultate usporediti s rezultatima reakcije RT-PCR. Analizirano je 11 uzoraka RNA (cDNA). Devet uzoraka bilo je pozitivno, a dva su bila negativna na fuzijski transkript BCR-ABL, što je dokazano pomoću RT-PCR (kvalitativno) te citogenetski. Za kvantitativno određivanje količine fuzijskog transkripta BCR-ABL u navedenim uzorcima primijenjen je LightCycler t(9;22) Quantification kit (Roche Applied Science). Kit omogućava osjetljivu kvantifikaciju i određivanje relativne razine izraženosti transkripata BCR-ABL u odnosu na razinu izraženosti kontrolnog gena G6PDH. Količina G6PDH i BCR-ABL (u fg) u pojedinom uzorku određena je usporedbom vrijednosti Ct (ciklus u kojem intenzitet fluorescencije prelazi granicu osjetljivosti) nepoznatog uzorka sa standardnom krivuljom. Za fuzijski transkript BCR-ABL raspon količina u uzorcima je bio od 0,08 fg do 97,64 fg. Za transkript G6PDH raspon količina u uzorcima je bio od 14,76 fg do 666,25 fg. Normalizirana vrijednost izraženosti fuzijskog transkripta BCR-ABL kretala se od 0,00097 do 0,146555. Od 11 testiranih uzoraka 8 ih je bilo pozitivno (s mjerljivom količinom fuzijskog transkripta BCR-ABL), dok su 3 uzorka ustanovljena kao negativna (bez mjerljive količine fuzijskog transkripta BCR-ABL). Rezultati analitičke procjene dokazali su prisutnost mjerljive količine fuzijskog transkripta BCR-ABL u 8 od 9 pozitivnih uzoraka (osjetljivost 89,9%). Mogući uzrok snižene osjetljivosti možda je u tome što su uvjeti za reakciju PCR iz izvornog protokola prilgođeni instrumentu LightCycler koji rabi staklene kapilare, dok Rotor-Gene ima plastične tubice. S obzirom na različit toplinski kapacitet stakla i plastike optimiranje vremena pojedinih koraka PCR vjerojatno bi dovelo do povećanja osjetljivosti, što zahtijeva daljnje ispitivanje u okviru proširene analitičke validacije.

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P16-6

Analytical evaluation of the Rotor-Gene 3000 real-time instrument

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The Rotor-Gene real time DNA amplification system is an open chemistry platform, allowing for the use of any real time chemistry. The software therefore aims to provide support to the analysis of data obtained, and absolute and relative quantification. The aim of the study was to perform analytical evaluation of the RotorGene 3000 by quantification of BCR-ABL fusion trancripts in patients with chronic myeloid leukemia in real time and to compare the results obtained with the results of RT-PCR reactions. Eleven samples of RNA (cDNA) were analyzed. Nine samples were positive and two samples were negative for BCR-ABL fusion transcript, which was proven by RT-PCR (qualitative) and cytogenetic studies. The LightCycler t(9;22) Quantification Kit (Roche Applied Science) was used. The kit enables sensitive quantification and determination of the relative level of BCR-ABL fusion transcript expression normalized with G6PDH housekeeping gene expression. The amount of G6PDH and BCR-ABL (in fg) in each sample was determined by comparison of Ct values (number of the cycle when the signal was above the threshold) of an unknown sample with the standard curve. The amount in the study samples ranged from 0.08 fg to 97.64 fg for BCR-ABL fusion transcript, and from 14.76 fg to 666.25 fg for G6PDH control gene. Normalized values of BCR-ABL fusion transcripts expression were from 0.00097 to 0.146444. From eleven samples tested, eight were positive (with a measurable amount of BCR-ABL fusion transcript) and three were negative (without a measurable amount of BCR-ABL fusion transcript). Results of the analytical evaluation showed the presence of a measurable amount of BCR-ABL fusion transcript in eight of nine positive samples (sensitivity 89.9%). The possible cause of this lower sensitivity might lie in the specific conditions of reagent kit for PCR reaction, which were adapted for the LightCycler instrument. It has glass capillaries, whereas RotorGene has plastic tubes. Considering the different thermal capacity of glass and plastic, the optimization of some PCR steps would probably increase the sensitivity, which calls for additional studies as part of extended analytical validation.

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P16-7

Značenje međulaboratorijskih usporedba u području molekularne dijagnostike – vlastita iskustva

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Zbog razvoja molekularne dijagnostike raste broj laboratorija koji rabe ovu metodologiju, što nameće potrebu za standardizacijom, a to znači i sudjelovanje u shemama vanjske procjene kvalitete. Prikazuju se rezultati sudjelovanja u međunarodnim programima kontrole kvalitete dobiveni uporabom standardiziranih metoda u molekularnoj dijagnostici. Ciljevi analitičke kvalitete obuhvaćaju standardizaciju potupaka: pohrane i rukovanja reagensima, postupka s uzorcima i izolaciju DNA/RNA, izvođenje reakcije PCR, analizu proizvoda PCR, unutarnju i vanjsku kontrolu. Zavod za kliničku kemiju KB Merkur potvrđen je prema međunarodnom standardu ISO:9001:2000, a od 2004. godine sudjeluje u dva programa vanjske procjene kvalitete: EQUAL-qual i EMQN. Od 2005. godine sudjeluje i u vanjskoj procjeni UKNEQAS u programu Molecular Diagnosis of Haematological Malignancies. Ovim kontrolama obuhvaćeni su svi aspekti analitičke kvalitete: izolacija DNA/RNA, određivanje čistoće i koncentracije, izvođenje reakcija PCR, analiza proizvoda, genotipiziranje te dokazivanje molekularnih biljega. Rezultati EQUAL-qual (2 ispitivanja) prikazani su u arbitrarnim jedinicama, prema analizi percentila, kao ukupan broj bodova i opis izvedbe. Odlični rezultati u oba navrata zabilježeni su u 7/25 (28%) laboratorija sudionika u oba ispitivanja. Naš rezultat: odlična izvedba oba puta. Rezultati EMQN (genotipiziranje gena HFE u 3 uzorka) izraženi u bodovima za genotipiziranje (2 boda/uzorku) i interpretaciju (2 boda/uzorku). Tako je 50/53 (94%) laboratorija sudionika ispravno genotipiziralo sva 3 uzorka, a 23/53 (43%) je dalo odgovarajuće tumačenje. Naši rezultati: genotipiziranje: 6/6 (100%), interpretacija: 0/6 (0%). Rezultati UKNEQAS izraženi su opisno: transkript BCR/ABL pozitivan (86% lab); preuredba gena IgH nije nađena (73% lab), što su i naši rezultati. Međulaboratorijske usporedbe u području molekularne dijagnostike imaju veliko značenje zbog različitosti metodologija rada od predanalitičkih, analitičkih do poslijeanalitičkih postupaka, u svrhu dobivanja točnih rezultata i pronalaženju uzroka mogućih pogrešaka. Tumačenje ima danas sve veće značenje, jer daje smjernice i pomaže kliničarima u donošenju relevantnih zaključaka i postavljanju dijagnoza na dobrobit bolesnika.

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P16-7

Role of inter-laboratory comparison in the field of molecular diagnosis – own experience

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Major developments in molecular diagnosis in recent years have led to an increased number of laboratories using this methodology, which requires standardization and participation in external quality control schemes. Results of participation in international quality control schemes, obtained using standardized methods in molecular diagnosis, are presented. Analytical quality goals include standardization of procedures: reagent storage and set-up, sample treatment and preparation of DNA/RNA, PCR performance, PCR product analysis, internal and external control. Since 2004, Department of Clinical Chemistry, Merkur University Hospital, has been certified according to ISO:9001:2000, and has participated in two external quality assessments: EQUAL-qual and EMQN scheme. Since 2005, it has participated in UKNEQAS in Molecular Diagnosis of Haematological Malignancies Scheme. With this quality controls all aspects of analytical quality are included: isolation of DNA/RNA, quality and quantity determination, PCR performance, PCR product analysis, genotyping, and identifying molecular markers. Results of EQUAL-qual (two surveys) are clustered in arbitrary units, according to percentile analysis, and expressed as a final score value. Seven of 25 (28%) participating laboratories in both surveys had excellent results. Our result: excellent performance on both surveys. Results of EMQN (HFE gene genotyping in three samples) are expressed in score for genotyping (2 marks/sample) and interpretation and reporting (2 marks/sample). Fifty of 53 (94%) laboratories identified all three genotypes accurately, and 23 of 53 (43%) produced an appropriate interpretation. Our results: genotyping 6/6 (100%); interpretation 0/6 (0%). UKNEQUAS results are descriptive: BCR/ABL fusion transcript positive (86% of labs); IgH gene rearrangements negative (73% of labs). Our results were the same. Accordingly, inter-laboratory comparison is of major importance in molecular diagnosis because of the wide range of different methodologies from preanalytical, analytical, and postanalytical procedures in getting accurate results and finding out the cause of possible errors. Interpretation of results is of great importance today because it can help clinicians draw relevant conclusions and make an accurate diagnosis to patient's benefit.

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P16-8

Usporedba osjetljivosti ugnježđenog PCR i kvantitativnog PCR u određivanju Bcr/Abl p210 transkripta kronične mijeloične leukemije

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Za otkrivanje minimalne ostatne bolesti (MRD) kod bolesnika s kroničnom mijeloičnom leukemijom (CML) koji su postigli potpunu kliničku remisiju i potpun citogenetički odgovor rabe se metode ugnježđene PCR (nested PCR) i kvantitativne PCR (Q-PCR). Budući da je cilj terapije postizanje molekularne remisije, od iznimne je kliničke važnosti postići što bolju osjetljivost molekularnog testiranja. Cilj studije bio je usporediti razinu osjetljivosti semikvantitativne, ugnježđene PCR s kvantitativnom PCR u dokazivanju transkripta Bcr/Abl p210 u pokusu razrjeđenja stanica Filadelfija-kromosom pozitivne stanične linije K562. Za određivanje razine osjetljivosti napravljena su serijska razrjeđenja stanica stanične linije K562 (Bcr/Abl pozitivna) sa stanicama stanične linije NB4 (Bcr/Abl negativna) u rasponu 10-3 (1:1000) – 10-7 (1:10 mil). Izolirana RNA iz pojedinih staničnih omjera prevedena je u cDNA te su uzorci testirani na p210 transkript metodama ugnježđene PCR (Biomed1, Leukemia 1999:13) i Q-PCR-Taqman (EAC, Leukemia 2003:17). Također su istim metodama usporedno testirani uzorci koštane srži (KS) i periferne krvi (PK) dvaju bolesnika s CML na terapiji Imatinib mesilatom. U dilucijskom testu metoda ugnježđene PCR pokazala je osjetljivost dokazivanja fuzijskog transkripta Bcr/Abl p210 od 10-4, dok je metoda Q-PCR pokazala osjetljivost 10-6. Obje metode pokazale su prisutnost transkripta p210 u koštanoj srži bolesnika. Metodom Q-PCR otkriven je transkript p210 i u uzorcima periferne krvi bolesnika sa značajno manjom količinom transkripta u odnosu na uzorke koštane srži. U ovom smo pokusu pokušali odrediti razine osjetljivosti dviju molekularnih metoda za dokazivanje minimalne ostatne bolesti kronične mijeloične leukemije. Za to smo rabili model rastuće dilucije onkogen-pozitivnih i negativnih stanica. Iako se često izvještava kako su ugnježđene metode za oko 1 log osjetljivije od metoda Q-PCR, u našem pokusu je Q-PCR prema protokolu Europe Against Cancer Program postigao višu osjetljivost dokazivanja tumorske stanice. Ovaj ćemo nalaz dodatno komentirati, a zanimljivo je da je i u kliničkim uzorcima bolesnika u remisiji bolesti Q-PCR bila u prednosti za dokazivanje MRD.

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P16-8

Comparison of sensitivity of nested PCR and quantitative PCR in Bcr/Abl p210 transcript detection in chronic myelogenous leukemia

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Nested PCR and quantitative real-time PCR (Q-PCR) can be used to detect minimal residual disease (MRD) in chronic myelogenous leukemia (CML) patients who have achieved complete clinical remission and complete cytogenetic response. Achieving molecular remission is the goal of therapy, so it is of critical importance for a clinical utility to attain high sensitivity of molecular testing. The aim of the study was to compare the level of sensitivity of nested PCR and Q-PCR in the detection of Bcr/Abl p210 transcripts in Bcr/Abl-positive cell dilution model. Serial dilutions of K562 cell line (Bcr/Abl-positive) in NB4 cell line (Bcr/Abl-negative) were made (range of positive cell dilution: 10-3 – 10-7) to determine the level of sensitivity. Isolated RNA samples were transcribed into cDNA and tested for p210 transcript by nested PCR (Biomed1, Leukemia 1999:13) and Q-PCR-Taqman (EAC, Leukemia 2003:17). Bone marrow and peripheral blood samples of two CML patients on Imatinib mesylate therapy were also tested by both methods. In the cell dilution test, nested PCR showed a sensitivity for detecting Bcr/Abl positive cell of 10-4, and Q-PCR showed a sensitivity of 10-6. Both methods detected p210 transcripts in bone marrow samples of CML patients. However, Q-PCR also detected transcripts in peripheral blood samples, with a significantly lower level of transcription in comparison to bone marrow samples. Although nested-PCR has been frequently reported as being by approximately 1 log more sensitive than Q-PCR, in our marker-positive cell line K562 dilution experiment we documented higher sensitivity for standardized Europe Against Cancer Program Q-PCR method.

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P16-9

Mutacija gena JAK2 u kroničnim mijeloproliferativnim bolestima

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Nedavno otkrivena mutacija gena JAK2 za tirozin kinazu staničnih receptora za citokine i faktore rasta čini se patogenom za skupinu kroničnih mijeloproliferativnih bolesti. To je prva značajna genska mutacija nakon otkrića Filadelfija kromosoma i preuredbe Bcr/Abl onkogena. Posljedica točkaste mutacije G/T ili V617F konstitucijski aktivira ovu kinazu, što ima za posljedicu staničnu proliferaciju. Ovdje prikazujemo učestalost JAK2V617F mutacije u velikoj skupni bolesnika s kroničnim mijeloproliferativnim bolestima: policitemija vera (PV), esencijalna trombocitemija (ET), primarna mijelofibroza (IMF) te njezinu diferencijalno dijagnostičku ulogu u obradi poliglobulija-eritrocitoza. Mutacija smo određivali alel specifičnom PCR prema Baxter *et al.* (Lancet 2005;365), koja je modificirana i za određivanje mutacije na molekulama RNA. DNA i RNA izolirane su iz nukleiranih stanica krvi. Osjetljivost metode za dokazivanje mutiranog alela je oko 1%. U skupni od 85 bolesnika s PV (prema kretierijima WHO) 6 ih nije imalo mutaciju (93% pozitivnih). Od 22 bolesnika s ET 10 ih je bilo negativnih (55% pozitivnih). Od 15 bolesnika s IMF 54% ih je bilo pozitivnih. Od tri KMML bolesnika jedan je imao mutaciju, dok ju u skupini od 5 MDS-RA bolesnika nismo našli. Zanimljivo je da je od 10 bolesnika koji su obrađivani zbog nalaza eritrocitoze samo jedan bio pozitivan, dok je negativan test za druge značio usmjerenje dijagnostike ka traženju razloga sekundarne poliglobulije. Molekularna dijagnostika JAK2V517F danas je postavljena na sam početak algoritma obrade poliglobulija i drugih kroničnih mijeloproliferacija.

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P16-9

JAK2 gene mutation in chronic myeloproliferative disorders

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Mutation in the JAK2 tyrosine kinase gene has recently been shown as the major molecular genetic deffect in non-Philadelphia chronic myeloproliferative disorders. G/T point mutation in the autoinhibitory region of the gene constitutively activates its kinase activity, which leads to cell proliferation. We sought to investigate the frequency of this mutation in a large cohort of chronic myeloproliferative patients with particular interest in its role in guiding differential diagnosis of polyglobulia – erythrocytosis patients. We used allele specific PCR test as described by Baxter *et al*. (Lancet 2005;365), which we also modified for amplification of mutated RNA molecules of JAK2 gene.
Of 85 PV patients 6 were negative for the mutation (7%) (PV diagnosis according to WHO criteria). Of 22 ET patients, 45% were negative and so were 7/15 (46.6%) IMF patients. We also found one of three CMML patients positive for mutation, but did not detect it in any of the five MDS-RA patients. In the work-up of erythrocytosis patients, the test was positive in only one of 10 patients, which fitted well their later diagnosis of secondary polycythemia. Today, V617F JAK2 mutation search has been considered the first test in the differential diagnosis of patients with incerased hematocrit and red blood cell count.

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P16-10

Pankreatitis i genske varijante lipoprotein lipaze u ispitanika hrvatskoga podrijetla

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Lipoprotein lipaza (LPL) je ključni enzim metabolizma triacilglicerolom bogatih lipoproteina. Polimorfizmi gena za LPL mogu utjecati na patogenezu nekih bolesti kao što je pankreatitis. Cilj studije je bio pretražiti mutacije i polimorfizme u genu za LPL te ispitati utjecaj različitih polimorfizama na razvoj pankreatitisa. U studiju je bilo uključeno 77 odraslih ispitanika s pankreatitisom različite etiologije i 98 kontrolnih ispitanika. Petogodišnji dječak s hilomikronemijom i njegovi roditelji prikazani su kao slučaj. Pretraživanje dijelova gena za LPL koji se prepisuju načinjeno je pomoću PCR-SSCP. Polimorfizmi LPL-gena D9N, N291S, S447X i rijetka mutacija W86R potvrđeni su analizom nukleotidnog slijeda i metodom RFLP. Polimorfizmi dijelova gena za LPL koji se ne prepisuju analizirani su metodom RFLP. U ispitanika s pankreatitisom učestalost X alela genske varijante LPL-S447X bila je značajno manja u odnosu na kontrolnu skupinu (p<0,05). Omjer rizika za povezanost istog polimorfizma s pankreatitisom iznosio je 0,39 (95% CI 0,176-0,866; p<0,05). U skupini s pankreatitsom koncentarcije HDL-kolesterola su bile značajno više u nositelja 447X alela (p<0,05). Koncentracije triacilglicerola su bile znatno više u nositelja LPL-Pvu II P+ alela (p=0,05) u ispitanika s pankreatitisom. Petogodišnji dječak i njegovi roditelji su nositelji rijetke mutacije W86R. Polimorfizam LPL-S447X bi mogao imati zaštitnu ulogu od pankreatitisa. Kod osoba koje boluju od pankreatitisa polimorfizmi LPL-Pvu II i Hind III pokazuju značajan utjecaj na lipidni profil. Genske varijante LPL, a osobito rijetka mutacija W86R mogu biti od velike važnosti u razumijevanju genski uvjetovane sklonosti za obolijevanje od pankreatitisa različite etiologije.

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P16-10

Pancreatitis and lipoprotein lipase gene variants in subjects of Croatian descent

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Lipoprotein lipase (LPL) is a key enzyme involved in lipoprotein triglyceride-rich lipoproteins. LPL gene polymorphisms may contribute to the pathogenesis of some diseases such as pancreatitis. The aim of the study was to screen for the mutations and polymorphisms of the LPL gene as well as to investigate the influence of different polymorphisms on the development of pancreatitis. The study included 77 adults with pancreatitis of different etiology and 98 control subjects. A 5-year-old male with chylomicronemia and his parents were also included in the study as a case report. The screening of the coding region was performed by PCR-SSCP. LPL gene polymorphisms D9N, N291S, S447X, and the rare W86R mutation were confirmed by DNA sequence analysis and RFLP. Intronic polymorphisms Hind III and Pvu II were analyzed by RFLP. The X allele of the LPL S447X genetic variant was less frequent in the individuals with pancreatitis (p<0.05). Odds ratio for the association between LPL-S447X and pancreatitis was 0.39 (95% CI 0.176-0.866; p<0.05). In the patient group, HDL-C levels were significantly higher in carriers of 447-X allele (p<0.05). TG levels were significantly higher in carriers of LPL-Pvu II P+ allele (p=0.05) in the patient group. The 5-year-old male and his parents were carriers of the W86R mutation. Accordingly, LPL-S447X polymorphism may have a protective role against pancreatitis. LPL-Pvu II and Hind III polymorphisms can contribute to the lipid profile differences in individuals with pancreatitis. LPL genetic variations, especially the rare W86R mutation, may be of considerable importance for the understanding of the genetic predisposition to pancreatitis of different etiology.

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P16-11

Učestalost mutacije V617F u genu za JAK2 u mijeloproliferativnim bolestima

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Mijeloproliferativne bolesti obuhvaćaju skupinu poremećaja obilježenih sličnim kliničkim slikama te klonalnom proliferacijom jedne ili više staničnih loza, što je posljedica transformacije na razini pluripotentne matične stanice. Toj skupini pripadaju i policitemija vera (PV), esencijalna trombocitemija (ET) i idiopatska mijelofibroza (IM), za koje je tek odnedavno poznata molekularna podloga bolesti. Točkasta mutacija gvanina u timin u genu za Janus kinazu 2 (JAK2), koja kodira zamjenu valina fenilalaninom na poziciji 617 (V617F), javlja se u značajnom postotku bolesnika s dijagnozom PV, ET i IM. Mutacija V617F smještena je u domeni JH2 gena za JAK2, a uključena je u autoinhibiciju vlastite tirozin kinazne aktivnosti. Mutirani protein se neprekidno iznova fosforilira aktivirajući signalne putove, a stanice postaju neovisne ili preosjetljive na citokine te pojačano proliferiraju. Cilj ovoga istraživanja bio je odrediti učestalost mutacije V617F u genu za JAK2 kod bolesnika s dijagnozom PV, ET ili IM. Obrađeno je ukupno 47 bolesnika, od kojih 17 s uputnom dijagnozom PV (skupina PV), 20 s dijagnozom ET (skupina ET) te 10 s uputnom dijagnozom IM (skupina IM). Iz stanica periferne krvi ili koštane srži izolirana je DNA. Za dokazivanje točkaste mutacije V617F u genu za JAK2 primijenjena je metoda alel-specifične PCR (prema Baxter i sur., Lancet 2005.). Svim bolesnicima napravljena je i analiza PCR na fuzijski gen BCR/ABL. U skupini PV dokazana je učestalost mutacije V617F u genu za JAK2 od 59%, u skupini ET 70%, a u skupini IM 30%. Prisutnost fuzijskog gena BCR/ABL nije dokazana ni u jednom analiziranom uzorku. Nadalje, skupine PV i ET podijeljene su svaka na dvije podskupine s obzirom na prisutnost mutacije V617F u genu za JAK2 te su u svakoj podskupini analizirani parametri krvne slike. Utvrđeno je da ne postoji statistički značajna razlika u prosječnom povišenju broja eritrocita i hematokrita u skupini PV s obzirom na prisutnost mutacije. U skupini ET nije bilo statistički značajne razlike u povišenju broja trombocita kod bolesnika s dokazanom mutacijom V617F, ali je dokazana statistički značajna razlika u povišenju broja leukocita (p=0,007) i hematokrita (p<0,005). U zaključku, učestalost točkaste mutacije V617F u genu za JAK2 značajna je u PV i ET, a njeno dokazivanje metodom alel-specifične PCR pomaže pri dijagnozi bolesti i praćenju terapije na molekularnoj razini.

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P16-11

The prevalence of JAK2 V617F mutation in myeloproliferative diseases

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Myeloproliferative disorders include a group of diseases with clinical and biological similarities sharing the major common feature of being clonal hematopoietic disorders arising from the transformation of pluripotent hematopoietic progenitor cell. This group, among others, includes polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM). Recently discovered guanine-to-thymine point mutation encoding valine-to-phenylalanine substitution at position 617 (V617F) in the autoinhibitory JH2 domain of JAK2 gene is present in most patients with PV, ET and IM. The mutant protein is constitutively phosphorylated, activating its downstream signaling pathways in the absence of any cytokine. Cells expressing JAK2 V617F become independent and hypersensitive to cytokines causing abnormal cell proliferation. The aim of this study was to determine the prevalence of JAK2 V617F mutation in patients with PV, ET and IM. The study included 47 patients: 17 patients with PV (PV group), 20 patients with ET (ET group) and 10 patients with IM (IM group). DNA was isolated from bone marrow cells or peripheral blood cells. The detection of JAK2 V617F was performed by allele specific PCR (published in Baxter *et al*., Lancet 2005). All patients were also analyzed for BCR/ABL fusion gene. The presence of JAK2 V617F mutation was detected in 59% of patients in PV group, 70% in ET group, and 30% in IM group. All samples analyzed were negative for BCR/ABL fusion gene. Additionally, PV and ET groups were each divided in two subgroups. The presence or absence of mutation was correlated to the erythrocyte count and hematocrit value in PV subgroups, and to platelet and leukocyte count and hematocrit value in ET subgroups. No statistically significant difference was found between PV subgroups in the mean increment in erythrocyte count and hematocrit value. In ET subgroups there was no statistically significant difference in platelet count while a statistically significat difference was found in the mean increment in leukocyte count (p=0.007) and hematocrit value (p<0.005) in patients with JAK2 V617F mutation. The prevalence of JAK2 V617F is significant in patients with PV, ET and IM. Detection of this point mutation can be easily performed by allele specific PCR at diagnosis and used for therapy monitoring at molecular level.

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P16-12

Probiranje za mutacije u genu GALT kod zdrave hrvatske populacije

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Gen GALT kodira enzim galaktoza-1-fosfat uridil-transferazu (GALT) koji katalizira pretvorbu galaktoze-1-fosfat u uridil difosfat (UDP)-galaktozu. Do danas su otkrivene brojne mutacije u genu GALT koje na različite načine utječu na aktivnost GALT i mogu uzrokovati galaktozemiju. Kod ovoga poremećaja prisutna je značajna heterogenost alela kod različitih populacija i etničkih skupina. Dvije najučestalije mutacije kod pripadnika bijele rase su Q188R i K285N. Osobe koje imaju ove mutacije na oba alela obolijevaju od tzv. klasične galaktozemije obilježene potpunim gubitkom enzimske aktivnosti i različitim teškim simptomima. Blaži oblik galaktozemije, poznat kao varijanta Duarte, često je povezan s mutacijom N314D u genu GALT, ali i s drugim genetičkim promjenama poput varijacije u slijedu introna V (IVS5-24G<A) (Duarte-2) ili tihe mutacije L218L (varijanta Duarte-1 ili Los Angeles). Homozigoti s Duarte-2 imaju oko 50%, a heterozigoti oko 75% ostatne aktivnosti GALT, dok Duarte-1 obilježava povećana aktivnost GALT. Iako heterozigoti za klasičnu galaktozemiju nemaju izraženih simptoma pri rođenju, a Duarte galaktozemija se čini dosta bezazlenom, neka su istraživanja ukazala na to da kod osoba s ovim poremećajima postoji povećani rizik za razvoj određenih bolesti tijekom života. Cilj je ovoga istraživanja bio utvrditi prisutnost i učestalost pojedinih mutacija u genu GALT (Q188R, K285N, IVS5-24G<A i N314D) kod zdrave hrvatske populacije. DNA smo izolirali iz pune krvi prikupljene od 166 zdravih dobrovoljaca, umnožili smo određene fragmente DNA lančanom polimeraznom reakcijom, digestirali smo ih pomoću specifičnih restrikcijskih endonukleaza (PCR-RFLP) i razdvajali smo dobivene proizvode na 4%-tnom agaroznom gelu. Učestalost alela s mutacijom Q188R, K285N, IVS5-24G<A ili N314D bila je 0%, 0%, 3,6% i 6,6 %. Među osobama kod kojih smo utvrdili prisutnost mutacija IVS5-24G<A i/ili N314D bilo je 30,3% varijanta Duarte-2. Dobiveni rezultati ukazuju na to da je mutacija N314D najučestalija kod zdrave hrvatske populacije. Naši rezultati dobro koreliraju s onima objavljenim za zdravu slovensku populaciju.

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P16-12

Screening for GALT gene mutations in a healthy Croatian population

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The GALT gene codes for the galactose-1-phosphate uridyl transferase (GALT) enzyme that catalyzes the conversion of galactose-1-phosphate to uridyl diphosphate (UDP)-galactose. Numerous mutations in the GALT gene have been found to impair GALT activity to a varying extent, causing galactosemia. This disorder exhibits considerable allelic heterogeneity in different populations and ethnic groups. Two most common mutations among Caucasians are Q188R and K285N. Individuals homoallelic for these mutations have a severe phenotype, named classic galactosemia, with complete loss of enzyme activity. A milder form of galactosemia, known as Duarte variant, is caused by the N314D mutation in the GALT gene. Along with the N314D mutation, Duarte variants of galactosemia depend on other genetic changes such as intronic sequence variation G1391A in intron V (IVS5-24G<A) (Duarte-2) or silent mutation L218L (Duarte-1 or Los Angeles variant). In Duarte-2 variant, homozygotes have approximately 50% and heterozygotes 75% of residual GALT activity, whereas Duarte-1 is characterized by increased GALT activity. Although heterozygotes for classic galactosemia are asymptomatic at birth and Duarte galactosemia appears to be quite benign, there are some indications that these disorders can increase the risk of developing certain diseases later in life. The aim of our study was to analyze a healthy Croatian population for the frequencies of Q188R, K285N, IVS5-24G<A and N314D mutations within GALT gene. DNA samples from 166 healthy individuals were analyzed for all four mutations by polymerase chain reaction and digestion with restriction enzymes (PCR-RFLP). Allele frequencies for Q188R, K285N, IVS5-24G<A and N314D were found to be 0%, 0%, 3.6% and 6.6%, respectively. There were 30.3% of Duarte-2 variant among the persons carrying IVS5-24G<A and/or N314D mutations. Study results showed N314 to be the most frequent mutation in the healthy Croatian population. Our results correlate well with those reported for a healthy Slovenian population.

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P16-13

Molekularna dijagnostika mitohondrijskih bolesti – MELAS

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MELAS, mitohondrijska encefalomiopatija, laktoacidoza s epizodama nalik apopleksiji je sindrom uzrokovan mutacijama u mitohondrijskom genu MTTL1 koji kodira tRNA za leucin (tRNALeu). Opisano je više točkastih mutacija ovoga gena od kojih je A3243G utvrđena u 80%, a T3271C u 10% bolesnika. Molekularne promjene u genu za MTTL1 imaju za posljedicu smanjenu sintezu mitohondrijskih enzima te smanjenu proizvodnju energije potrebne za normalno odvijanje staničnih procesa. Kako svaka stanica ima nekoliko mitohondrija u kojima se nalazi veći broj kopija mitohondrijske DNA (mtDNA), broj mutiranih kopija unutar stanice ili organa može biti varijabilan (heteroplazija), o čemu ovisi i klinička slika. Simptomi se javljaju najčešće već u dječjoj dobi u organima koji najviše troše energiju: mozak, skeletni i srčani mišić. Cilj je bio postaviti metodu lančane reakcije polimeraze (PCR) za određivanje mutacije T3271C te ispitati prisutnost mutacija A3243G i T3271C u bolesnika sa sumnjom na MELAS. Ispitivanjem je obuhvaćeno 41 dijete sa simptomima koji upućuju na poremećeno stvaranje stanične energije. mtDNA je izolirana iz leukocita periferne krvi. Pretraživanje na mutacije A3243G i T3271C je provedeno metodom PCR uz cijepanje umnoženih ulomaka s restrikcijskim enzimima (PCR/RFLP). Čimbenici reakcije PCR/RFLP za mutaciju T3271C su optimirani i provjereni uz pozitivni kontrolni uzorak. Mutacije A3243G i T3271C nisu utvrđene u ispitivanih bolesnika. Zaključuje se kako određivanje molekularnih promjena u mtDNA može pomoći u diferencijalnoj dijagnostici mitohondrijskih bolesti, a zbog heteroplazije bi mtDNA trebalo analizirati i u uzorcima zahvaćenih tkiva.

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P16-13

Molecular diagnosis of mitochondrial diseases – MELAS

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MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, is a syndrome caused by mutations in mitochondrial MTTL1 gene coding tRNA for leucin (tRNALeu). A number of point mutations of this gene have been described, of which A3243G and T3271C were established in 80% and 10% of patients, respectively. Molecular changes in MTTL1 gene lead to reduction in both mitochondrial enzyme synthesis and production of energy required for normal development of cellular processes. As each cell has several mitochondria with many copies of mitochondrial DNA (mtDNA), the number of mutated copies within a cell or an organ may vary (heteroplasia) and thus affect the clinical picture. Most frequently, the disease symptoms occur already during childhood in organs that are the highest energy consumers: the brain, skeletal and cardiac muscle. The aim was to establish a method of polymerase chain reaction (PCR) to determine T3271C mutation and to examine the presence of A3243G and T3271C mutations in patients with suspected MELAS. The study included 41 children with symptoms indicating impaired cellular energy production. mtDNA was isolated from peripheral blood leukocytes. Screening for A3243G and T3271C mutations was conducted by the PCR-restriction fragment length polymorphism methods (PCR-RFLP). Factors of PCR/RFLP reaction for T3271C mutation were optimized and verified against a positive control sample. A3243G and T3271C mutations were not detected in the patients observed. Determination of molecular changes in mtDNA may aid in the differential diagnosis of mitochondrial diseases. Due to heteroplasia, mtDNA analysis should also include affected tissue samples.

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P16-14

Neinvazivno određivanje spola fetusa iz majčine plazme – usporedba metoda multipleks PCR i PCR u stvarnom vremenu

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Pouzdano utvrđivanje spola fetusa tijekom rane trudnoće zahtijeva uzorkovanje fetalnih stanica invazivnim metodama (amniocenteza, biopsija korionskih resica itd.), koje sa sobom nose i određeni rizik kako za majku tako i za fetus. U obiteljima s X-vezanim nasljednim bolestima osobito je važno rano utvrditi spol fetusa. Zbog svega navedenog nastoje se razviti neinvazivne metode prenatalne dijagnostike. Svrha ovoga rada bila je usporediti pouzdanost metoda multipleks PCR i PCR u stvarnom vremenu za rano dokazivanje fetalnog Y kromosoma (*cell free fetal DNA*) u majčinoj plazmi.

Cell free DNA je izolirana iz 80 uzoraka majčine plazme uporabom QIAamp® DNA Blood Midi Kit, a zatim je: A) umnožena pomoću AmpFl STR® Identifiler™ kita (15 STR lokusa I amelogenin) i nakon toga analizirana kapilarnom elektroforezom na genetičkom analizatoru ABI PRISM 310; B) amplificirana i analizirana uporabom Quantifiler™ Y Human Male DNA Quantification kita (SRY gen) na uređaju ABI PRISM 7000 Sequence Detection System. Spol fetusa je potvrđen citogenetičkom analizom. Amplifikacija paternalnih STR alela trebala je poslužiti kao pozitivna kontrola prisutnosti fetalne DNA u majčinoj plazmi, osobito u slučajevima ženskih fetusa. No, u većini slučajeva amelogenin je bio jedini umnoženi fetalni lokus. Od ukupno 41 muškog fetusa prisutnost Y kromosoma u majčinoj plazmi je dokazana u 37 (90%) uporabom metode multipleks PCR, dok je primjenom PCR u stvarnom vremenu dokazano u 39 (95%) uzoraka. Nije bilo lažno pozitivnih uzoraka. Rezultati su u nekim slučajevima bili neuvjerljivi, jer je postojala mogućnost da je uzrok izostanka amelogeninskog/SRY gena bila neodgovarajuća količina fetalne DNA u uzorku ili u izolatu DNA. Daljnja ispitivanja na ovu temu trebala bi se usredotočiti na pronalaženje novih biljega koji bi bili sigurniji pokazatelji prisutnosti fetalne DNA u majčinoj plazmi.

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P16-14

Noninvasive determination of fetal sex from maternal plasma – comparison of multiplex PCR and real-time PCR

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Reliable determination of fetal gender in early pregnancy generally requires sampling of fetal cells by invasive procedures that are associated with low but definite risks for both the fetus and the mother. In families with X-linked recessive genetic disorders it is important to early determine the sex of the fetus, thus the development of safer methods is needed. The purpose of this study was to compare the reliability of multiplex PCR and real-time PCR methods for the determination of the presence of fetal Y-chromosome DNA in maternal plasma. Cell-free DNA was extracted from 80 samples of maternal plasma using QIAamp® DNA Blood Midi Kit. The extracted cell free fetal DNA was: (a) amplified using AmpFl STR® Identifiler™ kit (15 STR loci and sex-specific amelogenin) and subsequently analyzed on a capillary electrophoresis system ABI PRISM 310 Genetic Analyzer; and (b) amplified and analyzed using Quantifiler™ Y Human Male DNA Quantification kit (SRY gene) on ABI PRISM 7000 Sequence Detection System. The sex of fetuses was confirmed by cytogenetic analysis. Amplification of paternal STR alleles was expected to serve as a positive control for the presence of fetal DNA in maternal plasma in case of female fetuses, but unfortunately in a vast majority of samples amelogenin was the only fetal locus that was successfully amplified. Out of 41 male fetuses, the presence of Y-chromosome DNA in maternal plasma was successfully determined in 37 (90%) using multiplex PCR method and in 39 of 41 (95%) using real time PCR method. There were no falsely positive detected Y-chromosomes in female fetuses, but the results were partly inconclusive because we were not able to eliminate the possibility that the lack of male amelogenin/SRY gene was simply due to inadequate amount of fetal DNA. Further work on this method should consider the use of different markers that will be more confirmative for the presence of fetal DNA in maternal plasma.

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P16-15

Molekularna dijagnostika nasljednih ataksija

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Nasljedne ataksije su heterogena skupina neurodegenerativnih bolesti kod kojih prevladava poremećaj koordinacije pokreta. Atrofija malog mozga i/ili njegovih veza s drugim strukturama živčanog sustava razvija se kao posljedica molekularnih promjena u više od 40 različitih gena. Osnovni genski poremećaj u većine ovih gena je povećanje broja ponavljajućih slijedova nukleotida (CAG, CTG, CGG, GAA ili ATTCT). Produljenje slijeda u kodirajućoj regiji gena rezultira sintezom nefunkcionalnog proteina, dok produljeni nukleotidni slijed u nekodirajućoj domeni gena uzrokuje promjenu regulacije transkripcije gena ili pogrešno izrezivanje molekula mRNA, što u konačnici završava aktivacijom apoptoze živčanih stanica. Prema načinu nasljeđivanja razlikujemo autosomno dominantne (spinocerebelarne ataksije, SCA), autosomno recesivne, spolno (X-vezane) te ataksije vezane uz mitohondrijske bolesti. Cilj studije bio je proširiti diferencijalnu dijagnostiku ataksija molekularnom analizom te ispitati učestalost pojedinih oblika nasljednih ataksija u našoj populaciji bolesnika. Ispitana su 73 bolesnika u kojih su isključeni stečeni oblici ataksije. Određivanje broja tripleta CAG u genu za ataksin-1 (SCA 1), ataksin-2 (SCA 2) i ataksin-3 (SCA 3) te broja tripleta GAA u genu za frataksin (Friedreichova ataksija, FRDA) provedeno je metodom lančane reakcije polimerazom (PCR). Proizvodi PCR su analizirani kvalitativno na 2%-tnoj agarozi i kvantitativno na 6%-tnom poliakrilamidnom gelu. Od 13 bolesnika sa sumjom na autosomno recesivni oblik ataksije FRDA je potvrđena u 3 (23%) bolesnika. U preostalih bolesnika otkrivene su dvije autosomno dominantne ataksije, podtipa SCA2 i SCA3. Nasljedne ataksije su potvrđene u samo 5 (7%) bolesnika. Uvedene metode za FRDA, SCA1, SCA2 i SCA3 su tek prva faza molekularne analize nasljednih ataksija kojima se potvrđuju najučestaliji oblici. U idućem razdoblju predviđa se proširenje dijagnostičkog slijeda.

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P16-15

Molecular diagnosis of hereditary ataxias

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Hereditary ataxias are a heterogeneous group of neurodegenerative diseases characterized by movement coordination disorder. Atrophy of the cerebellum and/or its connections with other nervous system structures develops as a consequence of molecular changes in more than 40 different genes. The basic gene disorder in most of these genes is an increased number of nucleotide repeats (CAG, CTG, CGG, GAA or ATTCT). Expansion of repeats in gene encoding region results in the synthesis of nonfunctional protein, while expanded nucleotide repeat in non-coding gene domain causes a change in gene transcription regulation or defective mRNA splicing, eventually leading to activated apoptosis of neuronal cells. Ataxias may be distinguished according to the manner of inheritance: autosomal dominant (spinocerebellar ataxias, SCA), autosomal recessive, X-linked, and ataxias related to mitochondrial diseases. The aim was to expand the differential diagnosis of ataxias by molecular analysis and to investigate the frequency of individual types of hereditary ataxias in our patient population. The study included 73 patients with the acquired types of ataxia excluded. Determination of the CAG triplet number in the frataxin gene (Friedreich's ataxia, FRDA) was performed by the method of polymerase chain reaction (PCR). PCR products were analyzed qualitatively on 2% agarose and quantitatively on 6% polyacrylamide gel. Of 13 patients suspected of autosomal recessive type of ataxia, FRDA was confirmed in 3 (23%) patients. Two autosomal dominant ataxias were detected in the remaining patients, i.e. subtypes SCA2 and SCA3. Hereditary ataxias were confirmed in only five (7%) patients. The methods introduced for FRDA, SCA1, SCA2 and SCA3 are only the first stage of the molecular analysis of hereditary ataxias used to confirm the most frequent types. Expansion of diagnostic range is anticipated in the forthcoming period.

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P16-16

Asocijacijska studija porasta tjelesne težine i terapijskog odgovora na olanzapin s polimorfizmom gena SERT u shizofrenih bolesnica

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U usporedbi s tipičnim antipsihoticima druga generacija antipsihotika (DGA) ima prihvatljiviji profil nuspojava, kao i moguću širu učinkovitost glede simptoma. Porast tjelesne težine kao jedan od najčešćih nepovoljnih učinaka liječenja atipičnim antipsihoticima, poglavito klozapinom i olanzapinom, povezan je, međutim, s brojnim medicinskim problemima, tj. dijabetesom, kardiovaskularnim i malignim bolestima, nepridržavanjem terapije, čime značajno ometa učinkovitost liječenja shizofrenije. Veći afinitet DGA prema serotoninskom sustavu u odnosu na uobičajene antipsihotike dovodi do zaključka da bi taj sustav mogao biti uključen kako u terapijskim učincima tako i u opsegu nuspojava. Transporter serotonina (SERT) regulira čitav serotoninergični sustav kroz prilagodbu izvanstaničnih koncentracija serotonina. Polimorfne alelske varijacije SERT čine se značajnima za izražaj i funkciju transportera serotonina. Karakterizirane su dvije polimorfne regije gena SERT, tj. insercijski/delecijski polimorfizam s 44 para baza u promotorskoj regiji (SERTPR) i promjenjivi broj motiva ponavljanja u drugom intronu (SERT-in2). Cilj studije bio je ispitati odnose između genetičkih varijanata promotora L/S (SERTPR) i introna 2 l/s polimorfizama gena SERT i porasta tjelesne težine potaknutog olanzapinom te odgovora na terapiju u 94 shizofrenične bolesnice liječene olanzapinom do 3 mjeseca. Genotipizacija SERT provedena je metodom PCR. Indeks tjelesne mase (BMI) je izračunat za svaku bolesnicu prije liječenja olanzapinom te tri mjeseca kasnije. Kako bi se procijenilo poboljšanje kliničkih psihotičnih simptoma i terapijski odgovor na antipsihotik, sve su bolesnice ocijenjene prema ljestvici za pozitivne i negativne sindrome (engl. *positive and negative syndrome scale,* PANSS). Općenito je prisutnost alelske varijante S, SERTPR i genotipa SS povezana sa značajno višim porastom težine u bolesnika koji nisu bili pretili kod prijma (p=0,04). Prisutnost varijante L SERTPR je povezana sa značajno boljim odgovorom na terapiju mjerenim prema ukupnoj kao i općoj podljestvici PANSS (p<0,05), dok je prisutnost varijante l, SERTin2 uzrokovala bolji tera-

P16-16

Association study of olanzapine-induced weight gain and therapeutic response with SERT gene polymorphisms in female schizophrenic patients

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Compared to typical antipsychotics, second generation antipsychotics (SGA) have more acceptable side effect profiles, and possibly broader symptom efficacy. However, weight gain as one of the most common adverse effects of treatment with atypical antipsychotics, especially clozapine and olanzapine, is associated with numerous medical problems, i.e. diabetes, cardiovascular disease, malignant disease, and noncompliance, thus significantly obstructing the therapeutic efficacy for schizophrenia. The higher SGA affinity for serotonin system as compared to typical antipsychotics leads to a conclusion that serotonin system could be implicated in both therapeutic effects and side effect spectrum. Serotonin transporter (SERT) regulates the entire serotoninergic system through modulation of extracellular serotonin concentrations. SERT polymorphic allelic variations seem to be significant for the SERT expression and function. Two polymorphic regions of SERT gene: a 44-base-pair (bp) insertion/deletion polymorphism in the promoter region (SERTPR), and a variable number of tandem repeats in second intron (SERT-in2) have been characterized. The aim of this study was to investigate the relationships between L/S promoter (SERTPR) and l/s intron2 (SERTin2) genetic variants of SERT gene polymorphisms with olanzapine-induced weight gain and treatment response in 94 female schizophrenic patients treated with olanzapine for up to 3 months. SERT genotyping was performed by PCR method. Body mass index (BMI) was calculated in each patient prior to olanzapine administration and three months afterwards. To assess and evaluate improvement of clinical psychotic symptoms and therapeutic response to the antipsychotic, all patients were rated using the Positive and Negative Syndrome Scale (PANSS). Overall, the presence of S SERTPR allelic variant and SS genotype was associated with a significantly greater weight gain in subjects who were nonobese at the time of admission (p=0.04). The presence of L SERTPR variant was associated with a significantly better treatment response measured with

pijski odgovor za samo neke simptome. Rezultati ukazuju na genetičke čimbenike povezane s porastom težine potaknutim olanzapinom i odgovorom na terapiju u shizofreničnih bolesnica.

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total PANSS and general PANSS subscale ($p<0.05$), while the presence of I SERTin2 variant was associated with better treatment response only in several items. These findings identify genetic factors associated with olanzapine-induced weight gain and treatment response in female schizophrenic patients.

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P16-17

Frekvencija polimorfizma S311C gena PON2 u bolesnika s kroničnim bubrežnim zatajenjem

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Genska obitelj za paraoksonazu (PON) smještena je na dugom kraku kromosoma 7q21.3-22.1 i predstavljena je s tri gena (pon1, pon2 i pon3) koji kodiraju enzime djelatne u zaštiti organizma od oksidativnog stresa. Paraoksonaza 2 (PON2) prisutna je u gotovo svim ljudskim stanicama. Smatra se da PON2 djeluje kao stanični antioksidant te mu se s tim u svezi pripisuje zaštitni učinak naspram razvoja ateroskleroze. Najčešći uočeni polimorfizam kodirajuće regije pon2 gena je S311C kod kojega dolazi do zamjene serina cisteinom na poziciji 311. Taj se polimorfizam povezuje s povećanim rizikom od nastanka ateroskleroze. Utvrđena je pojavnost polimorfizma S311C u populaciji bolesnika s kroničnim zatajenjem bubrega koji su podvrgnuti hemodijalizi (n=160) i zdravih ispitanika (n=167) te su dobiveni rezultati međusobno uspoređeni. Prisutnost polimorfizma S311C utvrđena je standardnim postupkom: Millerovom metodom izolirana je DNA iz pune krvi ispitanika, a analiza genotipa provedena je postupkom PCR-RFLP. Analiza polimorfizma gena pon2 kod bolesnika s kroničnim bubrežnim zatajenjem pokazala je slijedeće frekvencije: 88 (0,55) SS, 63 (0,39) CS, 9 (0,06) CC. U kontrolnoj skupini dobili smo vrlo slične frekvencije: 83 (0,50) SS, 80 (0,48) CS, 4 (0,02) CC. Frekvencije alela kod bolesnika s kroničnim bubrežnim zatajenjem bile su 239 (0,75) za alel S i 81(0,25) za alel C, a u kontrolnoj skupini 246 (0,74) za alel S alel i 88 (0,26) za alel C. Na temelju rezultata χ^2-testa zaključeno je da ne postoji statistički značajna razlika u distribuciji genotipa S311C (p=0,139) i frekvenciji alela (p=0,831) između bolesnika s kroničnim zatajenjem bubrega i zdravih dobrovoljaca.

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P16-17

Frequency of PON2 gene S311C polymorphism in patients with chronic renal failure

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The paraoxonase (PON) gene family, located on the long arm of chromosome 7q21.3-22.1, includes three members (pon1, pon 2, pon 3) that code for enzymes involved in the protection against oxidative stress. Paraoxonase 2 (PON2) is ubiquitously expressed in nearly all human tissues and acts as a cellular antioxidant. Due to the reduction of oxidative stress PON2 may also act as an antiatherogenic enzyme. Common polymorphism of pon2 gene related to the serine/cysteine substitution at position 311 (S311C) has been reported to be associated with the high risk of atherosclerosis. We determined S311C polymorphism of pon2 gene in patients with chronic renal failure undergoing hemodialysis (n=160) that have an increased risk of developing atherosclerosis, and compared the results obtained with a control group (n=167). For S311C polymorphism detection we used standard procedure: Miller's method for DNA isolation from whole blood, and PCR-RFLP method for polymorphism analysis. The analysis of pon2 S311C gene polymorphism in patients with chronic renal failure showed the following frequencies: 88 (0.55) SS, 63 (0.39) CS and 9 (0.06) CC. In the control group we found rather similar frequencies: 83 (0.50) SS, 80 (0.48) CS and 4 (0.02) CC. Allele frequencies in patients were 239 (0.75) for S allele and 81 (0.25) for C allele. In control group we found the following allele frequencies: 246 (0.74) for S allele and 88 (0.26) for C allele. According to statistical evaluation by χ^2-test we concluded that there was no significant difference in the distribution of S311C genotype (p=0.139) and allele frequency (p=0.831) between chronic renal failure patients and healthy controls.

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P17 – Imunologija, P17-1 (UP11-1)

Analiza stanica bronhoalveolarnog lavata protočnom citometrijom u sarkoidozi

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Limfocitozu u uzorku bronhoalveolarnog lavata (BAL) nalazimo u virusnim infekcijama, alveolitisu, sarkoidozi i idiopatskoj plućnoj fibrozi (IPF). Analiza limfocitnih T-staničnih subpopulacija može pomoći u razgraničavanju sarkoidoze od ostalih bolesti. U većini slučajeva sarkoidoze glavninu T stanične populacije čine CD4+ stanice i povećan je omjer CD4/CD8 te se smatra da ovakav nalaz ima dijagnostičko značenje za plućnu sarkoidozu. Limfociti u BAL su analizirani protočnom citometrijom u svrhu određivanja preciznog imunofenotipa, tj. površinskih biljega (CD3, CD4, CD8) i omjera CD4/CD8 u populaciji bolesnika sa sarkoidozom (n=26). Radi usporedbe nalaza dobivenih u sarkoidozi analizirali smo i skupinu bolesnika s IPF (n=14). Panel za imunofenotipizaciju limfocita sastojao se od FITC-CD3/PE-CD19, FITC-CD3/PE-CD4, FITC-CD3/PE-CD8, FITC-CD45/PE-CD14 i negativne kontrole FITC-IgG1/ PE-IgG2a. Primijenjena je direktna imunofluorescentna metoda dvostrukog bojenja po standardnom protokolu. Obojene stanice smo analizirali na protočnom citometru (FACSCalibur, BD). Analizirano je 10000 stanica po uzorku. Rezultati su izraženi kao postotak pozitivnih limfocita unutar limfocitne populacije. U uzorcima bolesnika sa sarkoidozom zabilježili smo značajan ($p<0,05$) porast CD3 i CD4 uz pad CD8. IPF pokazuje suprotnu tendenciju. Više vrijednosti omjera CD4/CD8 (8,89±7,06) su zabilježene kod bolesnika sa sarkoidozom u usporedbi s bolesnicima s IPF (0,86±0,75). Imunofenotipizacija limfocita u BAL od velikog je značenja za diferencijalnu dijagnostiku plućnih bolesti kao što su sarkoidoza i idiopatska plućna fibroza.

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P17 – Immunology, P17-1 (UP11-1)

Flow cytometry analysis of bronchoalveolar lavage cells in sarcoidosis

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Bronchoalveolar lavage (BAL) hyperlymphocytosis, observed in viral infections, alveolitis, sarcoidosis and idiopathic pulmonary fibrosis (IPF), may involve different T cell subsets. CD4+ cells represent the major T cell subset in most cases of sarcoidosis, and demonstration of an increased CD4/CD8 ratio has been proposed as a diagnostic tool for pulmonary sarcoidosis. BAL lymphocytes were analyzed by flow cytometry to determine cell surface markers (CD3, CD4, CD8) and CD4/CD8 ratio in a population of patients with sarcoidosis (n=26). A group of IPF patients (n=14) were included to compare the findings in sarcoidosis. The staining panel for lymphocyte typing included FITC-CD3/ PE-CD19, FITC-CD3/ PE-CD4, FITC-CD3/ PE-CD8, FITC-CD45/ PE-CD14 and negative control FITC-IgG1/ PE-IgG2a (BD). An two-color direct immunofluorescence assay was performed using a standard protocol. Stained cells were analyzed on a flow cytometer (FACSCalibur, BD). A total of 10000 cells *per* sample were analyzed. Results were expressed as percentage of positive lymphocytes within the lymphocyte population. Both CD3 and CD4 were significantly ($p<0.05$) increased with decreasing CD8 in sarcoidosis, while IPF showed a reverse tendency. A higher CD4/CD8 ratio (8.89±7.06) was detected in patients with sarcoidosis than in those with IPF (0.86±0.75). The phenotyping of lymphocytes in BAL fluid is of special value in the differential diagnosis of lung disorders such as sarcoidosis and IPF.

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P17-2

Stanični biljezi u sinusnom ispirku astmatičnih i neastmatičnih bolesnika

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Velik broj bolesnika s kroničnim rinosinusitisom (CRS) pati od respiracijskih alergija. Aktivacija eozinofilne, mastocitne i neutrofilne stanične aktivnosti dokazana je u mukozi sinusa kod CRS unatoč tome što se radi o gornjim dišnim putovima. Eozinofilna upala je u sinusnoj sluznici najizraženija kada postoji bolest jedinstvenih gornjih i donjih dišnih putova (*united airways disease*). Namjera je bila usporediti stanične biljege eozinofila (ECP), mastocita (triptaze) i neutrofila (MPO) u sinusnom ispirku kod bolesnika sa CRS i onih s astmom + CRS prije i nakon endosinusne terapije antibiotikom i steroidom. Skupinu astmatičara činilo je 19 bolesnika s astmom + CRS, a skupinu neastmatičara 17 bolesnika sa CRS bez astme (alergičari i nealergičari uključeni u skupinu). Bolesnici su bili podvrgnuti 7-dnevnoj terapiji 40 mg gentamicina i 2 mg deksametazona po sinusu. Terapija i uzimanje sinusnog ispirka (5 mL fiziološke otopine) provedeni su kroz polietilensku cjevčicu umetnutu antralnom punkcijom u lokalnoj anesteziji. Sinusni ispirci uzeti sinuskopski 2 sata su stajali na sobnoj temperaturi, centrifugirani 10 min na 1000 G te smrznuti na -20 °C. Mjerenje ECP, MPO i triptaza izvršeno je fluoroenzim imuno metodom, prema standardnim uputama za serumsku detekciju (ImmunoCAP, Phadia, Švedska). Nakon terapije astmatičara ECP se značajno smanjuje, sa 64±72,22 na 19,04±32,67 μg/L, ali se u skupini CRS povisuje s 25,36±61,77 na 48,34±72,2 μg/L. Vrijednosti triptaze kod astmatičara značajno se smanjuju s 15,64±30,89 na 2,31±2,66 μg/L, ali i u skupini CRS s 11,07 na 9,02±24,58 μg/L. MPO se mijenja s 456,0±488,0 na 277,08±95,12 μg/L kod astmatičara, te s 364,0±802,0 na 488,59±741,83 u bolesnika s CRS. ECP i MPO su bili značajno viši u sinusnom ispirku astmatičara prije terapije, dok triptaze ne pokazuju značajnu razliku između skupina. Astmatičari sa CRS pokazuju značajno višu aktivaciju eozinofila i mastocita u sinusnoj mukozi od neastmatičara sa CRS. Endosinusna terapija steroidima i antibioticima učinkovita smanjuje lokalnu eozinofilnu i mastocitnu aktivaciju kod astmatičara, te mastocitnu staničnu aktivaciju jedino kod neastmatičara sa CRS. Endosinusna terapija nema učinka na neutrofilnu aktivnost.

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P17-2

Cellular markers in sinus fluid of asthmatic and non-asthmatic patients

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Significant proportions of patients with chronic rhinosinusitis (CRS) suffer from respiratory allergy. Activation of eosinophils, mastocytes and neutrophils has been demonstrated in sinus mucosa in patients with CRS, despite evidence of lower airways disease. Eosinophilic inflammation in sinus mucosa is most pronounced in the presence of upper and lower airways disease (united airways disease). The study was so designed as to compare cellular markers of eosinophils (ECP), mastocytes (tryptase), and neutrophils (MPO) in sinus fluid between patients with CRS and those with asthma + CRS before and after endosinusal treatment with antibiotic and steroid for 7 days. Asthma group consisted of 19 asthmatics with asthma + CRS, and the group of non-asthmatics consisted of 17 patients with CRS without asthma (allergic and nonallergic included in both groups). Patients received 7-day treatment with 40 mg gentamicin and 2 mg dexamethasone *per* sinus. Therapy was administered and sinus lavage (5 mL of saline) performed through polyethylene tubes inserted by antral puncture under local anesthesia. Sinus samples were obtained by sinoscopy, stored at room temperature for 2 hours, centrifuged for 10 minutes at 1000 G, and stored at -20 °C. Samples were analyzed according to standard instructions for serum samples by fluoroenzyme immunoassay (ImmunoCAP, Phadia, Sweden). After the treatment, ECP decreased significantly from 64±72.22 to 19.04±32.67 μg/L in asthmatics, but increased insignificantly from baseline 25.36±61.77 to 48.34±72.20 μg/L in CRS group. Tryptase levels decreased significantly from 15.64±30.89 to 2.31±2.66 μg/L in asthmatics and from 11.07 to 9.02±24.58 μg/L in CRS group. MPO changed from 456.0 ±488.0 to 277.08±95.12 f μg/L in asthmatics and from 364.0±802.0 to 488.59±741.83 μg/L in CRS patients. ECP ($p<0.0001$) and MPO ($p<0.01$) at baseline were significantly higher in the asthma group, while tryptase showed no significant between-group difference. In both groups, ECP and MPO demonstrated significant correlation at baseline. Accordingly, asthmatics with CRS show a significantly higher activation of eosinophils and mastocytes in sinus mucosa than non-asthmatics with CRS. Endosinusal treatment with steroid and antibiotic is effective in reducing local eosinophil and mastocyte activation in asthmatics, but influence mast cells only in CRS non-asthmatics. Endosinusal treatment has no effect on neutrophils.

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P17-3

Interleukin-8 (IL-8) i interleukin-10 (IL-10) u bronhoalveolarnom lavatu (BAL) u bolesnika sa sarkoidozom: korelacija s kliničkim parametrima

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Sarkoidoza je sistemska granulomatozna bolest obilježena nakupljanjem T stanica i makrofaga. Razni citokini mogu imati ključnu ulogu u aktivaciji T stanica i makrofaga, a time i u stvaranju granuloma. Cilj rada bio je istražiti koncentracije proupalnih interleukina-8 (IL-8) i antiupalnih interleukina-10 (IL-10) u bronhoalveolarnom lavatu (BAL) bolesnika s plućnom sarkoidozom (PS) i korelirati ih s kliničkim parametrima. U rad je bilo uključeno 17 bolesnika s plućnom sarkoidozom (3 muškarca i 14 žena, dob 34-61 godina). Koncentracije IL-8 i IL-10 izmjerene su metodom ELISA (R&D Systems). BAL je učinjen pomoću 4 x 50 mL sterilne fiziološke otopine. Svi su bolesnici imali limfocitozu u BAL (36,1±21,2%) i porast omjera CD4/CD8 (8,42±7,13). IL-8 i IL-10 izmjereni su u svih bolesnika. Nađena je statistički značajna korelacija između ovih dvaju citokina i biljega oštećenja tkiva: za IL-8 pozitivna s alkalnom fosfatazom na razini statističke značajnosti $p<0,05$, a za IL-10 negativna s albuminom na razini statističke značajnosti $p<0,05$. Statistički značajna korelacija na razini statističke značajnosti $p<0,01$ nađena je između koncentracije IL-8 i interleukina-1 beta (IL-1 beta), kao i tumor nekrotskog faktora alfa (TNF alfa). Međutim, statistički značajna negativna korelacija na razini statističke značajnosti $p<0,05$ nađena je između IL-10 i angiotenzin konvertirajućeg enzima (ACE). Rezultati pokazuju da lokalno stvoreni IL-8 i IL-10 u plućnoj sarkoidozi koreliraju s aktivnošću ove granulomatozne plućne bolesti.

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P17-3

Interleukin-8 (IL-8) and interleukin-10 (IL-10) in bronchoalveolar lavage (BAL) of patients with sarcoidosis: correlation with clinical parameters

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Sarcoidosis is a systemic granulomatous disease characterized by accumulation of T cells and macrophages. Various cytokines may play crucial roles in the activation of T cells and macrophages, and thereby in the formation of granulomas. The aim of the study was to investigate the levels of proinflammatory interleukin-8 (IL-8) and anti-inflammatory interleukin-10 (IL-10) in bronchoalveolar lavage (BAL) of patients with pulmonary sarcoidosis (PS), and to correlate them with clinical parameters. The study included 17 patients with pulmonary sarcoidosis (PS; 3 male and 14 female, age 34-61). The concentrations of IL-8 and IL-10 were measured by ELISA method (R&D Systems). BALF was performed with 4 x 50 mL sterile saline solution. All patients presented BAL lymphocytosis (36.1±21.2%) and an increase of CD4/CD8 ratio (8.42±7.13). IL-8 and IL-10 were detected in all patients. These cytokines correlated with the markers of tissue damage: IL-8 positively ($p<0.05$) with alkaline phosphatase, and IL-10 negatively ($p<0.05$) with albumin. A strong correlation ($p<0.01$) was found between the concentration of IL-8 and interleukin-1 beta (IL-1 beta) as well as tumor necrosis factor alpha (TNF alpha). Moreover, IL-10 showed negative correlation ($p<0.05$) with ACE. These results suggested that locally derived IL-8 and IL-10 in PS correlated with the activity of this granulomatous lung disease.

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P17-4

Imunofenotipizacija stanica citološkog punktata metodom protočne citometrije

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Citološki punktat limfnog čvora predstavlja suspenziju stanica, ali i nerutinski, specifičan heterogeni analitički uzorak čija je primjena u dijagnostici limfadenopatija najčešće ograničena nedostatnim brojem stanica uzorka. Cilj je bio analitički i dijagnostički evaluirati metodu protočne citometrije, odrediti prikladnost citološkog punktata limfnog čvora za analizu, ocijeniti učinkovitost literaturnih graničnih vrijednosti omjera lakih lanaca, a moguće latentne povezanosti prikazati matematičkim modelom. Citološki punktati limfnog čvora (n=245) dobiveni aspiracijom analizirani su metodom protočne citometrije u Zavodu za kliničku kemiju KB Merkur potvrđenom prema međunarodnom standardu ISO:9001:2000. Analitičkom procjenom (prema preporukama Instituta za kliničko- laboratorijske standarde NCCLS) je određena nepreciznost, netočnost, linearnost i donja granica detekcije. Dijagnostička osjetljivost i specifičnost, pozitivna (PPV) i negativna (NPV) prediktivna vrijednost metode protočne citometrije su određene eksplorativnom statistikom neuronske mreže programske potpore Statistica Version 6. Klasifikacijom podataka metodom stabla odlučivanja izgrađen je matematički model predviđanja dijagnoze. Kontrola ispravnosti optičkog i protočnog sustava citometra, kvalitete reagensa, monoklonskih protutijela i specifičnih analitičkih uvjeta bila je dio unutarnje kontrola kvalitete. Za kontrolu pouzdanosti analitičkih rezultata primijenjena je međunarodna nezavisna procjena rezultata UKNEQAS. Prema kriteriju odgovarajućeg broja leukocita (0,35x10^9/L) iz analize su izuzeta 72 (22%) citološka punktata. Nepreciznost mjernog instrumenta je iznosila do 7%, a netočnost do 10%. Određena je dijagnostička osjetljivost (82%), specifičnost (72%), PPV (93%) i NPV (48%) metode protočne citometrije. Primjena jačeg kriterija određivanja klonalnosti limfocita B smanjila je osjetljivost, a povećala specifičnost metode. Pouzdanost analitičkih rezultata potvrđena je zadovoljenjem svih međunarodnih kriterija prihvatljivosti. Zaključeno je kako standardizacija metode protočne citometrije prema radnim protokolima osigurava pouzdanost analitičkih rezultata u citološkom punktatu limfnog čvora. Potencijalna primjena i učinkovitost

P17-4

Immunophenotyping of fine needle aspirate cells by flow cytometry method

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Fine-needle aspirate (FNA) represents a suspension of lymph node cells but also a nonroutine, specific and heterogeneous analytical specimen the use of which is usually limited by the inadequate number of cells for the flow cytometry method (FCM). The aim of the study was to perform critical analytical and diagnostic evaluation of FCM, to assess the adequacy of FNA for FCM analysis, to evaluate the usefulness of the light chain ratio borderline values reported in the literature, and to present the possible associations as a mathematical model. Upon collection, 245 FNA specimens were analyzed by FCM at Department of Clinical Chemistry, Merkur University Hospital, certified according to ISO:9001:2000. Brief analytical evaluation (imprecision, inaccuracy, linearity and lower detection limit) was done according to the evaluation guides of the Clinical and Laboratory Standards Institute (NCCLS). Diagnostic specificity and sensitivity as well as positive (PPV) and negative (NPV) predictive value of the FCM were determined using explorative statistics of the neural network (Statistica Version 6). Using the classification tree method for the classification of input data, a mathematical model of diagnosis prediction was generated. The instrument quality control assurance of the optic and fluid system, reagents and monoclonal antibodies as well as specific analytical control were performed. The reliability of our analytical results was evaluated by UKNEQAS independent international external quality control. According to the criteria of FNA leukocyte count (0.35x10^9/L), 72 (22%) FNA specimens were excluded from further analysis. For all parameters of the instrument (Coulter EPICS-XL), imprecision and inaccuracy were 7% and 10%, respectively. Diagnostic sensitivity, specificity, PPV and NPV for FCM were 82%, 72%, 93% and 48%, respectively. The use of the literature light chain ratio borderline values reduced diagnostic sensitivity but increased diagnostic specificity of FCM. Standardization of FCM in nonroutine, specific and heterogeneous analytical specimens like FNA is possible due to the sample preparation protocols and instrument quality control assurance. In routine classification of new data, the use of the gener-

izgrađenog matematičkog modela u klasifikaciji novih podataka i svakodnevnom rutinskom radu predstavljala bi krajnji rezultat istraživanja i unaprjeđenje klasifikacije limfadenopatija.

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P17-5

Empirijska metoda konverzije rezultata dvaju dijagnostičkih testova za anti-CMV IgG protutijela

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Određivanje citomegalovirus imunoglobulina G (IgG) ima za cilj praćenje imunog statusa bolesnika, dok praćenje promjene aktivnosti titra IgG protutijela tijekom bolesti značajno doprinosi postavljanju dijagnoze infekcije ovim virusom. Zbog nepostojanja međunarodnog standarda teško je uskladiti i usporediti rezultate različitih proizvođača, pa je tako otežana i longitudinalna procjena nalaza, što je od iznimne važnosti u serodijagnostici. Neusporedivost rezultata dvaju proizvođača koji izražavaju rezultate u različitim jedinicama (metode Abbott AxSYM CMV IgG i Behring Enzygnost AntiCMV/IgG) predstavlja ozbiljnu prepreku u racionalnoj dijagnostici infekcije ovim virusom i postavljanju dijagnoze. Razlog za prelazak s jednog dijagnostičkog sustava na drugi može biti financijske naravi, zbog prestanka proizvodnje pojedinog reagensa, parni serum za usporednu analizu nije dostupan ili pak treba usporediti rezultate različitih laboratorija. Predlažemo dvije empirijske formule utemeljene na linearnoj regresiji, prema kojima je moguća pretvorba vrijednosti metoda i međusobna usporedba rezultata sa zadovoljavajućom preciznošću. Analizirali smo 38 seropozitivnih uzoraka metodama Abbott AxSYM CMV IgG i Behring Enzygnost AntiCMV/IgG. Nakon logaritamske transformacije na osnovi 10 izvornih vrijednosti provedene su dvije linearne regresijske analize uz izračun jednadžba pravca regresije i visine Pearsonovog koeficijenta korelacije. Medijan i raspon vrijednosti u jedinicama AU/mL prema metodi Abbotta iznosili su: M=183,5 (30-821), a vrijednosti titra prema metodi Behringa iznosili su: M=7486 (352-67675). Vrijednost korelacijskog koeficijenta između transformiranih vrijednosti metoda iznosila je 0,87 (95%CI: 0,76-0,93), p<0,001. Obrazac za preračunavanje vrijednosti titra dobiven ovom metodom može predstavljati najnovije istraživanje u klasifikaciji limfadenopatija.

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P17-5

Empirical method for conversion of results obtained by two anti-CMV IgG diagnostic tests

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Determination of cytomegalovirus (CMV) immunoglobulin G (IgG) serves for monitoring patient immune status, whereas monitoring of changes in IgG titer activity during the course of disease helps in making the diagnosis of CMV infection. Because international standards are lacking, comparison of the results of diagnostic tests from different manufacturers is very difficult or impossible. Longitudinal asessment, which is of paramount importance in serodiagnosis, is thus greatly hampered. The impossibility to compare the results obtained by the methods developed by different manufacturers (Abbott AxSYM CMV IgG and Behring Enzygnost AntiCMV/IgG method) presents a serious obstacle for rational diagnosis of CMV infection. There are many potential reasons for switching diagnostic systems in use, e.g., inadequate resources, the manufacture of a particular reagent may be stopped, paired serum may be missing, or one may simply need to compare results from different laboratories. We propose two empirical formulas for conversion between values obtained by the two methods, based on linear regression technique and with a satisfactory level of conversion precision. We analyzed 38 seropositive samples with both Abbott AxSYM CMV IgG and Behring Enzygnost AntiCMV/IgG methods. After the original results had been log(10)-transformed, two separate linear regression analyses were performed. The corresponding regression line equations and Pearson correlation coefficient were calculated. The median and range of values in AU/mL obtained by Abbott method were: M=183.5 (30-821), and titer values obtained by Behring method were: M=7486 (352-67675). Correlation coefficient between the log-transformed values was r=0.87 (95%CI: 0.76-0.93),

bivenih metodom Behringa u približne vrijednosti AU/mL prema metodi Abbotta glasi 10^(1,255+1,136*log(10)X). Obrnuti obrazac za izračun vrijednosti titra iz AU/mL glasi 10^(-0,282+0,664*Log(10)X). Visoki koeficijent korelacije ukazuje na jaku statističku povezanost vrijednosti dobivenih u različitim analitičkim sustavima. Predložene formule za konverziju omogućuju longitudinalnu procjenu nalaza u slučajevima kada serum nije moguće usporedno analizirati istom metodom. Bez obzira na širinu granica regresije, klinički je značajno najmanje dvostruko povećanje aktivnosti protutijela, što izlazi iz granica pogreške regresije.

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p<0.001. The formula for converting the values of titer derived by Behring method into approximate values of AU/mL is: 10^(1.255+1.136*log(10)X), where X may be any value within the range. The inverse formula for converting AU/mL to titer is: 10^(-0.282+0.664*Log(10)X). The high correlation coefficient indicates strong statistical correspondence between transformed values across two different analytical systems and different units. The proposed conversion formula allows for longitudinal assessment in cases when serum analysis in parallel with the same method is not possible. Regardless of the regression uncertainity limits, at least twofold increase of antibody activity is clinically relevant, which is beyond the regression confidence intervals.

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P17-6

Usporedba dviju metoda određivanja imunoglobulina

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Određivanje koncentracije imunoglobulina daje važnu informaciju o humoralnom imunom statusu.
Cilj ovoga rada bio je usporediti rezultate dobivene usporednim određivanjem imunoglobulina G, imunoglobulina A i imunoglobulina M dvjema imunokemijskim metodama: imunonefelometrijski i imunoturbidimetrijski. Koncentracije imunoglobulina odredili smo u istim uzorcima seruma nasumce odabranih bolesnika (n=45) reagensima dvaju proizvođača: imunonefelometrijski (Dade Behring, nefelometar ProSpec) i imunoturbidimetrijski (Abbott, biokemijski analizator Architect c8000). Ispitivanje je obuhvatilo nepreciznost iz dana u dan, netočnost i usporedna određivanja. Mjerene su koncentracije imunoglobulina G, imunoglobulina A i imunoglobulina M. Za određivanje nepreciznosti iz dana u dan i netočnosti primijenjeni su kontrolni serumi (kod imunonefelometrijskog određivanja NIT Protein Control M tvrtke Dade Behring i kod imunoturbidimetrijskog Precinom U tvrtke Roche). Dobiveni rezultati usporednih određivanja statistički su obrađeni neparametrijskom linearnom regresijskom analizom po Passingu i Babloku. Nepreciznost iz dana u dan određena je mjerenjem imunoglobulina u kontrolnom serumu kroz 25 dana. Koeficijent varijacije dobiven imunonefelometrijskim mjerenjem iznosi za IgG 3,9%, IgA 4,1%, IgM 4,3%, a imunoturbidimetrijskim mjerenjem za IgG 4,4%, IgA 4,2%, IgM 5,1%. Određivanjem netočnosti s kontrolnim

P17-6

Comparison of two methods of immunoglobulin determination

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Quantitative determination of immunoglobulins provides important information on the humoral immune status. The aim of the study was to compare the results obtained by immunoglobulin G, immunoglobulin A and immunoglobulin M determination by two immunochemistry methods, immunonephelometric and immunoturbidimetric methods. Immunoglobulin concentrations were determined in the same serum samples of randomly selected patients (n=45) using two assays for determination of immunoglobulins in serum: immunonephelometric (Dade Behring, Nephelometer Pro Spec) and immunoturbidimetric (Abbott, Architect c8000 biochemistry analyzer). Day-to-day imprecision, inaccuracy and correlation were determined. Immunoglobulin G, immunoglobulin A and immunoglobulin M concentrations were also measured. Day-to-day imprecision and inaccuracy were measured using control serum (immunonephelometrically by N/T Protein Control M, Dade Behring, and immunoturbidimetrically by Precinorm U, Roche). Results of these parallel determinations were statistically processed by use of nonparametric linear regression Passing-Bablok analysis. Day-to-day imprecision was determined by measuring immunoglobulins in control serum over 25 days. The coefficients of variation yielded by immunonephelometric determination were for IgG 3.9%, IgA 4.1% and IgM 4.3%, whereas those recorded

serumima dobivene su vrijednosti kod nefelometrijskog određivanja za IgG 6,4%, IgA 1,9%, IgM 1,24%, a kod imunoturbidimetrijskog određivanja za IgG 4,3%, IgA 1,3%, IgM 2,9%. Usporedbom rezultata dobivenih na nefelometru i biokemijskom analizatoru dobiveni su koeficijenti korelacije za IgG r=0,9882; za IgA r=0,9953; za IgM r=0,9933. Određivanjem imunoglobulina imunonefelometrijski i imunoturbidimetrijski dobili smo rezultate koji su usporedivi i ukazuju da su obje metode prihvatljive za rutinski rad.

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by immunoturbidimetric determination were IgG 4.4%, IgA 4.2% and IgM 5.1%. Determination of inaccuracy with control serum by immunonephelometric method yielded the following values: IgG 6.4%, IgA 1.9% and IgM 1.24%, and with immunoturbidimetric method IgG 4.3%, IgA 1.3% and IgM 2.9%. Comparison of the results obtained by these two analyses (nephelometer and biochemistry analyzer) produced the following correlation coefficients: IgG r=0.9882, IgA r=0.9953 and IgM r=0.9933. The results obtained by immunonephelometric and immunoturbidimetric assays show that both methods are comparable and acceptable for routine work.

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P17-7

Dokazivanje plazmatskih proupalnih citokina i čimbenika rasta na mikročipu

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Citokini su važni biološki čimbenici u regulaciji imunoreaktivnosti i patofiziologiji raznih infektivnih bolesti, te bolesti uzrokovanih upalom, poremećajem staničnog rasta i stvaranja novih krvnih žila. Mjerenjem pojedinačnih citokina enzimoimunotestom (ELISA) opažena je povišena koncentracija različitih proupalnih citokina u plazmi bolesnika s teškim infekcijama, autoimunim bolestima i nekim karcinomima. Otuda i zanimanje za ispitivanje moguće dijagnostičke i prognostičke vrijednosti proupalnih citokina u raznim bolestima. Nove tehnologije, kao što su mikrokuglice ili mikročipovi s monoklonskim antitijelima specifičnim za razne citokine, omogućuju istodobno dokazivanje više različitih citokina u istom uzorku plazme. U istraživanju smo rabili mikročip s dvanaest različitih citokina i čimbenika rasta. Koncentraciju citokina u plazmi odredili smo u 11 novorođenčadi s različitim bakterijskim infekcijama, 7 novorođenčadi s raznim urođenim grješkama bez popratne infekcije, te u 7 odraslih bolesnika s raznim histološkim tipovima i lokalizacijama karcinoma. Razinu proupalnih citokina odredili smo mikročipom koji omogućuje istodobno mjerenje IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-gama, TNF-alfa, IL-1 alfa, IL-1 beta, MCP-1 i EGF. Koncentracija kemokina IL-8 i MCP-1 povišena je u svih ispitanika. Povišena je i koncentracija IL-6 i TNF-alfa u 75%

P17-7

Detection of plasma proinflammatory cytokines and growth factors by biochip array

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Cytokines are major biologic factors in the modulation of immune responses and in the pathophysiology of various infections and diseases related to inflammation, growth deregulation and vascular remodeling. Determination of a single cytokine by ELISA has shown elevated concentrations of various proinflammatory cytokines in plasma of patients with serious infections, autoimmune diseases and cancer. These data motivated further investigations to determine the possible diagnostic and prognostic value of proinflammatory cytokine measurement. New technologies such as microbeads or biochip array platform with monoclonals specific for various cytokines allow for simultaneous detection of multiple cytokines in the same plasma sample. In this study, we used a biochip array platform for proinflammatory cytokine determination in the plasma of newborn infants and adults with cancer. Plasma cytokine levels were determined in 11 newborns with various bacterial infections, 7 infants without infection, and 7 adults with different histologic types and localization of cancer. Plasma cytokine levels were evaluated by biochip array for simultaneous detection of IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-gamma, TNF-alfa, IL-1 alfa, IL-1 beta, MCP-1 and EGF. Plasma levels of IL-8 and MCP-1 chemokines were elevated in all patients.

ispitanika. Nasuprot tome, razina IL-1 alfa i IL-1 beta nije bitno povišena (0-28%). Koncentracija ostalih citokina (IL-4, IL-10, VEGF, EGF, IFN-gama) sporadično je povišena samo u nekih ispitanika (0-40%), s iznimkom IL-2 koncentracija kojega je povišena u oko 85% ispitanika. Navedeni rezultati pokazuju kako istodobno dokazivanje brojnih citokina može biti vrlo informativno, ali interpretativno vrlo složeno.

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Elevated concentrations of IL-6 and TNF-alfa were found in 75% of infants and adults. At the same time, the concentrations of IL-1 alfa and IL-1 beta were not significantly elevated (0-28%). Plasma levels of other cytokines (IL-4, IL-10, VEGF, EGF, IFN-gamma) were only sporadically elevated (0-40%), with the exception of IL-2. Plasma levels of this cytokine were elevated in 85% of patients. These data suggest that simultaneous determination of multiple proinflammatory cytokines and growth factors by biochip array may be very informative but very complex in terms of interpretation.

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P17-8

Uspoređivanje testa AtheNA Multi-Lyte ANA za određivanje autoantitijela s indirektnom imunofluorescencijom i testom ELISA

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Laboratorijski testovi koji se rabe u obradi bolesnika s autoimunim bolestima služe za dijagnosticiranje bolesti, kliničko praćenje bolesnika i procjenu uspješnosti liječenja. Test AtheNA Multi-Lyte ANA rabi metodu homogene fluoroimunokemije gdje su polistirenske kuglice obložene s više različitih antigena. Stoga ovaj test služi za polukvantitativno dokazivanje IgG klase antitijela za SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, centromera i histona, te za kvantitativno dokazivanje IgG klase antitijela na dsDNA i kvalitativno dokazivanje IgG klase antitijela na ANA. Cilj rada je bio usporediti test AtheNA Multi-Lyte ANA s konvencionalnim metodama indirektne imunofluorescencije (IIF) i enzimimunoanalize (ELISA) koje rabimo u našem svakodnevnom radu. Analizirali smo 152 uzorka seruma zaprimljenih u laboratorij sa zahtjevima za određivanje slijedećih antitijela: 97 ANA, 59 SS-A, 56 SS-B, 11 Sm, 9 Sm/RNP, 39 Scl-70, 17 Jo-1, 39 dsDNA, 16 histona i 19 centromera bez obzira na dijagnozu. Svi uzorci usporedno su određivani i mjereni testom AtheNA Multi-Lyte ANA (Zeus Scientific, Inc.) na instrumentu Luminex 100 IS, komercijalnim testom ANA Hep-2 (BioSystems) na fluorescentnom mikroskopu, komercijalnim testom ELISA za dokazivanje ENA antitijela (SS-A, SS-B, Sm, Sm/RNP, Scl-70, Jo-1), dsDNA (Hycor test) te histona i centromera (DiaSorin) na automatskom analizatoru miniBos, Biomedica. Svaki pojedinačni rezultat

P17-8

Comparative analysis of Multiplex AtheNA Multi-Lyte ANA system with conventional laboratory methods of autoantibody detection

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Measurement of autoantibodies provides supporting evidence in the diagnosis and monitoring of systemic autoimmune diseases. The AtheNA Multi-Lyte ANA Test System (Zeus Scientific, Inc.) is a homogeneous, multiplexed, fluorescence-based microparticle immunoassay intended for semi-quantitative detection of IgG class antibody to 8 separate analytes (SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, centromere B and histone), quantitative detection of IgG class antibody to dsDNA, and qualitative detection of IgG class antibody to ANA in human serum. The objective of this study was to determine the performance of the AtheNA Multi-Lyte ANA test relative to the established, commercial indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) currently in use in our laboratory. The study population consisted of 152 serum specimens referred to our laboratory for autiommune testing: 97 ANA requests, 59 SS-A, 56 SS-B, 11 Sm, 9 Sm/RNP, 39 Scl-70, 17 Jo-1, 39 dsDNA, 16 histone and 19 centromere. Diagnosis was not included in the study. All specimens were tested on the AtheNA Multi-Lyte ANA Test System and a multitude of conventional immunoassays including commercial ANA Hep-2 test systems (BioSystems), commercial ELISA test systems for detection of antibodies to ENA (SS-A, SS-B, Sm, Sm/RNP, Scl-70, Jo-1), dsDNA (Hycor test, miniBos analyzer,

dobiven testom AtheNA Multi-Lyte ANA uspoređivan je s rezultatima dobivenim određivanjem IIF ANA Hep-2 i s rezultatima dobivenim metodom ELISA. Ovim metodama postotak usporedivih pozitivnih rezultata bio je za: ANA 83%, SS-A 96%, SS-B 98%, Sm 97%, Sm/RNP 85%, Scl-70 87%, Jo-1 67%, dsDNA 88%, histone 79%, centromere 89%. Postotak negativnih rezultata bio je za: ANA 86%, SS-A 98%, SS-B 94%, Sm 96%, Sm/RNP 85%, Scl-70 85%, Jo-1 67%, dsDNA 79%, histone 84%, centromere 87%. Rezultati koji se nisu slagali provjereni su ponovljenim određivanjem. Iz dobivenih rezultata možemo zaključiti da rezultati dobiveni testom AtheNA Multi-Lyte ANA daju u kratkom vremenu pouzdane rezultate s devet različitih antitijela, što ubrzava put do postavljanja dijagnoze.

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Biomedica), histone and centromere (DiaSorin, miniBos analyzer, Biomedica). Each individual reportable result from AtheNA Multi-Lyte ANA test was compared with the established methods. For most analytes evaluated, positive agreement was high: ANA 83%, SS-A 96%, SS-B 98%, Sm 97%, Sm/RNP 85%, Scl-70 87%, Jo-1 67%, dsDNA 88%, histone 79%, centromere 89%. For negative results agreement was: ANA 86%, SS-A 98%, SS-B 94%, Sm 96%, Sm/RNP 85%, Scl-70 85%, Jo-1 67%, dsDNS 79%, histone 84%, centromere 87%. When results were discrepant, analysis were repeated. The AtheNA Multi-Lyte ANA technology provides a fast, flexible and cost effective tool for measuring multiple disease markers from a single sample.

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P17-9

Učestalost antikardiolipinskih, antinuklearnih i anti-beta2 glikoproteinskih protutijela u djece s epilepsijom

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Visoka učestalost epilepsije u specifičnim imunim bolestima upućuje na to da imuni sustav može igrati ulogu u patogenezi epilepsije ili može biti povezan s tim. Ispitali smo učestalost antikardiolipinskih protutijela (aCL), antinuklearnih protutijela (ANA) i anti-beta2-glikoproteinskih protutijela (anti-beta2-GPI) kod 40 djece s epilepsijom i u kontrolnoj skupini opće populacije bez kliničkih dokaza za imune poremećaje ili akutnu infekciju, kako bismo procijenili prisutnost aCL, ANA i anti-beta2-GPI te njihovu povezanost sa spolom, vrstom epilepsije, težinom i trajanjem epilepsije. Studija je provedena u Klinici za pedijatriju, Klinička bolnica Split. Uključeno je 40 bolesnika u dobi do do 18 godina s dijagnosticiranom epilepsijom, bez znakova i simptoma sukladnih kliničkim poremećajima imunog sustava, vezivnog tkiva ili reumatskih bolesti te akutnih infekcija. Kontrolna skupina se sastojala od 38 zdrave djece. U svakog bolesnika su zasebno provedena enzimimuno mjerenja za protutijela aCL i anti-beta2-GPI. Uzorci bolesnika s optičkom gustoćom većom od prijelomne vrijednosti kontrole smatrali su se pozitivnima. ANA smo određivali indirektnom imunofluorescencijom. Kad je fluorescencija bila u omjeru 1/40 ili veća, smatrali smo

P17-9

Frequency of anticardiolipin, antinuclear and anti-beta2 glycoprotein antibodies in children with epilepsy

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The high prevalence of epilepsies in specific immune diseases suggests that immune system may play a role in the pathogenesis of epilepsy or might be associated with it. We studied the frequency of anticardiolipin antibodies (aCL), antinuclear antibodies (ANA) and anti-beta2-glycoprotein antibodies (anti-beta2-GPI) in 40 consecutive children with epilepsy and in matched control subjects from the general population without any clinical evidence of immune disorder or acute infection, in order to evaluate the presence of aCL, ANA and anti-beta2-GPI, and their association with various factors including sex, type of epilepsy, severity and duration of epileosy, and anticonvulsants. The study was conducted at Department of Pediatric Neurology, University Department of Pediatrics, Split University Hospital. The study included patients with the diagnosis of epilepsy, no signs or symptoms consistent with clinical immune system disorders, connective tissue or rheumatic disease, acute infection, and aged 18 years or younger. Forty consecutive patients with these criteria were enrolled. Control group consisted of 38 healthy children. Commercially available immunometric enzyme immunoassays were used for measurement of

da su antinuklearna protutijela pozitivna. Prema učestalosti protutijela aCL i ANA kod djece s epilepsijom (44% i 16%) i kod zdrave djece (10% i 0%) izračunali smo da se kod 40 bolesnika trebala otkriti razlika s 90%-tnom točnošću i 5% značajnih nivoa. Upotrijebili smo Fisherov test za usporedbu razlika između bolesničke i kontrolne skupine. Statistička značajnost je utvrđena na razini p<0,05. Nismo našli značajne statističke povezanosti s godinama starosti, spolom, godinom početka epilpsije, trajanjem epilepsije i vrstom epilepsije, učestalosti epileptičnih izbijanja ili specifičnih antiepileptičnih lijekova s prisutnošću bilo kojeg izmjerenog protutijela.

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aCL and anti-beta2-GPI antibodies. Patient samples exhibiting optical densities higher than the optical density of the cut-off control were considered positive. ANA were detected by the standard indirect immunofluroescence method. Serum showing nuclear fluorescence at 1/40 or higher was considered positive. With the reported prevalence of aCL and ANA antibodies in children with epilepsy (44% and 16%) and in healthy children (10% and 0%), we calculated that a difference with 90% certainty and 5% significance should have been recorded in 40 patients.Fisher's exact test was used to compare differences between the study and control groups. Statistical signifiacance was set at p<0.05. There was no statistically significant correlation of age, sex, age at onset of epilepsy, duration of epilepsy, type of epilepsy, seizure frequency or specific antiepileptic medications with the presence of any antibody determined.

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P17-10

Intolerancija na hranu

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Hrana koju svakodnevno uzimamo sadrži različite spojeve, od kojih neki mogu uzrokovati neželjene posljedice na zdravlje. Nepoželjne reakcije na hranu mogu se podijeliti u 2 skupine: toksične i netoksične reakcije. Toksične reakcije posljedica su uzimanja hrane zaražene bakterijama ili toksinima. Netoksične reakcije javljaju se u preosjetljivih osoba kao alergijska reakcija i intolerancija na hranu. U slučaju alergije alergen (specifični protein iz hrane) izaziva specifični imuni odgovor organizma, povećavajući razinu specifičnog IgE antitijela. Pri alergijskoj reakciji nastaje brza uzročno-posljedična reakcija unutar nekoliko sati od unošenja određene hrane, s izraženim kliničkim simptomima. Drugi proces, intoleranciju hrane, teže je otkriti. Kod nje izostaje brza uzročno-posljedična reakcija, a kliničke manifestacije su često manje jasne, neprimjetne, ponekad teško uočljive i nepredvidive. Antigeni iz hrane koji mogu izazvati štetne reakcije su proteini ili glukoproteini manje molekularne mase, otporni na želučanu kiselinu i aktivnost probavnih enzima. Denaturaliziraju se uslijed topline. Bivaju obuhvaćene Peyerovim pločicama M stanica gornjeg sloja sluznice crijeva i fagocitiraju se pomoću makrofaga. U određenim slučajevima pojavljuje se imuna osjetljivost koja rezultira stvaranjem IgA antitijela u prvoj fazi, odnosno IgG antitijela nakon višestruke stimulacije. Hrana koja potiče proizvodnju specifičnih IgG antitijela

P17-10

Food intolerance

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The food taken daily is made of various compounds, some of which may cause undesirable effects on human health. Undesirable reactions to food can be divided into two groups: toxic and nontoxic reactions. Toxic reactions are caused by the consumption of food contaminated by bacteria or toxins. Nontoxic reactions occur in hypersensitive persons in the form of allergic reactions and food intolerance. In case of allergy, the allergen (a specific food protein) induces specific immune response of the body, thus raising the concentration of the specific IgE antibody. Allergic reaction is characterized by an immediate cause and effect reaction, within only a few hours of the specific food consumption, with visible clinical symptoms. The other process, food intolerance, is more difficult to detect. Unlike allergies, food intolerance does not provoke an immediate cause and effect reaction, and clinical manifestations are often less clear, obscure, hard to notice and unpredictable. Food antigens that can provoke adverse consequences are food proteins or glucoproteins that have smaller molecular weight, and are resistant to gastric juice and digestive enzyme activity. They are denatured at high temperatures. These molecules are taken up by Peyer's patches of M cells of the upper layer of intestinal epithelium and are phagocytosed by macrophages. In some cases, immune susceptibility develops,

u pojedinim slučajevima izaziva probavne tegobe, dok je u drugim slučajevima manifestacija neprimjetna i vrlo je teško povezati ju s hranom, jer dolazi do promjena u patologiji. Cilj je bio utvrditi koja hrana ili više vrsta hrane štete zdravlju bolesnika uzrokujući probavne tegobe, dermatološke procese, neurološke smetnje, respiracijske tegobe, psihološke smetnje, artritis, pretilost. Provedeno je mjerenje koncentracije IgG antitijela na 93 različita antigena u serumu metodom ELISA. Koncentracije IgG antitijela na 93 različite vrste namirnica određene su u serumu 400 osoba različite dobi, spola i zdravstvenog stanja. Kao najčešći uzročnici intolerancije pokazale su se žitarice, mliječni proizvodi, jaja, soja, ananas i Coca Cola. Ukidanjem jedne ili više vrsta namirnica s povećanom koncentracijom IgG dobivene testom intolerancije na hranu u više od dvije trećine slučajeva došlo je do poboljšanja između 20 i 60 dana primjene odgovarajuće dijete.

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resulting in the formation of IgA antibodies in the first phase, and of IgG antibodies upon multiple stimulations. The food that provokes the production of specific IgG antibodies in some cases induces gastrointestinal disorders, whereas in other cases manifestations are invisible and it is very difficult to connect them with food, while altering the pathology. The aim was to determine which food, or kinds of food, are detrimental to patient health, causing gastrointestinal disorders, dermatologic processes, neurologic disorders, respiratory problems, psychological disorders, arthritis, and overweight. Serum concentration of IgG antibodies to 93 different antigens from food was measured by ELISA in 400 patients of different age, sex and health condition. Cereals, dairy products, eggs, soy, pineapple and Coca Cola proved to be the most common causes of food intolerance. By excluding one or several kinds of food provoking an increase in IgG concentration measured by the food intolerance test, improvement was observed in more than two-thirds of cases within 20-60 days of introducing a diet.

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P17-11

Dijagnostička točnost protutijela na mutirani citrulinirani vimentin (anti-MCV) za reumatoidni artritis

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Novi terapijski pristup u reumatoidnom artritisu (RA) podrazumijeva primjenu antireumatske terapije prije ireverzibilnih oštećenja zglobova kada još nisu prisutne sve znakovite kliničke manifestacije. Stoga je neophodan specifičan i osjetljiv biljeg koji će pomoći u diferencijalnoj dijagnozi. Protutijela na citrulinirane peptide (CP) pokazala su se izrazito specifičnim serološkim biljegom RA. Citrulinirani vimentin, prisutan u reumatoidnom sinovijskom tkivu, jedan je od potencijalnih autoantigena u RA. Cilj je bio ispitati dijagnostičku točnost novoga testa ELISA (Orgentec Diagnostica, Njemačka) za otkrivanje protutijela na mutirani citrulinirani vimentin (anti-MCV) za RA i usporediti ju s dijagnostičkom točnošću testa anti-CCP ELISA dru-

P17-11

Diagnostic accuracy of antibodies against mutated citrullinated vimentin (anti-MCV) for rheumatoid arthritis

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According to the new therapeutic approach in rheumatoid arthritis (RA), antirheumatic therapy should be initiated before irreversible joint damage has set in. Since in this early stage symptoms are equivocal, a highly specific and sensitive marker is needed for differential diagnosis. Antibodies against citrullinated peptides (CP) have emerged as a highly specific serologic marker of RA. Citrullinated vimentin, present in rheumatoid synovial tissue, is a potential autoantigen in RA. The aim of the study was to evaluate diagnostic accuracy of the new ELISA (Orgentec Diagnostica, Germany) for detection of antibodies against mutated citrullinated vimentin (anti-MCV) for RA and to compare it with diagnostic accuracy of the second

ge generacije. Protutijela anti-MCV i anti-CCP određena su u serumima 264 ispitanika: 92 s RA i 172 ispitanika kontrolne skupine (38 s degenerativnim ili drugim upalnim bolestima zglobova, 27 s bolestima vezivnog tkiva ili vaskulitisom i 107 zdravih osoba). ROC analizom rezultata za anti-MCV utvrđena je AUC=0,874 (95% CI=0,828-0,911). Za graničnu vrijednost >20 U/mL, koju predlaže proizvođač, osjetljivost je bila 63,0% a specifičnost 97,1%. Za anti-CCP utvrđena je AUC=0,897 (95% CI=0,854-0,931), a osjetljivost i specifičnost za preporučenu graničnu vrijednost >5 RU/mL 70,6% odnosno 97,7%. AUC za anti-MCV i anti-CCP nisu se statistički značajno razlikovale (p=0,298). U skupini RA 11 uzoraka je bilo negativno na MCV (3 s visokom koncentracijom anti-CCP), dok su 4 uzorka bila negativna na CCP (1 s visokom koncentracijom anti-MCV). U kontrolnoj skupini 2 uzorka su imala visoke koncentracije obaju protutijela, 3 uzorka su bila pozitivna samo na MCV (1 s visokom koncentracijom), dok su 2 uzorka bila pozitivna samo na CCP (1 s visokom koncentracijom). Optimalna granična vrijednost za MCV dobivena ROC analizom je >10 U/mL uz osjetljivost od 71,7% i specifičnost od 95,3%. Rezultati naše studije pokazali su da test anti-MCV ima podjednaku dijagnostičku točnost za RA kao i anti-CCP, iako je osjetljivost nešto niža. Primjenom optimalne granične vrijednosti koju smo dobili ROC analizom osjetljivost se značajno povećava zadržavajući još uvijek visoku specifičnost.

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generation anti-CCP ELISA. Anti-MCV and anti-CCP antibodies were determined in 264 serum samples: 92 from RA patients and 172 from control patients including 38 with degenerative and other inflammatory joint diseases, 27 with connective tissue diseases or vasculitis, and 107 healthy subjects. ROC analysis of anti-MCV results yielded AUC=0.874 (95% CI=0.828-0.911). For the cut off >20 U/mL, recommended by the manufacturer, the sensitivity was 63.0% and specificity 97.1%. Anti-CCP revealed AUC=0.897 (95% CI=0.854-0.931) with a sensitivity of 70.6% and specificity of 97.7% for the recommended cut off >5 RU/mL. The AUC for anti-MCV and anti-CCP were not significantly different (p=0.298). In the RA group 11 samples were anti-MCV negative (3 with high anti-CCP concentration) and 4 were anti-CCP negative (1 with high anti-MCV concentration). Among controls, 2 samples had high antibody concentrations in both assays, 3 samples were only anti-MCV positive (1with high concentration) and 2 were only anti-CCP positive (1 with high concentration). The optimal cut-off for anti-MCV yielded by ROC analysis was >10 U/mL with a sensitivity of 71.7% and specificity of 95.3%. According to our results, the anti-MCV assay has a comparable diagnostic accuracy as anti-CCP assay for RA, although the sensitivity was somewhat lower. Using optimal cut-off yielded by ROC analysis, the sensitivity became significantly higher without a considerable decrease of specificity.

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P18 – Hitna laboratorijska dijagnostika, P18-1

Spektrofotometrijsko određivanje karboksihemoglobina – usporedba vrijednosti dobivenih na analizatoru NOVA CO-oksimetar i spektrofotometrijskom metodom po Bruckneru

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P18 – Emergency clinical chemistry, P18-1

Spectrophotometric carboxyhemoglobin determination – comparison of values obtained on a NOVA CO-oximeter and by spectrophotometric method according to Bruckner

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Karboksihemoglobin (COHb) nastaje reverzibilnim vezanjem ugljičnog monoksida na hemoglobin. Određivanjem udjela COHb u stanjima akutnog trovanja ugljičnim monoksidom (CO) procjenjuje se izloženost bolesnika i jačina intoksikacije. Referentna metoda mjerenja COHb je plinska kromatografija, dok se u rutinskom radu zbog analitičke jednostavnosti i brzine rabi spektrofotometrijska metoda. Analizom uzoraka krvi bolesnika nepuša-

Carboxyhemoglobin (COHb) is produced by reversible carbon monoxide binding to hemoglobin. Determination of COHb values in conditions of acute poisoning by carbon monoxide (CO) is used to assess patient exposure and the degree of intoxication. The reference method for COHb measurement is gas chromatography, while spectrophotometric method is used in routine practice due to analytical simplicity and rapidity. The values rang-

ča koji nisu bili u kontaktu s izvorima CO na analizatoru NOVA CO-oksimetar dobivane su vrijednosti od oko 3%-5% ukupnog hemoglobina. Kako su postojeće referentne vrijednosti ispod navedenih granica (<1,5% nepušači, <5% pušači), cilj istraživanja bio je ispitati osjetljivost analizatora NOVA CO-oksimetar, usporediti vrijednosti s ručnom spektrofotometrijskom metodom i ustanoviti primjenjivost postojećih referentnih intervala. Analiziran je ukupno 81 uzorak pune krvi u duplikatu. Ispitanici su podijeljeni u 3 skupine: akutno intoksicirani (I.), bez kontakta s izvorima CO (II.) i pušači (III.). Srednje izmjerene vrijednosti COHb dobivene na instrumentu NOVA i "ručnim" postupkom za pojedinu skupinu ispitanika bile su (χ±SD): za I. skupinu 25±11% i 14±10%, za II. skupinu 5±1% i 0±0%, te za III. skupinu 8±2% i 1±1%. Koeficijenti korelacije bili su: za I. skupinu 0,998, II. skupinu 0,973 i III. skupinu 0,423. Referentni interval za COHb uz primjenu NOVA CO-oksimetra nešto je viši od intervala koji se uobičajeno nalaze u literaturi, te iznosi <5% za nepušače, odnosno <10% za pušače. U kliničkom otkrivanju akutnog trovanja ugljičnim monoksidom (>20% ukupnog hemoglobina) oba spektrofotometrijska postupka mogu se primijeniti s podjednakom pouzdanošću.

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ing from 3% to 5% of total hemoglobin were obtained by blood sample analysis of non-smoking patients not in contact with the sources of CO, performed on a NOVA CO-oximeter. As these results were above the current reference values (<1.5% non-smokers, <5% smokers), the aim of the study was to investigate the sensitivity of the NOVA CO-oximeter, to compare it with manual spectrophotometric method, and to establish applicability of the current reference intervals. A total of 81 whole blood samples were analyzed in duplicate. Subjects were divided into three groups: acutely intoxicated (I), without contact with CO sources (II), and smokers (III). The mean COHb values determined on the NOVA instrument and by manual procedure were (χ±SD): 25±11% and 14±10% in group I, 5±1% and 0±0 in group II, and 8±2% and 1±1% in group III, respectively. Correlation coefficients were 0.998, 0.973 and 0.423 in groups I-III, respectively. Accordingly, the reference intervals of COHb obtained by using NOVA CO-oximeter were higher than the intervals commonly found in the literature, i.e. <5% and <10% for nonsmokers and smokers, respectively. Both spectrophotometric procedures are equally reliable for clinical detection of acute carbon monoxide intoxication (>20% of total hemoglobin).

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P18-2

Analitička procjena semikvantitativnog određivanja psihoaktivnih supstancija analizatorom Olympus AU 640

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Uporaba mokraće kao biološkog uzorka izbora pri pretraživanju na psihoaktivne supstancije (droge) ima ograničenu vrijednost zbog velikih intra- i inter-individualnih varijacija samog uzorka, ali i zbog ograničene specifičnosti imunokemijskih metoda pretraživanja, pa se rezultati izražavaju kvalitativno (pozitivan/nije dokazan). Novija generacija imunokemijskog određivanja reagensima Olympus omogućuje izdavanje semikvantitativnog (brojčanog) rezultata. Cilj rada bila je analitička procjena nove generacije reagensa za određivanje koncentracije droga (benzodiazepini, barbiturati, amfetamini, kokain, opijati, THC i metadon), mjerena spektofotometrijskom metodom na analizatoru Olympus AU 640. Načelo metode se

P18-2

Analytical evaluation of semi-quantitative determination of psychoactive substances on Olympus AU 640 analyzer

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The use of urine as a biological sample of choice on screening for psychoactive substances (drugs of abuse) is of limited value because of great intra- and inter-individual variation within the sample as well as for limited specificity of the immunochemistry methods of screening, with qualitatively expressed results (positive/not detected). The new generation of immunochemistry determination by use of Olympus reagents allows for generation of semi-quantitative (numerical) result. The aim of the study was to perform analytical evaluation of the new generation of reagents for determination of drug concentration (benzodiazepines, barbiturates, amphetamines, cocaine, opiates, THC, and methadone)

zasniva na bakterijskoj beta-galaktozidazi koja je sintetizirana u dva inaktivna fragmenta, čijim spontanim spajanjem nastaje aktivan enzim koji cijepa supstrat (ciljnu drogu), pričem se spektrofotometrijski mjeri promjena boje supstrata. Analitička procjena novog semikvantitativnog određivanja droga prema ECCL obuhvaća nepreciznost u seriji određivanjem svake droge u mokraći deset puta tijekom dva dana, nepreciznost iz dana u dan određivanjem svake droge u mokraći tri puta tijekom deset dana, dok se netočnost određivala u uzorcima svih droga u mokraći s niskim, srednjim i visokim koncentracijama tri puta. Kao uzorci mokraće rabljeni su komercijalni kontrolni uzorci tvrtke Olympus, kao i nasumični pozitivni uzorci ovisnika. Rezultati analitičke procjene prema ECCL: nepreciznost u seriji iznosila je za THC, barbiturate, amfetamine/metamfetamine, benzodiazepine, kokain, opijate i metadon 6,7%, 3,6%, 3,2%, 3,4%, 5,4%, 2,2% i 2,4%, dok je reproducibilnost bila 2,3%, 2,1%, 3,6%, 3,8%, 2,7% , 3,6% i 2,4%, a netočnost 1,5%, 2,0%, 1,5%, 2,2%, 1,0%, 2,2% i 2,1%. Na temelju dobivenih rezultata zaključak je kako reagensi za određivanje droga zadovoljavaju gore navedene kriterije pretraživanja mokraće brzinom, jednostavnošću izvedbe i većom točnošću u odnosu na stariju generaciju imunokemijskog određivanja.

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by spectrophometric method on an Olympus AU 640 analyzer. The principle of the method is based on bacterial beta-galactosidase that is synthesized in two inactive fragments, their spontaneous coupling resulting in the production of active enzyme which splits the substrate (target drug), whereby the change in the substrate color is being spectrophotometrically measured. Analytical evaluation of the new semi-quantitative drug determination according to ECCL includes serial imprecision by determination of each drug in urine on ten occasions during two days, day to day imprecision by determination of each drug in urine on three occasions during ten days, and inaccuracy by determination of all drugs tested in urine samples with low, intermediate and high concentrations on three occasions. Commercial control samples provided by Olympus and random positive samples from drug addicts were used as urine samples. Analytical evaluation according to ECCL produced the following results: serial imprecision 3.4%, 5.4%, 2.2% and 2.4%; reproducibility 2.3%, 2.1%, 3.6%, 3.8%, 2.7%, 3.6% and 2.4%; and inaccuracy 1.5%, 2.0%, 1.5%, 2.2%, 1.0%, 2.2% and 2.1% for THC, barbiturates, amphetamines/metamphetamines, benzodiazepines, cocaine, opiates and methadone, respectively. Study results indicated the reagents for drug determination to meet the above criteria for urine screening, offering a rapid, simple to perform and more accurate tool as compared with the previous generation of immunochemistry drug determination.

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P18-3

Analitička procjena aparata GEM Premier 3000

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Poremećaji acido-bazične ravnoteže i elektrolita česti su u kliničkoj praksi. Brza i točna dijagnoza, te odgovarajuće liječenje takvih poremećaja od velikog su značenja. Razvoj tehnologije, a naročito senzorske tehnologije, doveo je do izrade analizatora koji iz uzorka pune krvi brzo i jednostavno određuju spomenute analite ne samo u laboratoriju nego i uz bolesnka (POCT). GEM Premier 3000 je prijenosni aparat za brzu analizu uzoraka pune krvi uz bolesničku postelju (POCT). Aparat određuje vrijednosti pH, pCO_2, pO_2, Na^+, K^+, Ca^{++}, glukoze, laktata i hematokrita. Cilj rada bila je analitička procjena aparata GEM Premier

P18-3

Analytical evaluation of the GEM Premier 3000 instrument

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Acid-base balance and electrolyte impairments are frequently encountered in clinical practice. Rapid and accurate diagnosis followed by appropriate treatment for these disturbances are of utmost importance. Technological advancement, in sensor technology in particular, has led to the advent of analyzers for fast and simple determination of these analytes from whole blood samples not only in a laboratory but also as the point-of-care testing (POCT). GEM Premier 3000 is a portable instrument for rapid POCT analysis of whole blood samples. The following analytes can be determined on the instrument: pH, pCO_2,

3000. Ispitivanja su provedena u skladu s preporukama ECCLS. Analitička procjena obuhvatila je nepreciznost u seriji, nepreciznost iz dana u dan, netočnost i usporedna uspoređivanja u uzorcima bolesnika. Nepreciznost u seriji i netočnost ispitane su višekratnim određivanjem (20 puta) analita u jednom danu u kontrolnim uzorcima Critical Care QC Control 9 (Level 1, 2 i 3) proizvođača Instrumentation Laboratory. Nepreciznost iz dana u dan ispitana je određivanjem analita tijekom 20 dana u kontrolnim uzorcima Rapid QC Complete (Level 1, 2 i 3) proizvođača Bayer HealthCare. Analizirano je 160 heparinziranih uzoraka pune krvi na ispitivanom aparatu i referentnom aparatu Ciba Corning 865 (Bayer HealthCare) koji je uključen u program vanjske procjene kvalitete rada, ali ne može određivati glukozu, laktat i hematokrit. Maksimalne vrijednosti koeficijenata korelacije (%) za pojedini analit za sve tri razine za nepreciznost u seriji iznosile su: pH 0,03; pCO_2 1,87; pO_2 2,47; Na 1,01; K 1,83; Ca^{++} 1,09; glukoza 1,89 i laktat 3,46.
Maksimalne vrijednosti koeficijenata korelacije (%) za pojedini analit za sve tri razine za nepreciznost iz dana u dan iznosile su: pH 0,07; pCO_2 3,80; pO_2 4,65; Na 0,54; K 0,72; Ca^{++} 1,03; glukoza 2,70 i laktat 9,35. Rezultati ispitivanja netočnosti (R%, maksimalna vrijednost): pH –0,14; pCO_2 4,19; pO_2 –4,16; Na 0,59; K –2,24; Ca^{++} 2,92; glukoza 4,53 i laktat 13,59. Rezultati usporednih određivanja uzoraka bolesnika obrađeni su linearnom regresijskom analizom (Passing Bablok). Izračunati su koeficijenti korelacije za svaki analit i iznosili su: pH 0,991; pCO_2 0,985; pO_2 0,988; Na 0,921; K 0,992; Ca^{++} 0,973. Statistička obrada dobivenih podataka pokazala je zadovoljavajuću nepreciznost u seriji za sve analite (KV <3,46%), dok je nepreciznost iz dana u dan bila zadovoljavajuća za sve analite (KV <4,65%) osim za laktat (KV=9,35%). Rezultati ispitivanja netočnosti bili su zadovoljavajući za sve analite (R <4,53%) osim za laktat (R=13,59%). Koeficijenti korelacije pokazali su visoku podudarnost rezultata svih određivanih analita (r=0,921-0,992). Temeljem dobivenih rezultata zaključujemo da je GEM Premier 3000 jednostavan i pouzdan analizator (osim analize laktata). Visoka podudarnost sa standardnom metodom čini ga pogodnim za rad na bolničkom odjelu, jer omogućava brz i siguran rad.

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pO_2, Na^+, K^+, Ca^{++}, glucose, lactate and hematocrit. The aim of the study was analytical evaluation of the GEM Premier 3000 device. Testing was performed according to ECCLS recommendations. Analytical evaluation included serial imprecision, day to day imprecision, inaccuracy and parallel comparisons in patient samples. Serial imprecision and inaccuracy were tested by multiple determinations (20 times) of the analytes during a day in the control samples Critical Care QC Control 9 (Level 1, 2 and 3) manufactured by Instrumentation Laboratory. Day to day imprecision was tested by analyte determination for 20 days in the control samples Rapid QC Complete (Level 1, 2 and 3) manufactured by Bayer HealthCare. A total of 160 heparinized whole blood samples were analyzed on the instrument evaluated and on the reference device Ciba Corning 865 (Bayer HealthCare), included in the External Quality Assessment program; glucose, lactate and hematocrit cannot be determined on the latter. The maximal correlation coefficients (%) for serial imprecision at all three levels for particular analytes were as follows: pH 0.03; pCO_2 1.87; pO_2 2.47; Na 1.01; K 1.83; Ca^{++} 1.09; glucose 1.89; and lactate 3.46. The maximal correlation coefficients (%) for day to day imprecision at all three levels for particular analytes were as follows: pH 0.07; pCO_2 3.80; pO_2 4.65; Na 0.54; K 0.72; Ca^{++} 1.03; glucose 2.70; and lactate 9.35. Results of testing for inaccuracy (R%, maximal value): pH -0.14; pCO_2 4.19; pO_2 -4.16; Na 0.59; K -2.24; Ca^{++} 2.92; glucose 4.53; and lactate 13.59. Results of parallel determinations in patient samples were processed by linear regression analysis (Passing Bablok). The calculated coefficients of correlation for particular analytes were as follows: pH 0.991; pCO_2 0.985; pO_2 0.988; Na 0.921; K 0.992; Ca^{++} 0.973. Statistical analysis of the data obtained yielded a satisfactory serial imprecision for all analytes (CV <3.46%), whereas day to day imprecision was satisfactory for all analytes (CV <4.65%) except for lactate (CV=9.35%). Testing for inaccuracy yielded satisfactory results for all analytes (R <4.53%) except for lactate (R=13.59%). Coefficients of correlation revealed a high result concordance for all the analytes determined (r=0.921-0.992). Study results have indicated that GEM premier 3000 is a simple and reliable analyzer (with the exception of lactate). The high concordance with the standard method makes it suitable for work at hospital ward for its rapid and reliable performance.

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P19 – Pedijatrijska laboratorijska dijagnostika, P19-1 (UP6-1)

Enzimske aktivnosti AP, AST, ALT, LDH i GGT u novorođenčadi i babinjača

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Cilj studije je bio procijeniti enzimsku aktivnost alkalne fosfataze (AP), aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), laktat dehidrogenaze (LDH) i gama-glutamil transpeptidaze (GGT) u babinjača i njihove novorođenčadi. U studiju smo uključili skupinu babinjača (n=93) i skupinu novorođenčadi (n=94; uzorci krvi uzeti su iz pupčane vene) na Klinici za ginekologiju i porodništvo u Skopju, te kontrolnu skupinu zdravih studenata u dobi od 18-22 godine (n=77). Enzimsku aktivnost AP, AST, ALT, LDH i GGT određivali smo standardnim kinetičkim metodama prema IFCC na biokemijskom analizatoru Cobas Mira Plus.

	AP U/L	AST U/L	ALT U/L	LDH U/L	GGT U/L
Kontrole (n=77)	61,31 ±18,45	24,89 ±6,38	16,57 ±4,99	185,83 ±49,84	10,91 ±5,09
Babinjače (n=93)	153,19 ±53,89	24,23 ±21,18	18,84 ±19,22	199,40 ±61,81	64,75± 42,42
Novo--rođenčad (n=94)	132,03 ±51,06	35,06 ±15,13	17,67 ±5,55	347,19 ±107,25	64,75 ±42,42

Sve skupine ispitanika bile su negativne na HBsAg i anti HCV. Rezultati su prikazani u tablici (χ±SD):
Statistička analiza enzimske aktivnosti pokazala je značajno povišenje aktivnosti AP i GGT (p=0,000 oboje) u skupini babinjača u usporedbi s kontrolnom skupinom. Tome je razlog fiziološka kolestaza tijekom trudnoće. Iako statistički povišene, enzimske aktivnosti u skupini novorođenčadi nisu bile usporedive s onima u kontrolnoj skupini. Povišenje enzimskih aktivnosti u ovoj skupini vjerojatno su bile uzrokovane prilagodbom na novu životnu sredinu.

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P19 – Pediatric laboratory diagnostics, P19-1 (UP6-1)

Enzyme activities of AP, AST, ALT, LDH and GGT in newborns and puerperae

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The aim of the study was to estimate enzyme activities of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and gamma- glutamyltranspeptidase (GGT) in women in puerperium and their newborns. The study included a group of puerperae (n=93) and a group of newborns from Skopje University Department of Gynecology and Obstetrics (n=94) (blood was taken from umbilical vein); and a control group of healthy students aged 18-22 (n=77). Enzyme activities of AP, AST, ALT, LDH and GGT were determined by standard kinetic methods according to IFCC (International Federation of Clinical Chemistry) on a Cobas Mira Plus biochemistry analyzer. All study groups were HBsAg and anti HCV negative. Results are presented in table (χ±SD):

	AP U/L	AST U/L	ALT U/L	LDH U/L	GGT U/L
Controls (n=77)	61.31 ±18.45	24.89 ±6.38	16.57 ±4.99	185.83 ±49.84	10.91 ±5.09
Puerperae (n=93)	153.19 ±53.89	24.23 ±21.18	18.84 ±19.22	199.40 ±61.81	64.75± 42.42
Newborns (n=94)	132.03 ±51.06	35.06 ±15.13	17.67 ±5.55	347.19 ±107.25	64.75 ±42.42

Statistical evaluation of enzyme activities showed a significant elevation in the activities of AP and GGT (p=0.000 both) in the group of puerperae as compared with control group. This is due to physiological cholestasis during pregnancy. Although statistically elevated, enzyme activities in the group of newborns were not comparable with the control group. The elevation of enzyme activities in this group was probably caused by adaptation to the new life environment.

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P19-2

Određivanje koncentracije kalprotektina u stolici u diferencijalnoj dijagnostici kroničnih upalnih crijevnih bolesti kod djece

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Kalprotektin je S100 protein koji kod upale nastaje iz aktiviranih ili odumrlih neutrofilnih granulocita, monocita, makrofaga i drugih epitelnih stanica. Brojna istraživanja potvrđuju da je koncentracija kalprotektina u stolici značajno povišena kod kroničnih upalnih crijevnih bolesti (KUCB) i kolorektalnega karcinoma, kod upotrebe određenih lijekova (NSAID, PPI) te kod drugih bolesti (karcinom želuca, polipi kolona, akutni enterokolitis, ciroza jetre). Kalprotektin je vrlo koristan novi parametar u dijagnostici KUCB. Određivanje koncentracije kalprotektina u stolici uvodi se kao rutinska pretraga. To je neinvazivan test za određivanje upale u donjem dijelu probavnog sustava kod djece i odraslih. Granična vrijednost za djecu staru od 4 do 17 godina je 50 µg/g stolice. Cilj našega istraživanja je bio utvrditi ulogu testa Calprest: 1) u diferencijalnoj dijagnostici KUCB kod djece s gastrointestinalnim simptomima i 2) u razlikovanju Crohnove bolesti (CB) od ulceroznog kolitisa (UK). Koncentracija kalprotektina u stolici određena je metodom ELISA (Calprest, Eurospital, Italija). U ispitivanje smo uključili djecu s endoskopski i histološki potvrđenom KUCB (n=25), od toga 18 s CB i 7 s UK, te kontrolnu skupinu djece bez KUCB (n=18). Rezultati su prikazani u tablici:

	KUCB	Kontrolna skupina	p
Koncentracija u stolici (µg/g)	138,7 (9,4-452,5)	27,4 (9,4-371,3)	0,000
	CB	**UK**	**p**
	275,4 (12,2-452,5)	100,6 (9,4-452,3)	0,717

Vrijednosti su izražene kao medijan (raspon), uz razinu vjerojatnosti p<0,05. Razlika u koncentraciji kalprotektina između KUCB i kontrolne skupine bila je statistički značajna. Rezultati su pokazali da test Calprest za određivanje KUCB kod djece s probavnim simptomima ima osjetljivost 92%, specifičnost 66%, pozitivnu prognostičnu vrijednost 79%, negativnu prognostičnu vrijednost 86% i točnost 81%. Kod djece sa CB i UK nismo dobili statistički značajne razlike u koncentraciji kalprotektina. Test Calprest je

P19-2

Fecal calprotectin in the differential diagnosis of childhood inflammatory bowel disease

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Calprotectin is an S100 protein which is released in inflamed tissues by activation or lysis of neutrophils, monocytes, macrophages and some epithelial cells. Increased fecal calprotectin levels have been found in inflammatory bowel disease (IBD) and colorectal cancer as well as in patients using drugs like NSAID and PPI or being diagnosed with gastric cancer, colonic polyps, infectious gastroenteritis, liver cirrhosis and some other diseases. Calprotectin is a highly useful new parameter in the diagnosis of IBD, and determination of fecal calprotectin has been introduced as a routine test. It is a noninvasive test to indicate inflammation in lower gastrointestinal tract in children and adults. The cut-off level in children aged 4 to 17 years is 50 µg/g feces. The aim of this study was to evaluate the use of Calprest in (1) differential diagnosis of IBD in children with gastrointestinal symptoms; and (2) distinguishing between Crohn's disease (CD) and ulcerative colitis (UC). Stool samples were obtained from children who underwent endoscopy and had histopathologic findings suggesting IBD (n=25; CD: n=18, UC: n=7) and from a control group of children without IBD (n=19). Fecal calprotectin was measured using a simple ELISA test, Calprest (Eurospital, Italy). Results are presented in table below:

	IBD	Controls	p
Fecal calprotectin (µg/g)	138.7 (9.4-452.5)	27.4 (9.4-371.3)	0.000
	CB	**UC**	**p**
	275.4 (12.2-452.5)	100.6 (9.4-452.3)	0.717

Values are expressed as median (range); p<0.05=statistically significant difference.
The difference in median fecal calprotectin concentrations between IBD and control group was highly significant. According to study results, Calprest test has a 92% sensitivity 68% specificity, 79% positive predictive value, 86% negative predictive value and 81% accuracy to detect IBD in children with gastrointestinal symptoms. There was no statistically significant difference between

obilježen velikom osjetljivošću i niskom specifičnošću za određivanje KUCB kod djece. Pozitivan rezultat (>50 μg/g stolice) kod djece s gastrointestinalnim simptomima upućuje na dodatnu endoskopsku dijagnostičku obradu. Razlikovanje između CB i UK ovim testom nije moguće.

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the values in CD and UC. In conclusion, Calprest is a test of high sensitivity and low specificity for detecting childhood IBD. A positive test (>50 μg/g) in a child with gastrointestinal symptoms will facilitate the decision to proceed with additional studies including endoscopy. The test cannot be used to distinguish between CD and UC.

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P19-3

Vrijednosti kolesterola u djece predškolske dobi

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Nepravilna prehrana u najranijoj dobi udružena s nasljednim čimbenicima uvelike povećava rizik za razvoj nekih kroničnih bolesti. Cilj istraživanja bila je usporedba izmjerenih vrijednosti kolesterola kao značajnog rizičnog čimbenika s preporučenim vrijednostima za dobnu skupinu predškolske djece (manje od 4,7 mmol/L), te usporedba s indeksom tjelesne mase. Ispitivanjem je obuhvaćena skupina od 458 djece (243 dječaka i 215 djevojčica) rođenih 1999. i 2000. godine, s područja grada Pule i okolice, koja su u laboratorij upućena radi redovnog sistematskog pregleda za upis u prvi razred osnovne škole. Nakon izrade KKS iz uzoraka plazme izmjerene su vrijednosti ukupnog kolesterola na automatskom analizatoru Architect c8000 (Abbott, SAD). U slučajevima kada su vrijednosti kolesterola bile iznad preporučenih (4,7 mmol/L) određene su vrijednosti HDL i LDL kolesterola. Izmjerene vrijednosti statistički su obrađene i iskazane srednjom vrijednosti (SV), medijanom (m), standardnom devijacijom (SD), koeficijentom varijacije (KV). Odnos prema preporučenim vrijednostima iskazan je značajnošću razlike (p). Medijan vrijednosti kolesterola u cijeloj skupini djece iznosio je 4,16 mmol/L; djevojčice su imale vrijednost kolesterola 4,29 mmol/L, a dječaci 4,09 mmol/L. Kolesterol viši od preporučenih 4,7 mmol/L imalo je 22% djece kod kojih je medijan za HDL iznosio 1,81 mmol/L, a za LDL 3,38 mmol/L; 11,5% djece imalo je kolesterol viši od preporučenih vrijednosti za odrasle, uz HDL 1,80 mmol/L i LDL 3,41 mmol/L. Indeks tjelesne mase u skupini djece s povišenim vrijednostima kolesterola pokazao je visok koeficijent korelacije (r=0,966). Iako su vrijednosti kolesterola u plazmi za oko 3% niže nego u serumu, relativno velik postotak djece ove ispitne skupine s povišenim vrijednostima kolesterola i indeksom tjelesne mase ukazuje na pojačanu potrebu re-

P19-3

Cholesterol values in preschool children

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Unhealthy nutrition in the earliest age combined with inherited factors may increase the risk for the development of some chronic diseases. The purpose of the study was to compare the measured values of cholesterol (as a significant risk factor) with recommended values for preschool children (less than 4.7 mmol/L) and to compare it with body mass index. The study included 458 children (243 boys and 215 girls) born in Pula and its surroundings in 1999 and 2000. The children were referred to the laboratory as part of the regular medical check-up prior to school enrolment. Upon complete blood count, total cholesterol values were measured in plasma samples on an Architect c8000 (Abbott, USA) autoanalyzer. In cases when cholesterol values were above the recommended level (4.7 mmol/L), HDL and LDL cholesterol values were also measured. Measured values were statistically processed and expressed as mean (M), median (m), standard deviation (SD), and coefficient of variation (CV). Relation to the recommended values was shown by the significance of difference (p). The median for cholesterol values in the whole group was 4.16 mmol/L; cholesterol value was 4.29 mmol/L in girls and 4.09 mmol/L in boys; 22% of the children had cholesterol higher than the recommended level of 4.7 mmol/L. In this group, the median for HDL was 1.81 mmol/L and for LDL 3.38 mmol/L; 11.5% of the children had cholesterol higher than the recommended level for adults, with HDL 1.80 mmol/L and LDL 3.41 mmol/L. There was a high correlation coefficient (r=0.966) for body mass index in children with high cholesterol values. Although plasma cholesterol values are by some 3% lower than in those in serum, a relatively large percentage of study children had high cholesterol values and high body mass index. Study results pointed to the need of regular control

dovite kontrole ovih parametara već u najranijoj životnoj dobi, zajedno sa sticanjem navika pravilne prehrane.

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of these parameters in the earliest age and of upgrading the general awareness of the healthy and balanced diet.

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P19-4

Kondenzat izdaha u djece – neinvazivna metoda za praćenje biomarkera u djece s respiracijskim bolestima

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Cilj je bio odrediti biomarkere u kondenzatu izdaha (KI) i usporediti ih s FENO te plućnom funkcijom u djece s astmom i/ili gastroezofagusnim refluksom (GER). Ispitivanje je provedeno u djece s astmom i/ili GER. KI je uzorkovan uređajem ECoScreen, Jaeger. U KI su izmjereni pH, pCO_2, koncentracija CO_2, koncentracija željeza. FENO je mjeren uređajem NIOX, Aerocrine AB, a ventilacijski parametri spirometrom SanoScope, Schiller. Ventilacija pluća bila je u rasponu od urednih vrijednosti do srednje teških opstruktivno-restriktivnih smetnja. U 54% ispitanika dokazan je patološki GER. Utvrđena je statistički značajna korelacija između pH u KI i FENO u izdahu, te koncentracije željeza u KI i parametara plućne funkcije PEF, MEF75, MEF50. Kondenzat izdaha uzorkuje se neinvazivno, može se ponavljati, a prikladan je i za dječju dob. Nakon postupaka optimiranja i standardizacije, kondenzat izdaha mogao bi postati metodom izbora u dijagnostici i praćenju plućnih bolesti u djece.

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P19-4

Exhaled breath condensate in children – a noninvasive method for monitoring biomarkers in children with respiratory diseases

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The aim was to determine biomarkers in exhaled breath condensate (EBC) and to compare them with FENO and lung function tests in children with asthma and gastroesophageal reflux (GER). Children with asthma and GER were included in the study. EBC was sampled by ECoScreen, Jaeger. pH, pCO_2, CO_2 concentration and iron concentration were measured in EBC. Breath FENO was determined by use of a NIOX, Aerocrine AB. Lung function was evaluated using a SanoScope spirometer, Schiller. GER was found in 54% of children. Results of lung function tests ranged from normal to mild obstructive-restrictive changes. There was a statistically significant correlation between both EBC pH and breath FENO, and between EBC iron concentration and lung function tests PEF, MEF75 and MEF50. EBC is a noninvasively obtained sample, it is reproducible and thus applicable in childhood. Upon optimization and standardization, the analysis of EBC could be the method of choice in the diagnosis and monitoring of lung diseases in children.

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P19-5

Dijagnostička točnost C-reaktivnog proteina, ukupnog broja leukocita, broja trombocita i diferencijalne krvne slike u sepsi novorođenčadi

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Određivanje koncentracije C-reaktivnog proteina (CRP), ukupnog broja leukocita, broja trombocita i diferencijalne krvne slike (DKS) predstavljaju dijagnostički "*sepsa-screening*" pristup pri kliničkoj sumnji na novorođenačku sepsu. Cilj rada bio je ispitati dijagnostičku točnost navedenih parametara u novorođenčadi sa sepsom. U ispitivanje je bilo uključeno 58 novorođenčadi sa sepsom i 328 zdrave novorođenčadi. Koncentracija CRP određena je imunoturbidimetrijskom metodom povećane osjetljivosti (Olympus AU2700). Kompletna krvna slika određena je na automatskom hematološkom brojaču (Cell-Dyn 3200, Abbott Diagnostics). DKS određena je mikroskopskom metodom u obojenom razmazu periferne krvi. Dijagnostička točnost ispitana je analizom ROC (engl. *receiver operating characteristic*). Analiza ROC pokazala je izvrsnu diskriminacijsku učinkovitost CRP (AUC=0,970) uz optimalnu graničnu vrijednost (*cut-off*) 11,5 mg/L kojom se postiže dijagnostička osjetljivost (Os) 89,7% i specifičnost (Sp) 96,0%. Među parametrima DKS prihvatljivu dijagnostičku točnost pokazali su omjer nesegmentiranih i ukupnih neutrofilnih granulocita (I/T, AUC=0,789, Os=77,2%, Sp=73,2%, *cut-off* >0,11), omjer nesegmentiranih i segmentiranih neutrofilnih granulocita (I/M, AUC=0,774, Os=75%, Sp=73,6%, *cut-off* >0,13) te postotak nesegmentiranih neutrofilnih granulocita (AUC=0,784, Os=82,8%, Sp=66,2% *cut-off* >5%). Apsolutni broj neutrofilnih granulocita (AUC=0,607, Os=54,7%, Sp=66,1%) i limfocita (AUC=0,546, Os=45,6%, Sp=68%), postotak segmentiranih neutrofilnih granulocita (AUC=0,499, Os=51,7%, Sp=54,7%) i limfocita (AUC=0,659, Os=82,5%, Sp=47,9%), kao i ukupni broj leukocita (AUC=0,629, Os=65,5%, Sp=56,9%) i broj trombocita (AUC=0,542, Os=62,1% Sp=49,2%) pokazali su nezadovoljavajuću diskriminacijsku učinkovitost u dijagnostici novorođenačke sepse. Zaključuje se kako je koncentracija CRP parametar najveće dijagnostičke točnosti u sepsi novorođenčadi. Prihvatljivu diskriminacijsku učinkovitost u dijagnostici novorođenačke sepse pokazali su omjeri I/T i I/M te postotak nesegmentiranih neutrofilnih granulocita. Ukupni broj leukocita i broj trombocita nisu pouzdani dijagnostički pokazatelji novorođenačke sepse.

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P19-5

Diagnostic accuracy of C-reactive protein, total leukocyte count, platelet count and differential blood count in newborn sepsis

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Determination of C-reactive protein (CRP), total leukocyte count, platelet count and differential blood count represents a "sepsa-screen" diagnostic approach in newborns clinically suspected of sespis. The aim of the study was to assess diagnostic accuracy of these parameters in newborns with sepsis. The study included 58 newborns with sepsis and 328 healthy newborns. CRP concentration was determined by particle-enhanced immunoturbidimetric assay (Olympus AU2700). Complete blood count was determined on an automated blood counter (Cell-Dyn 3200, Abbott Diagnostics). Differential blood count was determined by microscopic method in stained peripheral blood smear. Diagnostic accuracy was evaluated using the receiver operating characteristic (ROC) analysis. ROC analysis showed excellent discriminating power of CRP (AUC=0.970) at optimal cut-off point of 11.5 mg/L, which achieves diagnostic sensitivity (Se) of 89.7% and specificity (Sp) of 96.0%. Among the parameters of differential blood count, acceptable diagnostic accuracy was obtained for immature to total neutrophil ratio (I/T ratio, AUC=0.789, Se=77.2%, Sp=73.2%, cut-off >0.11), immature to mature neutrophil ratio (I/M ratio, AUC=0.774, Se=75%, Sp=73.6%, cut-off >0.13) and percentage of band cells (AUC=0.784, Se=82.8%, Sp=66.2%, cut-off >5%). Absolute neutrophil count (AUC=0.607, Se=54.7%, Sp=66.1%) and lymphocyte count (AUC=0.659, Se=82.5%, Sp=47.9%), percentages of mature neutrophils (AUC=0.499, Se=51.7%, Sp=54.7%) and lymphocytes (AUC=0.659, Se=82.5%, Sp=47.9%) as well as total leukocyte count (AUC=0.629, Se=65.5%, Sp=56.9%) and platelet count (AUC=0.542, Se=62.1% Sp=49.2%) showed unacceptable discriminating power in the diagnosis of neonatal sepsis. Thus, the concentration of CRP showed the best diagnostic accuracy in the diagnosis of neonatal sepsis. Acceptable discriminating power in the diagnosis of neonatal sepsis showed I/T and I/M ratios and percentage of band cells. Total leukocyte count and platelet count are not reliable diagnostic indicators of neonatal sepsis.

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P19-6

Dijagnostička vrijednost "klasičnih" biljega neonatalne sepse

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Neonatalna sepsa ili bakteremija novorođenčadi, uz neusuglašenu terminologiju za sistemski odgovor na bakterijski infekt u novorođenčadi zasad nema ni savršene laboratorijske dijagnostičke pretrage temeljem koje će se i kod nesimptomatske novorođenčadi brzo uvesti antibiotska terapija. Potvrda infekcije nalazom hemokulture predugo traje, tako da se u sadašnjoj dijagnostici neonatalne sepse i u KB Sestre milosrdnice na Klinici za ginekologiju i porodništvo primjenjuju "klasični" biljezi. Oni obuhvaćaju određivanje kompletne krvne slike, omjera nesegmentiranih i segmentiranih granulocita (I/T) i CRP u sve novorođenčadi u prvih 24 sata života. U primjeni je i protokol SNAPII za procjenu fizioloških funkcija novorođenčadi. Cilj je bio utvrditi dijagnostičku i prognostičku vrijednost standardnih pretraga koje se rutinski rade kroz 24 sata za svu novorođenčad, nalazi kojih su bitni neonatolozima u svakodnevnom odlučivanju o pravodobnom uvođenju antibiotske terapije. U 69 novorođenčadi rođenih kroz mjesec dana na Klinici za ginekologiju i porodništvo određena je kompletna krvna slika na hematološkim analizatorima Beckman Coulter HmX i Micro Diff II. Omjer nesegmentiranih i segmentiranih granulocita određen je iz krvnog razmaza koji su pregledali medicinski biokemičari s iskustvom iz neonatalne hematologije. Koncentracija CRP određena je na analizatoru Olympus AU 640 uz uporabu visoko osjetljivog reagensa (HSCRP). U tumačenju i obradi rezultata slijedili smo važeći referentni raspon za zdravu novorođenčad u prvih 24 sata za sve određivane pretrage. Koncentraciju CRP iznad gornje granice (preporuka za bakteremiju je 10 mg/L) imalo je 83,7% novorođenčadi, 71,4% ih je imalo povišene leukocite (>17,8x10^9/L).Samo je 2,3% novorođenčadi imalo povišenu vrijednost omjera I/T. Na osnovi izmijenjenih fizioloških funkcija i laboratorijskih pretraga kod osmoro novorođenčadi učinjena je hemokultura, a pozitivan nalaz je zabilježen kod dvoje djece. U zaključku, "klasične" pretrage sepse, CRP i broj leukocita te omjer I/T, imaju vrlo dobru osjetljivost i visoku negativnu prediktivnu vrijednost u dijagnostici neonatalne sepse. Naši rezultati potvrđuju promišljanja da bi se za pravilnu interpretaciju graničnih vrijednosti za određivane biljege trebalo postaviti vrijednosti koje slijede fiziološku dinamiku njihovih promjena unutar prvih 48 sati života.

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P19-6

Diagnostic value of "classic" markers of neonatal sepsis

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There is an urgent need to know whether the newborn has developed sepsis, in order to introduce appropriate treatment as early as possible. Coniformation of the diagnosis (blood culture) may take time, and diagnostic tests are used to obtain quick indication of the infection status. These tests are not perfect. Some real cases of infection will produce negative test results, whereas some children free from infection will test positive. The potential usefulness of the test will depend on the clinical condition of the child. Current diagnostic approach in our hospital includes determination of "classic" markers of sepsis in all newborns: total leukocyte count, absolute neutrophil count, immature/total neutrophil count ratio (I/T) and C-reactive protein (CRP) within 24 hours of birth, and SNAP II physiology scores. The aim of the present study was to investigate the sensitivity and specificity of standard diagnostic tests for bacterial infection-sepsis of newborns that may be important for clinicians in their daily work at Department of Neonatology, Sestre milosrdnice University Hospital. We determined complete blood count (CBC) on the Cell Dyn Beckman Coulter HmX and Micro Diff II analyzers. The immature to total neuthrophil ratio (I/T) was determined by a hematology technologist. CRP concentration was measured by the HSCRP (high sensitive CRP) reagent on an Olympus AU 640 analyzer. The analysis was performed within the first 24 hours of life in all neonates born during a one-month period (n=69) at our hospital. On result interpretation, we followed reports on the upper marker limits within the first 24 h of life in healty newborns. CRP was above the reference range (10 mg/L) in 83.7%, WBC (>17.8x10^9/L) in 71.4%, and I/T (>0.25) in 2.3% of the newborns. Only two of eight newborns included in blood culture documentation had a "culture-proven" sepsis. In conclusion, the classic markers of neonatal sepsis, CRP and WBC, have a good sensitivity and negative prognostic value. Our results support the opinion that correct interpretation of cut-off values for the respective markers would require such values to be set that follow the physiological dynamics of their variation during the first 48 h of life.

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P19-7

Vrijednost mjerenja koncentracije masnih kiselina vrlo dugih lanaca u ranom otkrivanju bolesenika i heterozigota za X-vezanu adrenoleukodistrofiju

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X-vezana adrenoleukodistrofija (X-ALD) je nasljedna neurometabolična bolest obilježena centralnom ili perifernom demijelinizacijom i adrenalnom insuficijencijom. Gen za X-ALD normalno je odgovoran za sintezu peroksizomskog, transportnog adrenoleukodistrofičnog proteina (ALDP). Defektni ALDP uzrokuje, nepoznatim mehanizmima, poremećaj razgradnje masnih kiselina vrlo dugih lanaca (MKVDL), u najvećoj mjeri C26:0, a posljedica je patološko nakupljanje MKVDL u tkivnim i krvnim lipidima, osobito u korteksu nadbubrežne žlijezde i živčanom sustavu. Metabolična nepravilnost, povećana količina MKVDL i biokemijski poremećaj, te smanjena aktivnost acil-CoA sintetaze vrlo dugih lanaca u peroksizomima osnovne su osobine bolesti. Dok se adrenalna insuficijencija u ovih bolesnika liječi vrlo uspješno hormonskom nadomjesnom terapijom, još uvijek nema mogućnosti zaustavljanja i liječenja progredijentnih neuroloških poremećaja. Provođenje probiranja za otkrivanje nosilaca mutiranog gena na X kromosomu za ALD i prijenatalna dijagnostika od posebne su važnosti za prevenciju ove bolesti. Probiranje na nosioce gena za ALD provodi se kombinacijom nekoliko metoda: određivanjem koncentracije zasićenih MKVDL u serumu i kulturi kožnih fibroblasta, imunocitokemijskim određivanjem ALD proteina u kulturi kožnih fibroblasta te analizom DNA. Prikazujemo analitičku vrijednost i doprinos mjerenja MKVDL u otkrivanju nosilaca za X-ALD. Otkriće prvog bolesnika ponukalo nas je da u 18 dostupnih članova obitelji odredimo razine MKVDL. Koncentracije MKVDL u serumu mjerene su kao metilni esteri metodom plinske kromatografije-spektrometrije masa, uz interne standarde obilježene izotopima (stabilna izotopna dilucijska masena spektrometrija). U svim uzorcima određivano je pet parametara: C22:0, C24:0, C26:0, C24:0/C22:0, C26:0/C22:0. Određivanjem koncentracija MKVDL i njihovog međusobnog omjera te diskriminacijske vrijednosti Y iz seruma članova obitelji nađen je jedan oboljeli hemizigot, a za 3 žene je utvrđeno da su heterozigoti za ALD. Koncentracija C26:0 i omjer C26:0/C22:0 bili su značajno povišeni u serumu te su najvažniji, dijagnostički korisni parametri. Diskriminacijska vrijednost Y upućuje

P19-7

The value of very long chain fatty acid screening in the early detection of patients and heterozygotes for X-linked adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD) is an inherited neurometabolic disease presenting with central or peripheral demyelination and impaired function of adrenals. The responsible X-ALD gene normally encodes the peroxisomal adrenoleukodystrophy protein (ALDP). Defective ALDP causes, by unknown mechanisms, impaired degradation of very long chain fatty acids (VLCFAs), predominantly C26, resulting in pathognomonic accumulation of VLCFAs in tissues and blood lipids, notably in the adrenal cortex and nervous system. The metabolic abnormality, elevated levels of VLCFAs, and the biochemical defect, reduced peroxisomal very long-chain acyl-CoA synthetase (VLCS) activity, are the ubiquitous features of the disease. While adrenal dysfunction in X-ALD can be treated with adrenal hormone replacement therapy, there is no treatment for severe neurologic disabilities. Screening for carriers of mutated relevant gene and prenatal diagnosis are very important for the prevention of the disease. The carrier state can be investigated by determining the concentrations of saturated VLCFAs in serum or cultured skin fibroblasts, by looking for the presence of X-ALD protein in cultured skin fibroblasts, and by carrying out mutation analysis. The aim was to estimate the contribution of VLCFA measurement to the identification of the carrier state of X-ALD. Profiles of saturated VLCFAs were studied in 18 members of an affected family. VLCFA levels were analyzed as methyl esters by gas chromatography-mass spectrometry, using internal standards labeled with isotopes (stable-isotope dilution). Five parameters were determined: C22:0, C24:0, C26:0, C24:0/C22:0, C26:0/C22:0. Laboratory results revealed one patient to be hemizygous and three females heterozygous for ALD. The concentration of C26:0 and C26:00/C22:0 ratios were significantly increased in serum. Among VLCFAs, C26:0 (absolute and in proportion to C22:0) are the most important, diagnostically useful parameters. Discrimination value Y suggested women heterozygous for ALD. In conclusion, serum assay for VLCFAs has made it possible to perform large-scale screening of individuals at risk to identify asymptomatic patients with X-ALD.

na ženu heterozigota za ALD. U zaključku, metoda određivanja koncentracije MKVDL u serumu omogućila je probiranje širokog raspona rizičnih osoba u svrhu otkrivanja asimptomatskih bolesnika s X-ALD. Naglašava se potreba mjerenja MKVDL u serumu svih članova ne samo uže, već i šire obitelji bolesnika s ALD, kako bi se otkrili i drugi bolesnici i/ili heterozigoti za ALD. Otkrivanje heterozigota otvara mogućnost za prevenciju bolesti kroz gensko savjetovanje. Normalne koncentracije MKVDL u serumu u klinički suspektnih ispitanica ne isključuju heterozigotnost. U takvim slučajevima potrebno je odrediti koncentraciju MKVDL u homogenatu kulture kožnih fibroblasta te eventualno napraviti mutacijske analize gena ABCD1.

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Diagnostic tests should be offered to all at risk relatives of X-ALD patients and should include members of the extended family. Identification of heterozygotes provides an opportunity for disease prevention through genetic counseling. Normal concentration VLCFAs in clinically suspected females does not exclude carrier state. In these cases, the combined use of fibroblast VLCFA level analysis and possibly ABCD1 gene mutation analysis could detect X-ALD carriers correctly.

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P19-8

Usporedba reagensa dvaju proizvođača za određivanje vrijednosti C-reaktivnog proteina u nedonoščadi i novorođenčadi

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C-reaktivni protein (CRP) je reaktant akutne faze koji je u zdravih osoba prisutan u niskim koncentracijama. Patološka stanja kao što su bakterijske infekcije, upale ili razaranje tkiva praćena su povećanjem vrijednosti CRP uslijed otpuštanja upalnih citokina. Određivanje vrijednosti CRP vrlo je važno u dijagnostici i praćenju infekcije u nedonoščadi i novorođenčadi. U novorođenčadi sepsa može početi nespecifičnim simptomima i dijagnosticira se tek na osnovi patoloških laboratorijskih nalaza. Cilj rada bio je ispitati analitičku pouzdanost i reproducibilnost reagensa Full Range CRP tvrtke Randox za određivanje vrijednosti CRP u serumu/plazmi (mjerno područje 0,1-160,0 mg/L) u usporedbi s CRP reagensima tvrtke Olympus kao referentnim reagensima: Olympus CRP reagensom za koncentracijsko područje 5,0-300,0 mg/L i Olympus CRP Latex reagensom namijenjenom ranoj dijagnozi infekcije u nedonoščadi i novorođenčadi. Za Olympus CRP Latex reagens postoje dvije aplikacije: osjetljiva (SCRP, za koncentracijsko područje 0,5-20,0 mg/L) i visoko osjetljiva (HS-CRP, za koncentracijsko područje 0,05-2,00 mg/L). Za sve reagense primijenjene su izvorne baždarne i kontrolne otopine, kao i aplikacije za instrument Olympus AU 640. Ispitivanja su provedena u skladu s preporukama ECCLS.

P19-8

Comparison of reagents from two manufacturers for C-reactive protein determination in preterm and term neonates

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C-reactive protein (CRP) is an acute phase reactant, which is found in low concentrations in healthy individuals. Pathologic states such as bacterial infection, inflammation or tissue lesions are associated with an increase in CRP level due to the release of inflammatory cytokines. Determination of CRP is of utmost importance in the diagnosis and monitoring of infection in preterm and term neonates. In neonates, sepsis may begin with unspecific symptoms and is only diagnosed on the basis of pathologic laboratory findings. The aim of the study was to assess analytical reliability and reproducibility of the Full Range CRP reagent (Randox) for CRP determination in serum/plasma (measuring range 0.1-160.0 mg/L) in comparison with Olympus CRP reagents as reference reagents: Olympus CRP reagent for the concentration range of 5.0-300.0 mg/L and Olympus CRP Latex reagent intended for early diagnosis of infection in preterm and term newborns. The Olympus CRP Latex reagent has two applications: sensitive (SCRP, for concentration range of 0.5-20.0 mg/L) and highly sensitive (HSCRP, for concentration range of 0.05-2.00 mg/L). The original calibrated and control solutions and applications for the Olympus AU 640 instrument were used for all reagents. Tests were performed in line with

Analitička procjena obuhvatila je nepreciznost u seriji, nepreciznost iz dana u dan, netočnost i usporedna određivanja vrijednosti CRP u uzorcima bolesnika. Analizirano je 139 uzoraka seruma nedonoščadi i novorođenčadi. Uzorci s vrijednostima CRP manjim od 2,00 mg/L (skupina 1, n=35) analizirani su pomoću Olympus Latex reagensa (visoko osjetljiva aplikacija, mjerno područje 0,05-2,00 mg/L) i Randox Full Range CRP reagensa. Uzorci seruma s vrijednostima CRP manjim od 20,0 mg/L (skupina 2, n=63) analizirani su pomoću Olympus Latex reagensa (osjetljiva aplikacija, mjerno područje 0,5-20,0 mg/L) i Randox Full Range CRP reagensa, a uzorci seruma s vrijednostima CRP većim od 5,0 mg/L (skupina 3, n=41) analizirani su pomoću Olympus CRP reagensa (mjerno područje 5,0-300,0 mg/L) i Randox Full Range CRP reagensa. Dobiveni su zadovoljavajući rezultati za analitičku nepreciznost iz dana u dan (KV=1,64%), nepreciznost u seriji (KV <2,18%) i netočnost (R <3,67%). Rezultati usporednih određivanja vrijednosti CRP s reagensima dvaju proizvođača u tri skupine ispitanika (tri koncentracijska područja) statistički obrađeni lineranom regresijskom analizom pokazali su visok stupanj korelacije (r >0,99). Na temelju dobivenih rezultata može se zaključiti da je Full Range Randox reagens pouzdan i reproducibilan reagens za određivanje koncentracije CRP u nedonoščadi i novorođenčadi.

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ECCLS recommendations. Analytical evaluation included serial imprecision, day to day imprecision, inaccuracy, and parallel CRP determination in patient samples. A total of 139 serum samples from preterm and term babies were analyzed. Samples with CRP levels lower than 2.00 mg/L (group 1, n=35) were analyzed by use of Olympus Latex reagent (highly sensitive application, measuring range 0.05-2.00 mg/L) and Randox Full Range CRP reagent. Serum samples with CRP levels lower than 20.0 mg/L (group 2, n=63) were analyzed by use of Olympus Latex reagent (sensitive application, measuring range 0.5-20.0 mg/L) and Randox Full Range CRP reagent, whereas those with CRP level greater than 5.0 mg/L (group 3, n=41) were analyzed by use of Olympus CRP reagent (measuring range 5.0-300.0 mg/L) and Randox Full Range CRP reagent. Satisfactory results were obtained for analytical day to day imprecision (CV=1.64%), serial imprecision (CV <2.18%) and inaccuracy (R <3.67%). The results of parallel CRP determinations with the use of reagents from two different manufacturers in three study groups (three concentration ranges), statistically processed by linear regression analysis, showed a high degree of correlation (r >0.99). Based on the results obtained, it is concluded that the Randox Full Range reagent is a reliable and reproducible reagent for CRP determination in preterm and term newborns.

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P20 – Laboratorijski informacijski sustavi, P20-1

Analizator IL GEM Premier 3000 za pretrage uz bolesnika: umreženje i prednosti

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Razvojem biomedicinske i informatičke tehnologije rad na analizatorima koji su vezani uz bolesnika (engl. *point-of-care*, POC) znatno se pojednostavljuje. Uređaji se međusobno povezuju u lokalnu mrežu (engl. *Local Area Network*, LAN) koja omogućava nadzor nad umreženim analizatorima u jedinicama za pretrage uz bolesnika. Reorganizacijom laboratorijskog rada 2005. godine u Kliničkom zavodu za laboratorijsku dijagnostiku KBC Zagreb uvode se analizatori acidobaznog statusa tvrtke Instrumentation Laboratory (zastupnik tvrtka MDLab d.o.o.) GEM Premier 3000 na 11 lokacijskih jedinica (Klinika za anesteziologiju, reanimaciju i intenzivno liječenje, jedinice intenzivne skrbi i operacijska dvorana Klinike za neurokirurgiju, Klinike za kirurgiju, Klinike za kardijalnu kirurgiju, Klinike za

P20 – Laboratory information system, P20-1

IL GEM Premier 3000 POCT analyzer: networking and advantages

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Development of biomedical and computer technology has substantially contributed to increasing simplification of the operation of point-of-care (POC) analyzers. These instruments are interconnected in a local area network (LAN), which allows their surveillance in POC units. Following the restructuring of laboratory practice conducted during 2005 at Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, GEM Premier 3000 analyzers manufactured by Instrumentation Laboratory (represented in Croatia by MDLab d.o.o.) were introduced to determine acid-base status at 11 hospital locations: Department of Anesthesiology, Resuscitation and Intensive Care, intensive care units and operating rooms of University Departments of Neurosurgery, Surgery, Cardiac Sur-

pedijatriju; Zavod za dijalizu; Zavod za hitnu i intenzivnu medicinu; Klinika za ženske bolesti i porode, Zavod za perinatalnu medicinu, te hitni laboratoriji Kliničkoga zavoda za laboratorijsku dijagnostiku). Uporabom programa GEM Web koji je instaliran na dva računala smještena u hitnom i redovnom laboratoriju medicinskim su biokemičarima omogućeni 24-satni nadzor i praćenje udaljenih analizatora. Program omogućava dobivanje svih potrebnih izvješća za pretrage uz bolesnika koji uključuju: pregled svih rezultata bolesnika, aktivnu kontrolu kvalitete rada, uvid u pogrješke s identifikacijom analitičara, te uvid o broju izvršenih analiza i potrošnji reagensa. Program ima mogućnost povezivanja podataka u LIS i BIS. Tijekom 6 mjeseci na analizatorima za pretrage uz bolesnika KBC Zagreb obrađeno je 53 711 uzoraka krvi bolesnika i zabilježeno 511 prijeanalitičkih pogrješaka (ugrušak i/ili interferencija, 0,9%). Pojavnost prijeanalitičkih pogrješaka manja od 1% bila je moguća zahvaljujući trajnoj izobrazbi osoblja na odjelima koje provodi analize od strane laboratorijskih stručnjaka kojima umreženje uređaja omogućava stalan pregled nad analitičkim radom.
Vrijeme laboratorijskih djelatnika provedeno uz instrument svedeno je na minimum, a kontrole i rezultati retrogradno se mogu provjeriti za svakog bolesnika.

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gery, Pediatrics; Dialysis Unit, Department of Emergency and Intensive Care; University Department of Gynecology and Obstetrics, Department of Perinatal Medicine, and emergency laboratories of the Clinical Institute of Laboratory Diagnosis. GEM Web application has been installed on two computers located in emergency and central laboratory to allow medical biochemists 24-h surveillance and monitoring of remote analyzers. The application provides all the necessary POC test reports, comprising the list of all patient results, active quality management, survey of errors including analyst identification, and data on the number of analyses performed and reagent consumption. The application can also be integrated into LIS or HIS. A total of 53,711 patient blood samples were processed by POCT analyzers at Zagreb University Hospital Center during 6 months, and 511 preanalytical errors (clot and/or interference, 0.9%) were recorded. A less than 1% incidence of preanalytical errors was possible owing to continuous education of nurses that perform assays by laboratory professionals who can permanently monitor analytical performance due to analyzer integration. The time that laboratory staff spend by the analyzers has been reduced to minimum, and control and test results may be retrogradely checked for any patient.

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P20-2

Primjena informacijskih tehnologija u dostavi laboratorijskih nalaza

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Cilj rada bio je ispitati dostavu nalaza vanjskim korisnicima u Kliničkom zavodu za laboratorijsku dijagnostiku KBC Zagreb, te uvesti one metode koje će omogućiti brzu i pouzdanu dostavu nalaza te spriječiti nepotrebne troškove. U radu smo od metoda primijenili laboratorijski informacijski sustav (LIS), anketu, te statističku obradu nepodignutih nalaza. Glavne značajke primjene LIS su potpuna automatizacija u izradi laboratorijskih pretraga te brza razmjena informacija. Sam proces stvaranja informacija definira strukturne module LIS. Jedan od modula je izrada i dostava nalaza korisnicima, a uključuje mrežnu razmjenu podataka s bolničkim odjelima i ambulantama, te pretva-

P20-2

Use of information technologies in laboratory finding delivery

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The aim of the study was to investigate the delivery of laboratory findings to outpatients at Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, and to introduce methods that will allow for rapid and reliable laboratory result delivery and eliminate unnecessary costs. Regarding study methods, we used laboratory information system (LIS), survey, and statistical processing of uncollected results. Major LIS characteristics are complete automation of laboratory test performance and rapid information exchange. Structural models of LIS are defined by the actual information-producing process itself. One of the modules comprises preparation and

ranje validiranih nalaza u PDF-format (engl. *Portable Document Format*) i njihovu dostavu pomoću elektroničke pošte za vanjske korisnike. Kod takvog slanja nalaza osobito je važna zaštita podataka kod određenih genetičkih i drugih pretraga. Za anketno ispitivanje pripremljen je poseban upitnik na temelju kojega je proveden terenski dio istraživanja među posjetiteljima kliničkog laboratorija o njihovoj navici dobivanja nalaza, o poznavanju interneta, te o mogućnosti dobivanja nalaza elektroničkom poštom. Analiza zatvorenih pitanja iz upitnika pokazala je da 86% ispitanika sami dolaze u laboratorij po nalaze, 59% ispitanika se služi internetom, a 55% ispitanika je čulo za mogućnost slanja nalaza internetom. Jedna četvrtina ispitanika (25%) izjasnila se za dobivanje nalaza elektroničkom poštom i to pretežito oni iz dobne skupine od 25-50 godina. Otvorena su pitanja dala uvid o stavovima bolesnika kod odabira specijalističkih laboratorija. Statistička obrada nepodignutih nalaza kroz razdoblje od 6 mjeseci pokazala je kako svakog mjeseca ostaje oko 20% nepodignutih nalaza, od kojih su najbrojniji oni iz biokemije proteina i tumorskih biljega (33%), endokrinologije (23%), te imunologije (20%). Rezultati ispitivanja pokazuju da primjena informacijskih tehnologija (elektronička pošta ili elektronička razmjena podataka s liječnicima primarne zaštite) u dostavi laboratorijskih nalaza vanjskim korisnicima omogućuje smanjenje nagomilavanja nepodignutih nalaza, posjeta bolesnika i gužve u čekaonicama, ali i nepotrebnog rasipanja zdravstvenih sredstava. Također doprinosi racionalizaciji i uštedi u reorganizaciji zdravstvene zaište.

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delivery of laboratory reports to users, involving network data exchange with hospital wards and outpatient units, conversion of validated results into PDF (portable document format) files, and their delivery by electronic mail to outpatients. However, data protection during such delivery of laboratory reports is of particular importance for some genetic and other tests. A specific questionnaire was prepared for polling which was the basis for the field investigation among clinical laboratory users on the usual manner of receiving reports, their knowledge of internet, and the possibility of receiving laboratory results by e-mail. The analysis of closed-ended poll questions revealed that 86% of respondents collected their laboratory reports themselves, 59% confirmed the use of internet, and 55% of respondents had heard of the possibility of laboratory report delivery by the internet. One fourth of respondents (25%) declared in favor of receiving reports by e-mail, mainly those from the 25-50 age group. Open-ended questions provided information on the patient attitudes regarding selection of specialist laboratories. Statistical processing of reports uncollected over 6 months demonstrated that 20% of reports were left uncollected *per* month, mostly those on protein biochemistry and tumor markers (33%), endocrinology (23%), and immunology (20%). Study results showed that the use of information technologies, i.e. electronic mail or electronic data exchange with primary health care physicians, in delivery of laboratory findings to outpatients led to reduction in the number of uncollected reports, patient visits, waiting room queues, and unnecessary wasting of healthcare funds. The application of these technologies also contributes to rationalization and savings in healthcare restructuring.

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P21 – Automatizacija i robotika, P21-1

Automatizirani sustav za sveobuhvatnu analizu mokraće Iris IQ 200 – iskustva nakon godinu dana svakodnevnog rada

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Iris IQ 200 jedna je od dviju danas dostupnih automatiziranih alternativa tradicionalno "ručnoj" i subjektivnoj analizi mokraćnog sedimenta. Inicijalna procjena instrumenta ukazala je na dobru korelaciju nalaza dobivenih svjetlosnom mikroskopijom i upotrebom sustava Iris IQ

P21 – Automatization and robotics, P21-1

Iris IQ 200 fully automated urinalysis system – one-year experience in routine use

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Iris IQ 200 is one of the two currently available alternatives to traditionally manual urine microscopy. In our hands, initial instrument evaluation has indicated good correlation between light microscopy and Iris IQ 200 findings, provided all the elements were sorted manually by an

200, uz uvjet neizostavne preklasifikacije elemenata od strane iskusnog operatera za sve uzorke. Koeficijenti korelacije za tipične elemente sedimenta poput eritrocita, leukocita, različitih epitelnih stanica i cilindara kretali su se između 0,763 i 0,989. Nepreciznost brojanja bila je, prema očekivanjima, bitno manja nego za ručnu metodu i iznosila je, izražena kao CV%, od 5,4 do 9,7 za pojedine elemente unutar dana. Ukupno vrijeme potrebno za izdavanje nalaza pojedinačnog uzorka mokraće smanjeno je s prosječnih 10-15 na 2-5 minuta. Mogućnost izdavanja nalaza broja nađenih elemenata po vidnom polju učinila je prijelaz s tradicionalne na automatiziranu analizu mokraće jednostavnom za kliničare i bolesnike. Unutar inicijalne procjene instrumenta provjerili smo i prenošenje uzorak-uzorak (*carry-over*) za osnovne elemente sedimenta. Proizvođač se u tom smislu ograđuje od analize uzoraka s "velikom količinom krvi", što se, pretpostavljamo, odnosi na makrohematuriju. Ispitujući *carry-over* na uzorcima s mikrohematourijom (masa eritrocita, mokraća makroskopski uredna) ustanovili smo da zaista ne postoji značajno prenošenje uzorak-uzorak. Isto je ustanovljeno za leukocite i stanice pločastog i prijelaznog epitela. Međutim, tijekom rutinskog rada uočili smo slučajeve prenošenja uzorak-uzorak, i to za elemente koji nisu bili uključeni u inicijalnu validaciju – spermatozoide i gljivice. Kako ovakvo prenošenje nigdje nije opisano u literaturi vezanoj za Iris IQ 200, smatramo potrebnim izvijestiti o ovom problemu, kako bi se izbjeglo izdavanje pogrješnih nalaza, odnosno potencijalno neugodne situacije.

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experienced technician. Correlation coefficients obtained for typical elements such as erythrocytes, leukocytes, epithelial cells and casts were between 0.763 and 0.989. As expected, counting imprecision was considerably lower than the manual method with within day variation coefficients (CV%) between 5.4 and 9.7. Total average time needed for single urinalysis decreased from 10-15 to 2-5 minutes. The possibility of reporting the findings number/high power field has eased the transition from manual to automated urinalysis for both the clinicans and patients. Our initial evaluation protocol included sample to sample carry-over studies as well. However, we included only common sediment elements such as erythrocytes, leukocytes and squamous epithelial cells in these protocols. The manufacturer's instructions state "not to analyze samples with great quantities of blood", which presumably concerns macrohematuric samples. Indeed, we found no carry-over with microhematuria, i.e. in samples that were macroscopically normal but contained great quantities of erythrocytes. Similar results were obtained for leukocytes and squamous epithelial cells, with no carry-over detected. However, during routine use we spotted and documented isolated cases of sample to sample carry-over, which involved urine elements not included in initial evaluation studies – spermatozoa and fungi. Although occurring only with fairly high amounts of the mentioned elements, we think it is important to be aware of this possibility, in order to avoid false results and/or potentially embarrassing situations. Up to now, this kind of carry-over with Iris IQ System has not been reported in the literature.

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P21-2

Analitička procjena plinskog analizatora Gem® PremierTM 3000

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U novije vrijeme na tržištu se pojavljuju novi biokemijski analizatori glavna namjena kojih je određivanje analiza uz bolesnički krevet (POCT, *point of care testing*). Jedan od takvih analizatora je Gem® PremierTM 3000 (Inc. Lexington, MA, SAD), analitičku procjenu kojega smo proveli prema preporukama Europskog odbora za kliničko-laboratorijske standarde (ECCLS). Mjerili smo parametre: pH, pCO_2, pO_2, iCa^{2+}, Na^+, K^+, laktat, glukozu i hematokrit u uzorcima

P21-2

Analytical evaluation of the Gem® PremierTM 3000 analyzer

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New biochemical analyzers for point-of-care testing (POCT) have recently become available. One of these is Gem® PremierTM 3000 (Inc. Lexington, MA, USA). We conducted analytical evaluation of its performance based on the guidelines issued by the European Committee for Clinical Laboratory Standards (ECCLS). We measured pH, pCO_2, pO_2, iCa^{2+}, Na^+, K^+, lactate, glucose and hematocrit in whole blood samples. Analytical evaluation of analyz-

pune krvi. Analitička procjena analizatora obuhvaća ispitivanje nepreciznosti iz dana u dan, nepreciznost u seriji i usporedbu s konvencionalnim laboratorijskim metodama provedenim na biokemijskim analizatorima (Dimension RxL, Dade Behring Inc., Newark, DE, USA; Olympus AU 400, Olympus Corporation, Mishima, Japan), hematološkom (Cell Dyn 1700, Abbott Laboratories, SAD) i acidobaznom (Rapid 348, Bayer Corporation, SAD) analizatoru. Usporedna mjerenja pojedinih analiza pokazuju zadovoljavajuće koeficijente korelacije (r=0,8233-0,9843). Nepreciznost u seriji kod svih analiza ukazuje na vrlo nizak koeficijent varijacije (KV=0,00-3,73%). Nepreciznost iz dana u dan je bila dobra (KV=0,23-3,76%), osim za glukozu za koju je bila nešto veća (KV=5,57%) i za laktat (KV=9,29%). Analitičkom procjenom analizatora Gem® PremierTM 3000 ustanovili smo da je dovoljno pouzdan za svakodnevnu primjenu u sustavu POCT. Analizator je lagan za upotrebu, jednostavan za rukovanje, lako prenosiv, pouzdan, odgovarajući je za operacijske sale, za jedinicu intenzivne njege, za analize tijekom hitnih slučajeva, na udaljenim mjestima od centralnog laboratorija. Primjena ovakvih analizatora zahtijeva i dobru komunikaciju kliničkog i laboratorijskog osoblja, što je vrlo važno u organizaciji provođenja laboratorijskih pretraga uz bolesnika. Treba istaknuti da analizator ima mogućnost povezivanja s bolničkim, laboratorijskim informacijskim sustavom, što je budućnost svih naših laboratorija.

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P21-3

Analitička procjena analizatora ADVIA 1200

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ADVIA 1200 je automatizirani biokemijski analizator koji izvodi pretrage u serumu, plazmi i mokraći. Pretrage se obavljaju nasumice, u seriji i u STAT modu brzinom od 800 fotometrijskih testova na sat i 600 testova za elektrolite na sat. Cilj je prikazati analitičku procjenu biokemijskog analizatora ADVIA 1200, koja je provedena prema preporukama Europskog odbora za kliničko-laboratorijske standarde (ECCLS). Ispitivani su: glukoza, ureja, kreatinin, mokraćna kiselina, ukupni bilirubin, AST, ALT, ALP, GGT, CK, LDH, amilaza, kolesterol, trigliceridi i željezo. Procjena je obuhvatila nepreciznost unutar serije, nepreciznost iz dana u dan, netočnost i usporedno određivanje s analizaers includes assessment of within-run and between-run imprecision, and comparison with traditional laboratory methods of biochemical analyzers (Dimension RxL, Dade Behring Inc., Newark, DE, USA; Olympus AU 400, Olympus Corporation, Mishima, Japan), hematology (Cell Dyn 1700, Abbott Laboratories, USA) and blood gas analyzers (Rapid 348, Bayer Corporation, USA). Comparison measurements showed satisfactory correlation coefficients (r=0.8233-0.9843). Within-run imprecision for all parameters revealed a very low coefficient of variation (CV=0.00-3.73%). Between-run imprecision was good (CV=0.23-3.76%), except for glucose where it was slightly elevated (CV=5.57%), and for lactate (CV=9.29%). On the basis of data obtained by analytical evaluation of the Gem® PremierTM 3000 analyzer, we conclude that it is sufficiently reliable for daily use in POCT. The analyzer is easy to use, simple to operate, portable and reliable, therefore, it is suitable for operating rooms, intensive care units, emergency rooms, and decentralized locations. The application of such analyzers requires adequate communication between clinical and laboratory staff, resulting in an important link in the organization of point-of-care testing. It should also be stressed that the analyzer has the possibility of connection to hospital laboratory information system, what constitutes the future of all our laboratories.

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P21-3

Analytical evaluation of ADVIA 1200 analyzer

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The ADVIA 1200 Chemistry System is an automated clinical chemistry analyzer that can run tests on human serum, plasma or urine in random access, batch and STAT modes at a throughput rate of 800 photometric tests *per* hour and 600 electrolyte tests *per* hour. The objective is to present analytical evaluation of the ADVIA 1200 clinical chemistry analyzer, performed according to the guidelines of the European Committee for Clinical Laboratory Standards (ECCLS). The following analytes were tested: glucose, urea, creatinine, uric acid, total bilirubin, AST, ALT, GGT, ALP, CK, LDH, cholesterol, triglycerides, amylase and iron The evaluation consisted of determination of

torom Hitachi 911. Nepreciznost unutar serije određivana je u 30 uzastopnih mjerenja u kontrolnim serumima Bayer QC Control 1, Control 2, Precinorm U, Precipath U (Roche). Nepreciznost iz dana u dan određivana je u triplikatu u navedenim kontrolnim serumima tijekom 10 dana. Netočnost mjerenja prikazana je kao postotak odstupanja (R%) srednje izmjerenih vrijednosti od srednje deklariranih vrijednosti kontrolnih seruma. Usporedba analizatora provedena je pomoću 67 uzoraka u različitim koncentracijskim područjima. Rezultati su pokazali prihvatljiv koeficijent varijacije za nepreciznost unutar serije i nepreciznost iz dana u dan te zadovoljavajući stupanj točnosti u odnosu na kriterije temeljene na veličini biološke varijacije, osim za željezo u kontrolnom serumu Bayer QC Control 2 i visok stupanj korelacije s Hitachi 911 (koeficijent korelacije veći od 0,985). Provedena analitička procjena pokazala je da je ADVIA 1200 pouzdan biokemijski analizator koji osigurava brz i siguran rad uz uporabu minimalnih količina uzoraka i reagensa.

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between-day imprecision, within-run imprecision, inaccurcy and correlation with Hitachi 911. Within-run imprecision was determined on 30 samples of four control sera, Bayer QC Control 1, Control 2, Precinorm U, Precinorm P (Roche). Day-to-day imprecision was determined in triplicate in four control sera during 10 days. Inaccuracy was calculated as percentage of deviation of the mean determined value from the mean declared value of the control sera. Comparison of the analyzers was performed using 67 samples in various concentration ranges. The results of analycal performance were as follows: low within-run and day-to-day imprecision, a satisfactory level of accuracy according to the criteria based on biological variation except for iron in control sera, Bayer QC Control 2, and a high rate of correlation with Hitachi 911 (coefficient of corelation above 0.985). Analytical evaluation showed ADVIA 1200 to be a reliable biochemistry analyzer, which ensures rapid and safe work, using minimal quantities of samples and reagents.

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P22 – Procjena analitičkih sustava, P22-1 (UP4-1)

Epruvete različitih proizvođača i vrijednosti biokemijskih parametara

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Najčešći čimbenici koji utječu na promjenjivost laboratorijskih nalaza pripadaju skupini predanalitičkih metodoloških čimbenika. U toj skupini važno mjesto priprada pravilnom uzorkovanju krvi, odnosno uporabi odgovarajuće epruvete s podtlakom. Globalizacija tržišta donosi sa sobom i različite proizvođače ovoga potrošnog materijala koji u potpunosti ne uklanjaju interferenciju dodatka u epruvetama (antikoagulansi, konzervansi) s metodama različitih instrumenata koji se upotrebljavaju u laboratorijima. Poučeni vlastitim iskustvom o mogućnosti interferencije sastojka epruvete s rezultatima laboratorijskih nalaza (Crnokrak S. 16th IFCC-FESCC Congress, Glasgow, 2005.), ispitali smo utjecaj biokemijskih epruveta pet različitih proizvođača na vrijednosti biokemijskih parametara. Ispitivanje je izvršeno na 20 uzoraka seruma uzorkovanih u slijedeće biokemijske epruvete: Venosafe Vacuette, BD Vacutainer, Improve (s gelom i bez njega), Greiner-vacuette. U svim uzorcima izmjerene su vrijednosti 21 biokemijskog parametra (opća biokemija i hormoni štitnjače). Vrijednosti su izmjerene na biokemijskom analizatoru

P22 – Evaluation of analytical systems, P22-1 (UP4-1)

Use of different test tubes and values of biochemical parameters

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The most common factors affecting laboratory test results belong to the group of preanalytical methodology factors. Within this group, correct blood sampling, i.e. by use of appropriate vacuum test-tubes, has a very important role. Market globalization implies different manufacturers of this kind of expendable supplies. Some of them do not eliminate the interference between test-tube additives (e.g., anticoagulants, preservatives) and methods of various instruments that are used in laboratories.
Based on our own experience considering the possibility of test-tube interference with laboratory test results (Crnokrak S. 16th IFCC-FESCC Congress, Glasgow, 2005), we assessed the influence of biochemistry test-tubes by five different manufacturers on the values of biochemical parameters. Testing was conducted on 20 samples in the following biochemistry test-tubes: Venosafe Vacuette, BD Vacutainer, Improve (with and without gel), and Greiner-vacuette. In all samples the values of 21 biochemical parameters were measured (general biochemistry and thyroid hormones). Values were measured on an Archi-

Architect c8000 i imunokemijskom analizatoru Architect i2000 (Abbott, SAD) uz primjenu preporučene analitičke metode. Dobiveni rezultati statistički su obrađeni i iskazani srednjom vrijednosti, standardnom devijacijom (SD) i koeficijentom varijacije (KV), te koeficijentom korelacije (r). Vrijednosti općih biokemijskih parametara (glukoza, urea, kreatinin, ALT, AST, GGT, AP, ukupni bilirubin, CK, LDH, amilaza, željezo, kolesterol, trigliceridi, natrij, kalij, kloridi, kalcij) nisu pokazala značajna odstupanja u ovisnosti o vrsti uporabljene epruvete. Najniži koeficijent varijacije pokazale su vrijednosti natrija (KV=0,05%), a najviši vrijednosti GGT (KV=3,7%). Koeficijenti varijacije za izmjerene vrijednosti hormona štitnjače iznosili su od 0,84% za fT4 do 1,38% za T3. Najniži koeficijent korelacije pokazale su epruvete s gelom Improve (r=od 0,86 do 0,93) pri određivanju T3. S obzirom na statističke pokazatelje epruvete svih navedenih proizvođača smatraju se prihvatljivima za uzorkovanje krvi i ne pokazuju značajne interferencije s uporabljenom metodologijom.

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tect c8000 biochemistry analyzer and Architect i2000 (Abbott, USA) immunochemistry analyzer. We used the recommended analytical methods. The results obtained were statistically processed and expressed as mean, standard deviation (SD), coefficient of variation (CV) and correlation coefficient (r). The values of general biochemistry parameters (glucose, urea, creatinine, ALT, AST, GGT, AP, total bilirubin, CK, LDH, amylases, iron, cholesterol, triglycerides, sodium, potassium, chlorides, calcium) did not show any significant deviation related to the type of the test-tube used. Sodium showed the lowest and GGT highest coefficient of variation (CV=0.05% and 3.7%, respectively). The coefficients of variation for thyroid hormones were from 0.84% for fT4 to up to 1.38% for T3. The lowest correlation coefficient was recorded for the Improve test-tubes with gel (r=0.86-0.93) on T3 measurement. Considering statistical indicators, the test-tubes of all the above mentioned manufacturers were found acceptable for blood sampling and showed no significant interference with the tested methodology.

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P22-2 (UP4-2)

Određivanje hipoksantina, ksantina i mokraćne kiseline u likvoru tekućinskom kromatografijom visoke djelotvornosti (HPLC)

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Prikupljeni podatci ukazuju na to da oksidacijski stres igra glavnu ulogu u patogenezi mnogobrojnih neuroloških bolesti (moždani udar, traumatska ozljeda mozga, epilepsija, meningitis, neurodegenerativne bolesti, multipla skleroza, hipoksijsko-ishemijska encefalopatija novorođenčeta, preeklampsija itd.). Hipoksantin je proučavan kao biljeg hipoksije/ishemije budući da se nakuplja u tkivu kod pojačane razgradnje adeninskih nukleotida i kod inhibicije ksantin-oksidaze zbog nedostatka kisika. Smatra se da mokraćna kiselina ima važnu ulogu u uklanjanju endogenih radikala. Također se smatra da za vrijeme recirkulacije, nakon privremene ishemije ili nakon nepotpune ishemije, sustav hipoksantin-ksantin stvara slobodne radikale koji mogu izazvati novu ozljedu tkiva u razdoblju reoksigenacije (tzv. paradoks kisika). Cilj ovoga rada bio je uvesti u rutinsku likvorsku dijagnostiku određivanje

P22-2 (UP4-2)

Determination of hypoxanthine, xanthine and uric acid in human cerebrospinal fluid by high performance liquid chromatography (HPLC)

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Accumulating data indicate that oxidative stress plays a major role in the pathogenesis of numerous neurological diseases (stroke, traumatic brain injury, epilepsy, meningitis, neurodegenerative diseases, multiple sclerosis, perinatal hypoxic-ischemic injury, preeclampsia, etc.). Hypoxanthine has been studied as a marker of hypoxia/ischemia since it accumulates in the tissue due to the increased breakdown of adenine nucleotides and inhibition of xanthine oxidase activity because of the lack of oxygen. Uric acid has been implicated as an important endogenous radical scavenger. It has also been suggested that reactive oxygen species (ROS) produced by the hypoxanthine-xanthine oxidase system might trigger secondary injury in the reoxygenation period ("oxygen paradox") during recirculation after temporary ischemia or during incomplete ischemia. The aim of the study was

hipoksantina, ksantina i mokraćne kiseline metodom tekućinske kromatografije visoke djelotvornosti (HPLC). Hidrofilni analiti razdvojeni su izokratično metodom HPLC na koloni C18 pri temperaturi od 30 °C. Proteini iz likvora uklonjeni su ultrafiltacijom. Mobilna faza sastojala se je od 0,06 M fosfatnog pufera (pH 5,1) i metanola (97:3). Rabio se je protok 0,7 mL/min i UV detektor pri 254 nm. Kalibracijska krivulja bila je linearna u rasponu od 1-50 µmol/L (R2=0,996, R2=0,999) za hipoksantin i ksantin te 1-100 µmol/L (R2=0,997) za mokraćnu kiselinu. Ponovljivost izražena koeficijentom varijacije bila 6%, 5% i 4% za mokraćnu kiselinu, hipoksantin i ksantin (n=10). Reproducibilnost izražena koeficijentom varijacije bila je 6%, 9% and 5% za mokraćnu kiselinu, hipoksantin i ksantin (n=10). Rezultat testa iskorištenja za mokraćnu kiselinu, hipoksantin i ksantin bio je od 80% do 101% (n=9). Dobiveni rezultati pokazuju da opisana metoda HPLC ima zadovoljavajuću nepreciznost i točnost te se može rabiti za određivanje metabolita purina u likvoru.

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to introduce HPLC method for simultaneous determination of hypoxanthine (Hx), xanthine (Xa) and uric acid (UA) in routine cerebrospinal fluid (CSF) analysis. The hydrophilic analytes were separated isocratically by HPLC method using C18 column at a temperature of 30 °C. In this method, CSF proteins were removed by ultrafiltration. Mobile phase was composed of 0.06 M phosphate buffer (pH 5.1) and methanol (97:3). The flow rate was at 0.7 mL/min and UV detector at 254 nm. Calibration curves were linear at 1-50 µmol/L for Hx and Xa (R2=0.997, R2=0.999, respectively) and for UA at 1-100 µmol/L (R2=0.997). Within-day precision of the method defined by CV% for UA, Hx and Xa was 6%, 5%, and 4% (n=10), respectively. Between-day precison of the method defined by CV% for UA, Hx, and Xa was 6%, 9% and 5% (n=10), respectively. Recovery of the method for UA, Hx, and Xa was from 80% to 101% (n=9). These results indicate that the described HPLC method for purine metabolites in CSF has satisfactory precision and accuracy, so it can be introduced in laboratory practice.

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P22-3

Dugoročna procjena vanjske procjene kvalitete rada za srčane biljege

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Za određivanje srčanih biljega još uvijek ne postoje referentne metode, a referentni materijali još uvijek nisu uspostavljeni. Revizija preporuka Komiteta za standardizaciju srčanih biljega pri IFCC (engl. *IFCC Committee on Standardization of Markers of Cardiac Damage,* IFCC C-SMCD) je u tijeku. Sudjelovanje u vanjskoj procjeni kvalitete rada laboratorijima daje uvid u stupanj međulaboratorijskih razlika, što je preduvjet usklađivanja dobivenih rezultata. Cilj studije bio je evaluirati rezultate određivanja mioglobina i troponina I (cTnI) u vanjskoj procjeni kvalitete *Randox International Quality Assessment Scheme* (RIQAS EQA) potvrđenoj prema međunarodnom standardu ISO:9002 proizvođača Randox Laboratories. Zavod za kliničku kemiju KB Merkur potvrđen prema međunarodnom standardu ISO:9001:2000 sudjeluje u vanjskoj procjeni kvalitete rada za srčane biljege *RIQAS-Cardiac Programme* od 2001. godine. Liofilizirani kontrolni serumi humanog podrijetla se distribuiraju u 150 laboratorija, a rezultati se grupiraju prema metodama i procjenjuju u odnosu na srednju vri-

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Long-term evaluation of external quality assessment for cardiac markers

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No reference methods or reference materials have yet been established for cardiac markers. The IFCC Committee on Standardization of Markers of Cardiac Damage (IFCC C-SMCD) recommendations are still in the process of revision. Through their participation in the external quality assessment (EQA), laboratories are informed on the extent of inter-laboratory variation, which is a prerequisite for result harmonization. The aim of the study was to evaluate the results on myoglobin and cardiac troponin I (cTnI) in the Randox International Quality Assessment Scheme (RIQAS EQA) external quality assessment by Randox Laboratories. Department of Clinical Chemistry, Merkur University Hospital, certified according to ISO:9001:2000, participates in the RIQAS EQA-Cardiac Programme since 2001. Lyophilized control samples of human origin were distributed to 150 laboratories, results were grouped according to the method used and estimated by comparison with the group average. Results within ±2 standard deviation index (SDI) are considered

jednost grupe, pričem se prihvatljivima smatraju oni koji se nalaze unutar dozvoljenog odstupanja od ±2 relativne standardne devijacije (SDI). U Zavodu za kliničku kemiju koncentracija mioglobina se određuje imunoturbidimetrijskom metodom na analizatoru Olympus AU 400, a rezultati se procjenjuju prema grupi ostalih metoda (4 laboratorija). Koncentracija cTnI se određuje imunokemijskom (RPIA) metodom na analizatoru Dade Stratus CS, a rezultat se procjenjuje prema metodi RPIA (Dade Behring Stratus CS; 9 laboratorija). Procjena rezultata bez obzira na metodu je pokazala veliku varijabilnost (KV %): za mioglobin 26,6% i za cTnI 77,7%. Grupiranje rezultata prema metodama je pokazalo manju varijabilnost: za mioglobin 0,5–31,4% i za cTnI 3,6–20,7%. Varijabilnost rezultata grupe u kojoj se procjenjuju naši rezultati za mioglobin je bila 25,9%, što je očekivana vrijednost s obzirom na broj sudionika i heterogenost grupe. Varijabilnost rezultata za cTnI u grupi metoda RPIA je iznosila 6,1%, što je očekivana vrijednost s obzirom na homogenost grupe. S obzirom na visoku varijabilnost rezultata bez obzira na metodu neophodna je standardizacija metoda za cTnI primjenom metoda definiranih vezanjem protutijela isključivo na epitope stabilnoga dijela molekule cTnI i KV manjim od 10%. Iako su rezultati za mioglobin usklađeniji, varijabilnost unutar pojedinih metoda nameće standardizaciju metoda za mioglobin prema certificiranom referentnom materijalu.

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acceptable and satisfactory. Myoglobin concentrations were determined by immunoturbidimetric method (Olympus AU 400 analyzer) and results estimated within the group of other methods (4 participants). cTnI concentrations were determined by RPIA method (Dade Behring Stratus CS analyzer) and the results estimated within the group of RPIA method (9 participants). The results estimated irrespective of the method used showed high variability (CV %) for myoglobin and cTnI (26.6% and 77.7%, respectively). Variability of the results estimated within the group was lower: 0.5%-31.4% for myoglobin and 3.6%- 20.7% for cTnI. The variability of myoglobin results within the group of other methods (25.9%) was quite expected because of the small number of participants and group heterogeneity. The variability of cTnI results within the group of RPIA methods (6.1%) was lower as the result of group homogeneity. Accordingly, harmonization of cTnI results is necessary due to the high variability of results estimated regardless of the method. That is why cTnI methods with antibodies on epitopes on the stable part of the molecule are recommended. Although the results for myoglobin showed a higher degree of harmonization, the variability within the methods imposes the need of harmonization according to the certified reference material.

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P22-4

Prijeanalitičke pogrješke u laboratorijskoj medicini: vrsta i učestalost

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Kvaliteta analitičkog materijala kritični je čimbenik za kvalitetu rezultata laboratorijskih pretraga. Kako bi se liječnicima osigurali kvalitetni nalazi treba poštivati pravila dobre laboratorijske prakse i uvesti strategiju za prevenciju pogrješke odgovornim usvajanjem standardiziranog prijeanalitičkog postupka. Nepoštivanje prijeanalitičkih standarda uzrok je za oko 93% pogrješaka u dijagnostičkom laboratorijskom testiranju. Cilj rada bila je obrada ukupnih podataka o prijeanalitičkim pogrješkama na uzorcima bolesnika u svrhu dobivanja uvida u njihovu učestalost i broj te uspostava strategije za njihovo smanjenje. Istraživanje je obuhvatilo 237 600 uzoraka bolesnika od kojih je 3307 obilježeno prijeanalitičkim pogrješkama,

P22-4

Preanalytical errors in laboratory medicine: type and frequency

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The quality of analytical material is a factor critical for the quality of test results. To provide physicians with quality laboratory results, it is necessary to comply with the rules of good laboratory practice and to introduce strategy for error prevention by responsible adoption of standardized preanalytical procedure. Failure to comply with this standard is the cause of approximately 93% of errors in diagnostic laboratory testing. The aim of the study was to process all data on preanalytical errors in patient samples in order to get an insight in their frequency and number, and to establish a strategy for their reduction. The study included 237 600 patient samples; 3307 of them involved preanalytical errors, with 0.06%-2.4% relative frequency

što u relativnim frekvencijama za bolničke i izvanbolničke bolesnike iznosi od 0,06% do 2,4%. Na temelju klasifikacije prema specifičnim vrstama prijeanalitičkih pogrješaka najveća pojavnost pogrješaka zabilježena je kod hemoliziranih uzoraka (48%) te zgrušanih uzoraka (15%), dok ostale vrste pogrješaka bilježe pojavnost od 1% do 7%. Dokazano je kako je najčešći uzrok nepouzdanih laboratorijskih rezultata u neposrednoj svezi s nepoštovanjem prijeanalitičkog standardiziranog postupka od strane kliničkog osoblja. Zbog toga je potrebno provoditi sustavni program koji je usmjeren na uvažavanje prijeanalitičkog standarda za kvalitetu uzorka kroz izobrazbu, nadzor nad uzorcima i izvještavanje, te afirmaciju medicinsko-laboratorijske struke.

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for inpatients and outpatients. Classification of preanalytical errors according to specific error type revealed the highest error frequency in hemolyzed (48%) and clotted samples (15%), while the frequency of other error types was 1%-7%. The most common cause of unreliable laboratory results was demonstrated to be directly related to noncompliance with preanalytical standardized procedure by non-laboratory staff. Therefore, it is necessary to implement a systematic program focused on compliance with preanalytical standard of sample quality, which involves education, sample surveillance and reporting, and assertion of medical laboratory profession.

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P22-5

Određivanje koncentracije NT-pro BNP na analizatoru Dimension RXL MAX

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Određivanje N-terminalnog pronatriuretskog peptida mozga (NT-pro BNP) primjenjuje se u dijagnostici sumnjivih stanja koja mogu biti kongestivno zatajivanje srca, te u određivanju rizika u bolesnika s akutnim koronarnim sindromom i kongestivnim zatajenjem srca. Cilj rada bio je usporediti metode određivanja NT-pro BNP za Dimension RXL Max NT-pro BNP i Elescys 1010 NT-pro BNP prije primjene metoda u laboratoriju. U ovom radu procijenili smo dvije metode za mjerenje NT-pro BNP i izvršili korelaciju rezultata u plazmi 29 bolesnika s Odjela za kardiologiju naše Kliničke bolnice. Kontrolnu skupinu činilo je 62 zdravih ispitanika, 38 muškaraca i 24 žene, raspon dobi 20-71 godina. Mjerenje koncentracije NT-pro BNP izvršeno je pomoću enzimskog imuno testa na automatskom biokemijskom analizatoru Dimension RXL (Dade Behring) i elektrokemiluminiscentnog imuno testa na Diagnostics Elescys 1010 (Roche). Statističkom obradom između vrijednosti za Dimension RXL Max NT-pro BNP (x) i Elescys 1010 NT-pro BNP (y) dobivena je korelacija (y=0,94, x - 0,26, r=0,96, p<0,01). Kod zdravih ispitanika ženska skupina ispitanika (72,3±31,4 pg/mL) pokazala je značajno više vrijednosti nego muška skupina ispitanika (63,9±24,5 pg/mL). Kao što se i očekivalo, vrijednosti koncentracija NT-

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Determination of NT-pro BNP on a Dimension RXL MAX analyzer

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Determination of N-terminal pro-brain natriuretic peptide (NT-pro BNP) is used as an aid in the diagnosis of individuals suspected of having congestive heart failure as well as for risk stratification of patients with acute coronary syndrome and congestive heart failure. The aim was to compare Dimension RXL Max NT-pro BNT assay and Elescys 1010 NT-pro BNP assay before use in our laboratory. We evaluated two methods of NT-pro BNP measurement and correlated the results obtained in human plasma samples of 29 patients from Department of Cardiology of our Hospital. Control group included 62 healthy subjects, 38 men and 24 women, aged 20-71. NT-pro BNP was measured using one-step enzyme immunoassay on a Dade Behring Dimension RXL Max automated biochemistry analyzer and electrochemiluminescence immunoassay on a Roche Diagnostics Elescys 1010 instrument. The correlation using the least square method between Dade Behring Dimension RXL Max NT-pro BNP (x) and Elecsys NT-pro BNP (y) was very good (y=0.94 x-0.26, r=0.96). Healthy women showed significantly higher values (72.3±31.4 pd/mL) than men (63.9±24.5 pg/mL). As expected, the NT-pro BNP values were significantly higher in patients with heart failure than in normal subjects. In conclusion, the

pro BNP u bolesnika sa zatajivanjem srca bile su značajno više od vrijednosti koncentracija izmjerenih u zdravih ispitanika. Zaključuje se kako je metoda NT-pro BNP za Dade Behring pokazala odlične analitičke performanse koje su u suglasnosti s rezultatima metode NT-pro BNP dobivenim na instrumentu Elescys.

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NT-pro BNP Dade Behring assay demonstrated excellent analytical performance characteristics and agreement with NT-pro BNP results on Elescys instrument.

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P22-6

Iskustvo Zavoda za kliničku kemiju Kliničke bolnice Merkur u međunarodnoj procjeni kvalitete rada iz laboratorijske koagulacije

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Zavod za kliničku kemiju, atestiran prema standardu ISO 9001:2000 od 2003. godine, aktivni je sudionik međunarodne kontrole kvalitete u koagulaciji (IEQAS-Coagulation) pod pokroviteljstvom Svjetske zdravstvene organizacije (SZO) od 1992. godine. Cilj programa je provjera kvalitete vlastitih analitičkih sustava putem neovisnog sustava i usklađivanje prema međunarodnim standardima i preporukama uz obvezu organiziranja Nacionalne kontrole kvalitete. Provođenje programa kontrole se odvija kroz 3 ciklusa na godinu i sadrži određivanje protrombinskog vremena (PV, PV- INR) aktiviranog parcijalog tromboplastinskog vremena (APTV), APTV za praćenje heparinske terapije, fibrinogena, trombinskog vremena (TV) i antitrombina III (AT III). Rezultati 77 laboratorija iz 49 zemalja svijeta procjenjuju se kao postotak odstupanja od medijana referentne skupine koju sačinjavaju svi sudionici Nacionalne kontrole Velike Britanije koji rabe isti analitički sustav (*peer group*). Prihvatljivi su rezultati s odstupanjem <15% za sve parametre. Za određivanje PV-INR u oralnoj antikoagulantnoj terapiji prednost se daje reagensima visoke osjetljivosti. Kod testova probiranja (PV, APTV, TV) rezultati se izražavaju u omjeru, te se kao takvi uspoređuju i procjenjuju. Najčešće rabljene metode za određivanje fibrinogena su metoda po Claussu i određivanje deriviranog fibrinogena. Rezultati se procjenjuju prema medijanu pripadajuće metode. Rezultati Zavoda za kliničku kemiju u razdoblju 1992.-2006. godine zadovoljili su kriterije procjene uz udio pozitivnih rezultata od 80–100%. Rezultati potvrđuju važnost međulaboratorijske suradnje koja omogućuje razmjenu stručnih i praktičnih znanja u smislu

P22-6

Our experience in IEQAS-Coagulation: Department of Clinical Chemistry, Merkur University Hospital, Zagreb

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Department of Clinical Chemistry has been accredited on the basis of ISO 9001:2000 since 2003, and has participated in the WHO IEQAS-Coagulation for 16 years now, since 1992. The aim of the program is to provide external quality assessment for blood coagulation tests and to promote high standards of laboratory performance and practice. Additionally, the aim is to encourage participants to set up the National External Quality Assessment Schemes (NEQAS) in their countries. According to the scheme design, every participant receives control samples four times a year for INR-determination, PT for diagnosis, APTT for heparin dosage assessment, fibrinogen, APTT, TT and ATIII. There are 77 participants from 49 countries all over the world. Results for the scheme are compared to a reference group of results from UK NEQAS. Acceptable results are designated as "results within consensus" (<15%). For PT-INR most participants prefer thromboplastin reagents with low ISI. For other screening tests, PT for diagnosis, APTT and TT results must be expressed as the ratio of measurement clotting time/median of the reference interval. For fibrinogen determination, the Clauss and PT- derived fibrinogen method is most widely used. Results are evaluated according to the method-specific median. During the 1992-2006 period, our results were designated as "satisfactory performance" in 80%-100% of cases. Inter-laboratory collaboration on promoting high standards of Good Laboratory Practice is important for allowing useful exchange of professional and practical knowledge. The obligation of using high sensitivity reagents for PT and the unique form of reporting PTT

unapređivanja Dobre laboratorijske prakse, a to podrazumijeva primjenu visokoosjetljivih reagensa za određivanje PV i uniformnost izražavanja rezultata (PV-INR u oralnoj antikoagulantnoj terapiji i omjera za PV i APTV kao testova probiranja). Provođenje vanjske procjene kvalitete na nacionalnoj razini organizirano je od 1994. i provodi se neprekidno.

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P22-7

Imunofiksacija – automatizirani sustav Sebia Hydrasys

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Imunofiksacija je dvostupanjski postupak koji se sastoji od elektroforeze na agarozi i imunoprecipitacije. Proteini su razdvojeni elektroforetski na oštre vrpce u gelu. Na svaku vrpcu doda se specifični antiserum za molekulu pojedinačnih razreda imunoglobulina. Ako su specifični razredi laganog lanca imunoglobulina prisutni, nastati će netopivi kompleks s antiserumom koji se tada može obojiti i dokazati. Imunofiksaciju obilježava pojačana osjetljivost, laka interpretacija, brzi rezultati testiranja, vrhunsko razdvajanje, kao i mogućnost dokazivanja monoklonskog imunoglobulina koji nije vidljiv rutinskom elektroforezom. Pojačana osjetljivost testa jednim se dijelom postiže uporabom boje *acid violet* te jasnoćom gela, čime je olakšano tumačenje. Primarna upotreba ovoga testa je identifikacija i praćenje pojedinog monoklonskog imunoglobulina (IgG, IgM, IgA, IgD, IgE, laganih lanaca lambda i laganih lanaca kapa) koji su prisutni u multiplom mijelomu i Waldenstromovoj makroglobulinemiji. Imunofiksacijski test Sebia dizajniran je za identifikaciju gamopatija u humanom serumu ili ostalim humanim biološkim tekućinama, uključujući ukoncentriranu mokraću. Test rabi inovacijsku laboratorijsku metodu koja uvelike pojednostavljuje nanošenje antiseruma. Patentirana imunofiksacijska maska (IF Dynamic Mask) omogućuje znatno smanjenje ručnog pipetiranja antiseruma. Zamjenom višekratno upotrebljene IF maske Sebia je promijenila nanošenje antiseruma uvođenjem raspoloživog segmenta za svaki gel. Manji volumen antiseruma nanosi se na segment i tada premaže preko gela, čime je smanjena količina pipetiranog antiseruma, a istodobno je omogućeno nanošenje antiseruma na sve vrpce razdvojenih proteina. Upotrebom pojedinač-

P22-7

Immunofixation – Sebia Hydrasys automated system

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Immunofixation is a two-stage process combining agarose gel electrophoresis with immunoprecipitation. Proteins are separated electrophoretically in distinct tracks on the gel. Antisera specific to individual classes of molecules are added to each track. If specific classes of light chain immunoglobulins are present, insoluble complexes form with the antisera, which can then be stained and detected. Immunofixation is characterized by its enhanced sensitivity, ease of interpretation, quick test results, excellent resolution, and it may detect monoclonal immunoglobulins that are not visible on routine electrophoresis. The enhanced sensitivity of the assay, in part achieved by using an acid violet stain and gel clarity, facilitates interpretation. The primary use of this test is to identify and monitor certain monoclonal immunoglobulins (IgG, IgM, IgA, IgD, IgE, lambda light chain and kappa light chain) such as those present in multiple myeloma and Waldenstrom's macroglobulinemia. Sebia's immunofixation assay is designed for identification of gammopathies in human sera or other human biological fluids, including concentrated urine. An innovative labor saving method is used to greatly simplify antiserum application. The patented Dynamic Mask provides a significant reduction in manual antiserum pipetting. Replacing the reusable IF masks, Sebia has changed the method of antiserum application by introducing disposable segments for each gel. A smaller volume of antiserum is applied onto the segment and then spread across the gel, reducing the amount of antiserum pipetting and providing simultaneous application of antisera to all tracks. There is no template cleaning with the single use antiserum segments and so cross con-

nih segmenata za antiserume nije potrebno čistiti predloške te je uklonjena i mogućnost kontaminacije vrpca proteina. Obrađeni su serumi 30 bolesnika i verificirani imunofiksacijom kao: 18 monoklonskih imunoglobulina, 5 hipogamaglobulinemija, 3 poliklonske hipergamaglobulinemije i 4 normalna nalaza. Imunofiksacija je metoda izbora za dokazivanje monoklonskih proteina zbog njene brzine, specifičnosti, fleksibilnosti i lakoće u interpretaciji, naročito kada su monoklonski imunoglobulini prisutni u niskim koncentracijama u serumu i/ili u mokraći.

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tamination of tracks is eliminated. The sera of 30 patients were analyzed and verified by immunofixation as follows: 18 with monoclonal immunoglobulins, 5 hypogammaglobulinemias, 3 polyclonal hypergammaglobulinemias, and 4 normal results. Immunofixation is the method of choice for detecting monoclonal protein because it is fast, specific, flexible and easy to interpret, especially when monoclonal proteins are present in low concentration in serum and/or urine.

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P22-8

Procjena rezultata vanjske procjene kvalitete pH, plinova u krvi i ioniziranih elektrolita

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Cilj rada bila je procjena rezultata pH, plinova u krvi i ioniziranih elektrolita Zavoda za kliničku kemiju, KB Merkur u vanjskoj procjeni kvalitete koju provodi neovisni organizator Labquality – WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finska, tijekom 3 godine u svrhu utvrđivanja analitičke kvalitete. Dva puta na godinu se 3 različita uzorka pripremljena tonometrijski uvođenjem smjese CO_2, O_2 i N_2 u fiziološki puferirani matriks distribuiraju u 290 laboratorija iz 15 zemalja i analiziraju na 426 analizatora. Rezultati se procjenjuju u odnosu na dozvoljeni analitički bias koji iznosi ±0,5% za pH, ±2,0% za Na^+, ±3,0% za Ca^{++} i Cl^-, ±4,0% za pCO_2 i K^+, ±5,0% za Mg^{++}, ±6,0% za nisku i 8,0% za normalnu i visoku razinu pO_2. U Zavodu za kliničku kemiju KB Merkur analizirano je 12 uzoraka na analizatoru Stat Profile pHOx Plus (pHOx) i 9 uzoraka na analizatoru Stat Profile Critical Care Xpress tvrtke Nova Biomedical (SP CCX). Rezultati su pokazali da postoje značajne razlike u mjerenju pH i plinova u krvi između različitih tipova analizatora. SP CCX pokazuje negativan bias u mjerenju pH u odnosu na pHOx, te pozitivan bias u odnosu na Radiometrove (ABL 700-735, 800-835), Bayerove (248,348, 840-865) i ILove analizatore (1610-1640, Gem Premier 3000). Vrijednosti pCO_2 na analizatoru SP CCX sukladne su vrijednostima na Radiometrovim (ABL 700-735, 800-835) i Bayerovim analizatorima (840-865, Rapid Point 400-405), imaju pozitivan bias u odnosu na pHOx, te negativan bias

P22-8

Evaluation of external quality assessment results in acid base and ionized electrolyte analysis

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The aim of the study was to evaluate the results obtained at Department of Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia, in acid base and ionized electrolyte analysis in the external quality assessment schemes performed by an external, independent body, Labquality – WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finland, during 3 years, to determine analytical quality of the results. Two times a year, 3 different specimens prepared tonometrically by applying mixtures of CO_2, O_2 and N_2 into physiologically buffered matrix were distributed to 290 laboratories from 15 countries and analyzed on 426 instruments. Results were evaluated according to total analytical error limits of ±0.5% for pH, ±2.0% for Na^+, ±3.0% for Ca^{++} and Cl^-, ±4.0% for pCO_2 and K^+, ±5.0% for Mg^{++}, ±6.0% for low and 8.0% for normal and high level pO_2. We analyzed 12 samples on Stat Profile pHOx Plus (pHOx) and 9 samples on Stat Profile Critical Care Xpress from Nova Biomedical (SP CCX). The results showed marked bias in the measurement of pH, pCO_2 and pO_2 between the analyzers. SP CCX showed negative bias for pH *vs* pHOx, and positive bias *vs* Radiometer (ABL 700-735, 800-835), Bayer (248, 348, 840-865) and IL analyzers (1610-1640, Gem Premier 3000). The results for pCO_2 on SP CCX were comparable with Radiometer (ABL 700-735, 800-835) and Bayer analyzers (840-865, Rapid Point 400-405), showed positive bias *vs* pHOx and negative bias *vs* IL Gem Premier 3000 analyzer.

u odnosu na Gem Premier 3000. Vrijednosti pO_2 na analizatoru SP CCX sukladne su vrijednostima pO_2 na Radiometrovom ABL 500-555, imaju pozitivan bias u odnosu na Bayerove analizatore (248, 348, 840-865), ILov 1610-1640, te negativan bias u odnosu na Novin pHOx i ILov Gem Premier 3000. Naši rezultati na oba analitička sustava bili su 100% prihvatljivi za pH i Na^+, 80-90% za pO_2, pCO_2 i K^+, a manje od 80% za Ca^{++}, Mg^{++} i Cl^-, zbog male zastupljenosti analizatora SP CCX, malog broja sudionika (3 za Mg^{++}), te strogih kriterija prihvatljivosti rezultata. Rezultati ukazuju na problem kontrolnih materijala koji se različito ponašaju na različitim analitičkim sustavima. Ion selektivne elektrode vrlo su osjetljive na utjecaj matriksa, što znatno otežava usporedbu rezultata, poglavito kad se radi o malom broju sudionika.

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The results for pO_2 on SP CCX were comparable with Radiometer ABL 500-555, showed positive bias *vs* Bayer (248, 348, 840-865) and IL1610-1640 analyzers, and negative bias *vs* pHOx and IL Gem Premier 3000 analyzers. Our results from both analyzers were within the quality specifications, 100% for pH and Na^+, 80%-90% for pO_2, pCO_2 i K^+, and less than 80% for Ca^{++}, Mg^{++} and Cl^- due to the low proportion of SP CCX analyzers, small number of participants (3 for Mg^{++}) and strict criteria for the acceptability of results. The results obtained emphasize the problem of control materials, which behave differently on various blood gas analyzers of the same or different manufacturers. The ion selective electrodes are very sensitive to matrix effects, which makes the comparability of the results difficult, especially when a small number of laboratories participate in the assessment.

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P22-9

Procjena mjerne nesigurnosti pri određivanju standarda bakra spektrofotometrijskom metodom

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Kao dugogodišnji proizvođač dijagnostičkih reagensa Herbos Dijagnostika je s ciljem osiguranja standardne kvalitete proizvoda od siječnja 2004. godine uvela sustav upravljanja kvalitetom ISO 9001:2000. Naši zahtjevi za stalnim poboljšanjem kvalitete proizvoda provode se u proces proizvodnje vodenih standarada sljedljivih prema međunarodno potvrđenom referentnom materijalu (National Institute of Standards and Technology, NIST). Prvi uvedeni standard je standard bakra u sastavu našega dijagnostičkog testa TR-0171 Bakar. Cilj rada bio je kalibrirati vrijednost standarda bakra Herbos Dijagnostike prema ISO uputi za iskazivanje mjerne nesigurnosti (Guide to the Expression of Uncertainty in Measurement, GUM,1993.) preko NIST SRM (referentnog standardnog materijala). Bakar se određuje direktnom metodom sa specifičnim kromogenom 5-Br-PSAA u kiselom mediju. 5-Br-PSAA s bakrom tvori obojeni kompleks intenzitet boje kojega je proporcionalan koncentraciji bakra i mjeri se fotometrijski na 580 nm u kiveti 10 mm pri sobnoj temperaturi. Kvantifikacija standarda bakra provodi se pomoću kalibracij-

P22-9

Estimation of measurement uncertainty in the determination of copper standard by spectrophotometric method

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As a long-standing manufacturer of diagnostic reagents, Herbos Dijagnostika has introduced the ISO 9001:2001 quality management system as of January 2004 in order to ensure product quality standards. Our requirements for continuing improvements of product quality are implemented within the manufacturing process of water standards following the internationally certified reference material (National Institute of Standards and Technology, NIST). The first standard introduced was copper standard making part of our diagnostic test TR-0171 Copper. The aim of the study was to calibrate the value of the Herbos Dijagnostika copper standard on the basis of the Guide to the Expression of Uncertainty in Measurement (GUM) 1993, through the NIST SRM (standard reference material). Copper is determined using direct method with a specific chromogen 5-Br-PSAA in acid medium. Combined with copper, the 5-Br-PSAA forms a colored complex whose color intensity is proportional to copper concentration and is measured photometrically at 580 nm in a 10-mm cuvette at room temperature. The

skog pravca, a rezultati se izražavaju u μmol/L. Validacija metode provedena je kroz dvadeset dana po četiri mjerenja na dvjema razinama, te je validirana točnost testom iskorištenja. Za procjenu mjerne nesigurnosti i računanje sastavljene i proširene mjerne nesigurnosti rabljeni su podatci dobiveni validacijom metode (obnovljivost, točnost), kalibracijski pravac, priprema radnog standarda iz koncentrata. Usporedbom relativne standardne nesigurnosti je vidljivo da najveći doprinos mjerne nesigurnosti dolazi od kalibracijskog pravca i dugotrajne obnovljivosti, a najmanji od primarnog SRM. Mjerna nesigurnost za standard bakra za vrijednost 15,75 μmol/L iznosi 0,25 μmol/L s obuhvatnim faktorom k=2.

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copper standard quantification is carried out by use of calibration curve and results are expressed in μmol/L. The validation of the method is executed over 20 days, with four 2-level measurements *per* day, and the accuracy is validated based on the recovery test. For the purpose of assessing uncertainty in measurement and calculation of the combined and extended uncertainty in measurement the data obtained from the method validation (reproducibility, accuracy), calibration curve, preparation of working standards from the concentrate are used. The comparison of relative standard uncertainty makes it evident that the most significant input to measurement uncertainty derives from the calibration curve and long-term reproducibility while the least one comes from the primary SRM. Uncertainty in the measurement of copper standard for the value of 15.75 μmol/L is 0.25 μmol/L with the coverage factor k=2.

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P22-10

Iskustva Zavoda za kliničku kemiju Kliničke bolnice Merkur u programu međunarodne procjene kvalitete rada u kvalitativnoj analizi mokraće

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Od 1997. godine Zavod za kliničku kemiju KB Merkur sudjeluje u međunarodnom programu EQA Labquality, utemeljenom 1971. godine. U programu sudjeluje 3743 laboratorija iz 41 zemlje. Cilj je procjena kvalitete putem neovisnog sustava kvalitete potvrđenog standardom ISO 9001:2000 kao sustav provjere kvalitete vlastitih rezultata kako u kemijskoj analizi mokraće test trakom, tako i u prepoznavanju elemenata sedimenta mokraće te njihovo usklađivanje s Europskim preporukama kvalitete. Program se izvodi 3 puta na godinu za: 1) kemijsku analizu mokraće test trakom za glukozu, ketone, eritrocite, pH, proteine, nitrite, leukocite, relativnu volumnu masu i brojanje eritrocita (Erc) i leukocita (Lkc) i 2) prepoznavanje elemenata sedimenta mokraće pomoću 4 slike u boji priređene prema preporukama Europske grupe za analizu mokraće uz prikaz slučaja. Rezultati za test traku se obrađuju prema vrsti trake i načinu očitavanja, a pokazuju da se očitavanje izvodi isključivo instrumentalno uz uporabu 5 -7 vrsta traka. Iako je osjetljivost traka različita, ukupni rezultati

P22-10

Results of international EQA program for qualitative urinalysis at Department of Clinical Chemistry, Merkur University Hospital

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Since 1997, Department of Clinical Chemistry, Merkur University Hospital has participated in the International Labquality EQA Programme established in 1971 and encompassing 3743 participating laboratories from 41 countries. The aim is quality assessment by an independent quality system certified according to ISO 9001:2000 standard as a system of quality assessment of each laboratory's own results, both in chemical urinalysis using a test strip and in the identification of cell elements, and their harmonization with the European quality recommendations. The program is performed 3 times a year including the following: 1) chemical urinalysis using a test strip for glucose, ketones, erythrocytes (Erc), pH, proteins, nitrites, leukocytes (Lkc) relative volume mass, and Erc and Lkc counting; and 2) identification of urine sediment elements using 4 color pictures prepared according to the European Urinalysis Group with a case report. Results are analyzed for the type of test strip and way of reading. Five to seven types of strips are in use, and measuring is

pokazuju manji rasap vrijednosti za ista koncentracijska područja zbog primjene standardiziranokg postupka. Brojanje Erc i Lkc iskazuje se prema metodi brojanja stanica kao srednja vrijednost i razlika od ciljne vrijednosti izražene kao srednja vrijednost ± 50%. Dobiveni rasap vrijednosti unutar metoda, iako su matode standardizirane, pokazuje još uvijek prisutnu mogućnost utjecaja prije- i poslijeanalitičkih grješaka. Prepoznavanje elemenata sedimenta mokraće se izvodi isključivo tzv. shemom "e-mail" putem interneta. Mogućnost manipulacije slikom olakšava prepoznavanje elemenata. Rezultati su dostupni na web stranicama Labquality. Sudjelovanjem u međunarodnom programu EQA uspostavili smo stalnu provjeru vlastitih analitičikh sustava u kemijskoj analizi mokraće test trakom i u kvantificiranju i identifikaciji staničnih elemenata; time dobiveni rezultati postaju pouzdani nalaz koji zadovoljava europske ciljeve analitičke kvalitete.

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mainly instrumental. In spite of varying sensitivity of test strips, overall results show lower dispersion of values due to the use of standardized methods. Erc and Lkc count is expressed according to the method of cell counting as mean and difference from the target value expressed as mean ±50%. Despite standardization of the methods, dispersion of values still implies the possibility of pre- and postanalytical errors. Identification of urinary sediment elements is performed exclusively by use of the "e-mail" scheme *via* internet. The possibility of picture manipulation facilitates element analysis. Results are available on the Labquality web pages. By participating in the International EQA Program, we have established permanent control of our own analytical systems in chemical urinalysis with test strips and in quantification and identification of cell elements. In this manner, we have ensured the results obtained to be a relevant finding that meets the European standards in analytical quality.

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P22-11

Iskustva vanjske procjene kvalitete rada UKNEQAS u području imunofenotipizacije stanica

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Točnost određivanja apsolutnog broja i udjela subpopulacija limfocita i CD34 hematopoetskih matičnih stanica je važna za kliničku obradu imunokompromitiranih osoba i bolesnika sa zloćudnom bolesti koji se podvrgavaju transplantaciji perifernim matičnim stanicama. Sudjelovanje u vanjskoj procjeni kvalitete rada je stoga neophodno da bi se laboratorijski rezultati mogli usporediti i vrednovati radi povećanja vjerodostojnosti i njihove usporedivosti širom svijeta. Cilj je prikazati rezultate sudjelovanja u međunarodnoj kontroli UKNEQAS iz područja imunofenotipizacije dobivene upotrebom standardiziranih i danas preferiranih postupaka za kvantifikaciju stanica protočnom citometrijom.
Od kraja 2005. godine Zavod za kliničku kemiju KB Merkur atestiran prema međunarodnom standardu ISO: 9001:2000 sudjeluje u vanjskoj procjeni kvalitete rada UKNEQAS for Leukocyte Immunophenotyping u dva programa: Immune Monitoring (imunološki status) i CD34 Stem Cell (CD34+ stanice). Kao uzorak se rabi stabilizirana

P22-11

The experience in UKNEQAS External Quality Assessment Scheme for Immunophenotyping

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Accurate determination of absolute and percentage values for lymphocyte subsets and CD34 hematopoietic stem cells is important for clinical management of immunocompromised individuals and cancer patients undergoing peripheral blood stem transplantation. It is essential, therefore, that laboratory data dan be compared and evaluated through participation in an external quality assurance program to improve the credibility of results and their comparability worldwide. The objective of this presentation is to point to the results achieved in the UKNEQAS external quality control for immunophenotyping using the state-of-the-art flow cytometry methodologies for cell quantification.
Since the end of 2005, the Institute of Clinical Chemistry of the Merkur University Hospital certified according to ISO: 9001:2000 has participated in the UKNEQAS external quality assessment for leukocyte immunophenotyping in two programs: immune monitoring and CD34 stem cell. Stabilized whole blood was used as a specimen in

puna krv. Uzorci za čitanje na protočnom citometru EPICS XL obrađeni su amonij klorid (NH4Cl) lizatorom, postupkom "liziraj-ne ispiri". Za određivanje parametara imunološkog statusa rabili smo preporučenu analizu CD45/postranično raspršenje (SSC), a za mjerenje broja CD34+ stanica protokol ISHAGE. Apsolutni broj pozitivnih stanica je određen na protočnom citometru metodom s česticama definiranog broja (*Flow Count beads*). Svi rezultati relativnog i apsolutnog broja pozitivnih stanica imunološkog statusa bili su unutar dozvoljenih granica s tendencijom grupiranja u području ciljne vrijednosti (χ±1SD kod imunološkog statusa i 25.-75. centila za broj CD34+ stanica). Predobrada uzoraka za čitanje na protočnom citometru s lizatorom NH4Cl postupkom "liziraj-ne ispiri" je dominantan pripremni postupak i rabi ga više od trećine sudionika. Nadalje, upotrijebljena CD45/SSC analiza na protočnom citometru je najzastupljenija vrst analize (>75%) kod imunološkog statusa, dok je protokol ISHAGE najzastupljeniji (>90%) u programu CD34+ stanica. Metoda *Flow Count beads* za određivanje apsolutnog broja pozitivnih stanica na protočnom citometru druga je po zastupljenosti (>30%). Upotrebom preporučenih postupaka i protokola u imunofenotipizaciji stanica, koji uključuju pripremu uzoraka za mjerenje i analizu na protočnom citometru, moguće je osigurati visok stupanj međulaboratorijske usklađenosti uz potpunu pouzdanost dobivenih rezultata.

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all send-outs. For sample processing on an EPICS XL flow cytometer, the lyse/no wash preparation technique with ammonium chloride (NH4Cl) lysing reagent was employed. In the immune monitoring program, the CD45/sideward light scatter (SSC) proposed gating strategy was adopted for lymphocyte subsets, while ISHAGE protocol was used for CD34+ cell enumeration. Absolute count determination was performed on a flow cytometer using the Flow Count beads solution with the known number of particles. The relative and absolute enumeration results from both programs were within the tolerable limits, and tended to group within the region around the target value (value within χ±1SD, and 25^{th}-75^{th} centile in CD34+ in immune monitoring and stem cell program, respectively). Sample treatment in the lyse/no wash technique using NH4Cl lysing solution for acquisition on flow cytometer was a dominant procedure used by more than one-third of participants. Moreover, in immune monitoring and CD34+ cell determination the most frequent analyses were CD45/SSC gating strategy (>75%) and ISHAGE protocol (>90%), respectively, whereas the Flow Count beads method for absolute count enumeration on flow cytometer was the second of the bead-based techniques (>30%). The use of the recommended procedures and protocols for cell quantification, which include sample preparation and flow cytometric analysis, can ensure a high degree of interlaboratory harmonization accompanied by absolute reliability of the results obtained.

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P22-12

Vanjska procjena kvalitete u laboratorijskoj hematologiji (IEQAS-H) – dvadeset godina iskustva Zavoda za kliničku kemiju Kliničke bolnice Merkur

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Zavod za kliničku kemiju Kliničke bolnice Merkur od 1985. godine sudjeluje u međunarodnoj sponzoriranoj kontroli iz područja laboratorijske hematologije Shema za internacionalnu vanjsku procjenu kvalitete iz hematologije (International External Quality Assessment Scheme for Haematology, IEQAS-H) pod pokroviteljstvom Svjetske zdravstvene organizacije. Godine 1987. Zavod je dobio

P22-12

International External Quality Assessment Scheme for Hematology – 20-year experience of Department of Clinical Chemistry, Merkur University Hospital

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Department of Clinical Chemistry, Merkur University Hospital, has been involved in the International External Quality Assessment Scheme for Haematology (IEQAS - H) organized by the World Health Organization (WHO) since 1985. In 1987, the Department received a certificate with the obligation of active participation in external quality control at regional level, which was fulfilled. The aim

certifikat s obvezom aktivnog sudjelovanja u provođenju vanjske procjene kvalitete na regionalnoj, razini što je učinjeno. Cilj vanjske procjene kvalitete rada je usklađivanje rezultata u laboratorijskoj hematologiji neprekidnom izobrazbom zasnovanom na povratnim informacijama o rezultatima svakog pojedinog ciklusa kontrole. Kroz 6 kontrola na godinu prođe ukupno 12 uzoraka za hemoglobin, leukocite i trombocite na hematološkom brojaču (specijalni pripravci); 8 uzoraka za leukogram, 8 uzoraka za parazite (pripravci obojeni po Pappenheimu); i 8 uzoraka za retikulocite (pripravci obojeni briljant krezil modrilom). Rezultati za hemoglobin, leukocite, trombocite i retikulocite vrednuju se prema indeksu odstupanja DI (DI >3,0 neprihvatljiv). Rezultati za diferencijalnu krvnu sliku – leukogram uspoređuju se s deklariranim intervalima za pojedinu vrstu leukocita, a nađene morfološke promjene na krvnim stanicama opisno, do 5 najbitnijih morfoloških obilježja u razmazu.
Za retikulocite dozvoljeno odstupanje je ± 50%. Za točan nalaz krvnih parazita potrebno je definirati vrstu i podvrstu parazita. Prihvatljivost rezultata tijekom 20-godišnje kontrole: za hemoglobin 95,2% , za leukocite 80,5%, za trombocite 95,2%, za retikulocite 80,5%, za leukogram 97,0% i za vrstu parazita 99,0%. Različiti analitički sustavi u laboratorijskoj hematologiji postavljaju apsolutni zahtjev za usklađivanje rezultata. To se može postići kroz nekoliko stupnjeva: svakodnevnim provođenjem unutarnje kontrole kvalitete rada i statističkom obradom rezultata nepreciznosti i netočnosti; sudjelovanjem u nacionalnoj vanjskoj procjeni kvalitete rada i usklađivanjem rezultata među laboratorijima. U takovom ustroju treba predvidjeti i sudjelovanje u međunarodnim procjenama. Kontrola IEQAS pod pokroviteljstvom Svjetske zdravstvene organizacije je svakako jedna od najvažnijih.

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of external quality assessment is harmonization of the results in laboratory hematology by continuous education based on feedback on the results from each control step. Through 6 controls *per* year, 12 samples undergo EQA for hemoglobin, leukocytes and platelets on a blood counter (special preparations); 8 samples for leukogram, 8 samples for parasites (preparations stained according to Pappenheim); and 8 samples for reticulocytes (preparations stained with brilliant cresyl blue). The results on hemoglobin, leukocytes, platelets and reticulocytes are evaluated according to the deviation index DI (DI >3.0 is unacceptable). The results on differential blood count – leukogram are compared with declared intervals for each type of leukocytes, and morphological changes observed in blood cells are descriptively presented, including up to 5 most important morphological features *per* smear. For reticulocytes the allowed deviation is ± 50%. For accurate identification of blood parasites, it is required to define the parasite type and subtype. Acceptable results during 20 years of control: for hemoglobin 95.2%, for leukocytes 80.5%, for platelets 95.2%, for reticulocytes 80.5%, for leukogram 97.0%, and for parasites 99.0%. Different hematologic analytical systems require a system for harmonization of the results. This can be accomplished through several steps: daily conduction of internal quality control and statistical analysis of the results on imprecision and inaccuracy; and participation in national external quality control and assessment of the results between laboratories. The structure should also include participation in international controls. IEQAS control under WHO supervision is certainly one of the most important.

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P23 – Ostalo, P23-1

Oksidacijski stres u miševa: učinak kukuruznog ulja i željeza

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P23 – Other, P23-1

Oxidative stress in mice: effect of dietary corn oil and iron

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Antioksidacijski status predstavlja ravnotežu između nusproizvoda oksidacijskog stresa i zaštitnog antioksidacijskog sustava. Oksidacijski stres najčešće se mjeri indirektno preko enzimskih i neenzimskih antioksidacijskih pokazatelja. Unos velikih količina željeza (Fe) rezultira ok-

Antioxidant status represents the balance between by-products of oxidative stress and antioxidant defense system. Oxidative stress is most commonly measured indirectly *via* enzymatic and nonenzymatic antioxidant markers. High Fe ingestion may result in oxidative stress

sidacijskim stresom i štetnim reakcijama kao što su peroksidacija višestruko nezasićenih masnih kiselina i smanjena aktivnost antioksidacijskih enzima. Mehanizam kojim je Fe uključen u započinjanje ili poticanje oksidacijskog oštećenja nije sasvim jasan. Cilj ovoga istraživanja bio je ispitivanje učinka velikih količina Fe na antioksidacijski status miševa čija je hrana obogaćena kukuruznim uljem. Mužjaci miševa Balb/c hranjeni su 3 tjedna standardnom prehranom obogaćenom s 5% kukuruznog ulja i s optimalnom količinom Fe (skupina FCO), odnosno obogaćenom s 1% karbonilnog Fe (skupina FCOFe). Kontrolna skupina je hranjena standardnom prehranom. Količina Fe, bakra (Cu) i cinka (Zn) u tkivu jetre određeni su induktivno spregnutom plazma spektrometrijom. Aktivnosti Cu/Zn superoksid dismutaze (CuZnSOD) i glutation peroksidaze (GPx) određene su spektrofotometrijski u nadsloju homogenata jetre. Lipidna peroksidacija određena je mjerenjem količine reaktivnih spojeva s tiobarbiturnom kiselinom (TBARS). Prehrana s velikim udjelom Fe povećala je količinu Fe u jetri 2 puta. Međutim, porast količine Fe u timusu izazvan je isključivo masnoćama u hrani. Sadržaj Cu u jetri blago se smanjio u skupini FCO. U slezeni je porast količine Fe izazvan prehranom obogaćenom Fe negativno korelirao s količinom Cu. Na antiokidacijski status utjecali su oboje, i prehrambena masnoća i Fe. Miševi čija je hrana bila obogaćena kukuruznim uljem imali su povećanu količinu TBARS, s većim porastom u skupini FCOFe. Aktivnost CuZnSOD u jetri smanjena je u skupini FCO, dok je dodatak Fe u prehrani uzrokovao daljnje smanjenje aktivnosti enzima. Rezultati ukazuju na to da prehrana obogaćena kukuruznim uljem uzrokuje oksidacijsko oštećenje i smanjenje antioksidacijske enzimske zaštite. Dodatak velikih količina Fe dodatno je utjecao na antioksidacijski sustav povećavajući osjetljivost jetrenog tkiva na lipidnu peroksidaciju. Uz to, prehrana obogaćena kukuruznim uljem i Fe povećala je u miševa potrebe za Cu.

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and deleterious reactions such as peroxidation of polyunsaturated fatty acids and decreased antioxidant enzyme activities. The mechanism by which Fe is involved in initiating or promoting oxidative damage is not entirely clear. The aim of the study was to analyze the effect of high dietary iron (Fe) on liver antioxidant status in mice fed corn oil-enriched diet. Male Balb/c mice were fed for 3 weeks standard diets enriched with 5% by weight of corn oil, with adequate Fe (FCO group), or supplemented with 1% carbonyl Fe (FCOFe group). Control group was fed standard diet. Fe, copper (Cu), and zinc (Zn) levels in liver tissue were determined by inductively coupled plasma spectrometry. Cu/Zn superoxide dismutase (CuZnSOD) and glutathione peroxidase (GPx) activities were determined spectrophotometrically in liver homogenate supernatant. Lipid peroxidation was evaluated by measuring thiobarbituric acid-reactive species (TBARS). High-Fe diet induced 2-fold increase of hepatic Fe level. However, the increase of thymic Fe level was induced solely by dietary fat. Hepatic Cu level slightly decreased on FCO diet. In spleen, the high-Fe diet induced increase of Fe level correlated negatively with Cu level. The antioxidant status was influenced by both dietary fat and Fe. Mice fed corn oil-enriched diets had a higher concentration of TBARS, with a greater increase on FCOFe diet. Hepatic CuZnSOD activity was decreased in FCO diet, and Fe supplementation caused further decrease in the enzyme activity. These results suggest that feeding with corn oil-enriched diets increases oxidative damage by decreasing antioxidant enzyme defense. The high-Fe diet additionally affects the antioxidant defense system, further increasing the tissue susceptibility to lipid peroxidation. Additionally, both corn oil- and Fe-enriched diets increased Cu requirements in mice.

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P23-2

Povezanost koncentracije serumskog kolesterola i suicidnog ponašanja

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Ranije studije pokazale su povezanost niske koncentracije ukupnog kolesterola i suicidnog ponašanja. Cilj ispitivanja bio je ustanoviti postoje li razlike u koncentraciji serumskog kolesterola u bolesnika s određenim psihijatrij-

P23-2

Relationship between serum cholesterol and suicidal behavior

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Previous studies have pointed to a relationship between low level of total serum cholesterol and suicidal behavior. The aim of our study was to investigate the possible differences in cholesterol level between psychiatric patients

skim dijagnozama u odnosu na bolesnike koji su pokušali suicid. Uzorak se sastojao od 677 bolesnika raspoređenih u skupine s dijagnozama prema MKB-10 (F10 sindrom ovisnosti-alkoholizam, F20 shozifrenija, F23 psihotični poremećaji, F25 shizoafektivni potemećaji, F32 depresivni poremećaj, F43 reakcija na stres, F60 poremećaji ličnosti, X61 nenasilni pokušaj suicida, X70 nasilni pokušaj suicida), kojima je određena koncentracija ukupnog kolesterola standardnom metodom kod prijma na liječenje u Psihijatrijsku bolnicu Sveti Ivan u razdoblju od 1. siječnja do 1. travnja 2005. Razlike u koncentraciji kolesterola prema dijagnozama testirane su analizom varijance, budući da je test homogenosti varijance pokazao da se varijance statistički značajno ne razlikuju (Bartlett χ^2=11,12, st.sl=8, p=0,19). Analiza varijance pokazala je kako postoji statistički značajna razlika između skupina bolesnika s različitim dijagnozama (F=2,47, st.sl.=8, p=0,012). Višestrukim post hoc testom (LSD) pokazalo se da se skupina s dijagnozom X61 koja ima najmanju vrijednost kolesterola statistički značajno ne razlikuje od skupine s dijagnozama F10, F23 i X70, dok se statistički značajno razlikuje od skupina F20, F25, F32, F43 i F60. Dobiveni rezultati potvrdili su hipotezu o povezanosti niske koncentracije ukupnog kolesterola i suicidnog ponašanja. Određivanje koncentracije ukupnog kolesterola u serumu bolesnika s dijagnozama F10 i F23 može poslužiti kao koristan biološki biljeg pri procjeni rizika suicidnog ponašanja psihijatrijskih bolesnika.

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with particular diagnoses and patients with a history of suicide attempts. Total cholesterol level was determined in serum samples of 677 patients admitted to Sveti Ivan Psychiatric Hospital from January 1 till April 1, 2005, distributed into groups according to ICD10 diagnoses. The ICD-10 diagnoses were: F10, syndrome of alcohol dependence; F20, schizophrenia; F23, psychotic disorder; F25, schizoaffective disorder; F32, depressive disorder; F43, stress reaction; F60, personality disorder; X61, nonviolent suicide attempt; and X70, violent suicide attempt. Analysis of variance was used to analyze differences in cholesterol levels between patient groups because the test of homogeneous variance yielded no statistically significant difference. A significant difference was found between the groups with different diagnoses (F=2.47, p=0.012). Multiple post hoc test (LSD) showed the group of patients with X61 diagnosis, which had the lowest cholesterol level, not to differ from the groups of patients with the F10, F23 and X70 diagnoses, but to differ statistically significantly from the patient group with the F20, F25, F32, F43 and F60 diagnoses. In conclusion, our results approve the hypothesis on the relationship between low levels of total cholesterol in serum and suicidal behavior. It is also suggested that determination of total serum cholesterol in patients with the F10 and F23 diagnoses could provide a valuable biological risk marker on assessing suicidal behavior in these patients.

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ZR1 – Međulaboratorijske usporedbe, ZR1-1

Utjecaj harmonizacije općih medicinsko-biokemijskih pretraga na rezultate vanjske procjene kvalitete medicinsko-biokemijskih laboratorija

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Uloga vanjske procjene kvalitete rada medicinsko-biokemijskih laboratorija (MBL) je osigurati neovisnu i objektivnu procjenu dobivenih rezultata kako bi se utvrdio stupanj razvoja struke i potaknuo postupak standardizacije svih procesa laboratorijskog rada s ciljem povećanja stupnja međulaboratorijske usporedivosti i harmonizacije u području laboratorijske dijagnostike. Analizom rezultata vanjske procjene kvalitete HDMB provedene u 3 ciklusa tijekom 2005. g. uočeno je da su se prosječni koeficijenti varijacije (KV%) za pojedine pretrage unutar svih laboratorija sudionika značajno smanjili: za urate sa 6% na 3% neprihvaćanjem metode urikaza-PAP bez askorbat oksidaze, a obveznom primjenom metoda IFCC za enzime za LDH s 22% na 5%, za CK sa 7% na 3%, za ALP sa 14% na 8%, za CHS s 31% na 8%, za aminotransferaze sa 7% na 4%. Rezultati međulaboratorijskih usporedba laboratorijskih pretraga za 174 laboratorija sudionika još uvijek pokazuju značajna odstupanja rezultata pojedinih MBL od srednje vrijednosti grupe, što ima za posljedicu veliku raspodjelu dobivenih rezultata. To potvrđuje da na konačan rezultat laboratorijske pretrage ne utječu samo razlike u primijenjenim analitičkim metodama, nego i različiti kalibratori i kontrolni materijali. Zato u cilju globalne harmonizacije Europska direktiva 98/79 za "*in vitro* laboratorijsku dijagnostiku" zahtijeva mjeriteljsku sljedljivost kalibratora i kontrolnih materijala uz iskazanu mjernu nesigurnost. Godišnje izvješće o rezultatima vanjske procjene kvalitete HDMB za 3 provedena ciklusa tijekom 2005. g. pokazalo je da prosječno 30% MBL zadovoljava postavljene analitičke ciljeve kvalitete sa 100% prihvatljivih rezultata, više od 60% MBL s više od 80% prihvatljivih rezultata, a manje od 10% MBL nije zadovoljilo postavljene analitičke ciljeve kvalitete. Rezultati vanjske procjene kvalitete rada MBL pokazali su pozitivan utjecaj primjene preporučenih analitičkih metoda na harmonizaciju laboratorijskih rezultata. U daljnjem radu uvođenje međunarodnog standarda ISO 15189:2003 za medicinske laboratorije omogućiti će harmonizaciju svih faza laboratorijskog procesa i time

ZR1 – Interlaboratory comparisons, ZR1-1

Impact of harmonization of general medical biochemistry analyses on external quality assessment results

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One of the main purposes of external quality assessment in medical biochemisty laboratories is to provide an independent and objective evaluation of laboratory test results in order to promote standardization of the overall laboratory process and achieve a high degree of inter-laboratory comparability and harmonization of test results in the field of medical biochemistry. Long-term evaluation of the results obtained in three surveys during 2005 showed a significant improvement of analytical quality in medical biochemistry laboratories. The coefficients of variation (CV%) for some analyses showed a decreasing tendency: for urate CV decreased from 6% to 3% with exclusion of the uricase-PAP method without ascorbate oxidase; for enzymes the obligatory use of IFCC methods decreased the CV for LDH, CK, ALP, CHS and aminotransferase from 22% to 5%, from 7% to 3%, from 14% to 8%, from 31% to 8% and from 7% to 4%, respectively. The inter-laboratory comparisons of laboratory test results for 174 participant laboratories showed significant result deviation from the mean of the group for some laboratories, resulting in wide distribution of the results obtained. These results indicate that laboratory test results are influenced not only by the analytical method used but also by different calibrators and control materials. Therefore, the European Directive 98/79 on *in vitro* medical devices (IVD) requires metrological traceability for standards and control materials with stated uncertainties. The annual report for the three surveys in 2005 showed that almost 30% of medical biochemistry laboratories achieved analytical goals with 100% of acceptable results, more than 60% laboratories fulfilled the requirements with more than 80% of acceptable results, and less than 10% of the laboratories were not able to meet the required analytical quality specification for diagnostic testing. External quality assessment in medical biochemistry laboratories in Croatia showed a positive effect of the recommended methods on the harmonization of laboratory results. In the future, the availability of an international standard,

postizanje najviših ciljeva kvalitete u laboratorijskoj dijagnostici.

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ISO 15189:2003, specifically developed and released for medical laboratories, provides a unique opportunity to harmonize laboratory activity and to meet the requirements to achieve highest quality goals in all steps of the laboratory work.

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ZR1-2

Evaluacija rezultata u laboratorijskoj hematologiji

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Zavod za kliničku kemiju KB Merkur od 1985. g. sudjeluje u međunarodnoj sponzoriranoj kontroli iz laboratorijske hematologije – International External Quality Assessment Scheme for Haematology IEQAS-(H) pod pokroviteljstvom Svjetske zdravstvene organizacije (SZO), s obvezom aktivnog sudjelovanja u provođenju vanjske procjene kvalitete u Hrvatskoj. U okviru Povjerenstva za vanjsku procjenu kvalitete Hrvatskoga društva medicinskih biokemičara, od 1988. g. provodi Vanjsku kontrolu kvalitete iz laboratorijske hematologije. Cilj rada je pokazati rezultate nacionalne kontrole kvalitete iz područja laboratorijske hematologije tijekom 2005. godine s obzirom na postavljene ciljeve analitičke kvalitete. Na godinu se šalju 3 komercijalna pripravka krvi za određivanje hematoloških pretraga na hematološkim brojačima s trodijelnom krvnom slikom. Uzorak za retikulocite je razmaz napravljen iz venske krvi obojene briljantkrezil modrilom kao preporučenom metodom. Ciljevi analitičke kvalitete za hematološke brojače definirani su međunarodno prihvaćenim standardima koji obuhvaćaju sve parametre kompletne krvne slike. Po uzoru na međunarodnu procjenu IEQAS-(H) pod pokroviteljstvom SZO procjenjuju se hemoglobin (Hb), leukociti (Lkc), trombociti (Tr) i retikulociti (Rtc). Kriteriji za prihvaćanje rezultata su deklarirane vrijednosti kontrolnog uzorka unutar ±2SD prema primijenjenom hematološkom analizatoru i/ili ciljne vrijednosti prema veličini biološke varijacije izražene kao ukupna dozvoljena analitička pogreška. U 2005. g. u 3 ciklusa sudjelovalo je 177 laboratorija s 37 tipova brojača od 11 proizvođača. Prosječno je neprihvatljivih rezultata bilo: za Hb 2,6%, za Lkc 1,7%, za Tr 6,2% i za Rtc 22,7%. Zaključuje se kako različiti hematološki brojači zahtijevaju usklađivanje rezultata. Ne postoji ko-

ZR1-2

Evaluation of results in laboratory hematology

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Department of Clinical Chemistry, Merkur University Hospital, has been involved in the International External Quality Assessment Scheme for Haematology (IEQAS - H) organized by the World Health Organization (WHO) since 1985. In 1987, it received a certificate of participation in this control scheme. Department of Clinical Chemistry has been cooperating in the external quality assessment program in laboratory hematology, which has been continuously performed in Croatia since 1988 by the Committee for External Quality Assessment Schemes under the auspices of the Croatian Society of Medical Biochemists. The objective is to show the results of the national quality control in laboratory hematology during 2005, considering the set aims of analytical quality. Commercial blood preparations are sent 3 times a year for determination of hematology parameters on counters. The sample for reticulocytes is a blood smear stained with brilliant-cresyl blue as the recommended method. The aims of analytical quality for blood counters are defined by the internationally accepted standards for all parameters of complete blood count. Modelled by the international IEQAS-H evaluation under the sponsorship of WHO, the examined parameters are: hemoglobin (Hb), leucocytes (Lkc), platelets (Plt) and reticulocytes (Rtc). The criteria for accepting the results are declared values of the control sample ±2SD according to the blood counter used and/or target values according to the scale of biological variation expressed as total allowed analytical error. A total of 177 laboratories participated in 3 controls conducted in 2005. The parameters of complete blood count are determined on 37 types of counters from 11 different manufacturers. Average unacceptable results: Hb 2.6%, Lkc 1.7%, Plt 6.2% and Rtc 22.7%. It is concluded that different hematology

mercijalni kontrolni uzorak idealno primjenjiv na svim hematološkim brojačima. Stoga dozvoljene granice odstupanja, koje ostvaruje većina laboratorija, ne smiju biti cilj postizanja kvalitete u redovnom radu. Na brojanje Rtc utiče niz čimbenika, ali je dozvoljeno odstupanje od ±50% neprihvatljivo u svakodnevnom radu. Stoga je nužno provođenje unutarnje kontrole kvalitete rada, te ispravci na analitičkom sustavu. U vanjskoj procjeni treba težiti ka što boljim rezultatima, a ispitati uzroke loših rezultata. Možda se mogu postaviti zahtjevi za bolju opremu ili dodatnu izobrazbu.

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analytical systems require a system of result harmonization. There is no single commercial control sample applicable on all blood counters.The allowed limits of deviation in external evaluation, accomplished by the majority of laboratories, are too wide to be acceptable for achieving a high quality daily performance (for Rtc ±50%). Therefore, an internal quality control is required, and it assumes statistical processing of the imprecision and inaccuracy. The best possible results should be the aim during participation in the national external quality assessment scheme.

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ZR1-3

Procjena rezultata u laboratorijskoj koagulaciji

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Program harmonizacije laboratorijskih nalaza koji omogućuje racionalnu primjenu i pravilnu procjenu rezultata citira referentni interval za aktivirano parcijalno tromboplastinsko vrijeme (APTV) samo kao omjer. To je jedini usporedivi način izražavanja rezultata. Rezultati za APTV kroz 2005. godinu upućuju na djelomično pridržavanje preporuka, te se nameće hitna potreba za ujednačenim načinom izražavanja rezultata APTV. Trajno poticanje unapređenja visoke razine kvalitete laboratorijske dijagnostike kroz primjenu standardiziranih analitičkih sustava i standardiziranih visokoosjetljivih reagensa za protrombinsko vrijeme (PV) uz uvođenje standardiziranog načina izražavanja rezultata (uvođenje PV-INR, izražavanje rezultata za PV i APTV kao omjer) osigurati će dobru osnovu za harmonizaciju laboratorijskih nalaza. Kriterij provjere analitičke kvalitete pojedinog laboratorija je procjena rezultata koagulacijskih pretraga prema ciljnim vrijednostima koje se rabe u Međunarodnoj procjeni iz koagulacije (WHO-IEQAS-Coagulation) i iznosi 15% dozvoljenog odstupanja za PV i APTV i 20% za fibrinogen. Tijekom 2005. godine sudjelovalo je 145 laboratorija. Određivanje na automatskim koagulometrima primjenjuje se u 143 laboratorija. Za određivanje PV u 93% laboratorija rabe se standardizirani visokoosjetljivi reagensi. KV% prema skupinama reagensa je <9%. Uvidom u rezultate za APTV (podijeljeni u skupine prema reagensu) uočena je

ZR1-3

Evaluation of the NEQAS coagulation results

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The established program of laboratory test harmonization cites reference range for APTT as a ratio. This is the only way to overcome the wide variation of APTT results in seconds. An overview of APTT results through NEQAS in 2005 points out that APTT-ratio has not been readily accepted. Continuous promotion of high standards in laboratory performance and improvement of analytical quality as well as the use of standardized methods and highly sensitive reagents for PT along with a unique form of expressing results (PT-INR, APTT-ratio) will provide a good basis to achieve harmonization in coagulation tests. The accepted percentage deviation from group median for PT and APTT is 15% and for fibrinogen 20% (perfomance criteria in WHO IEQAS-Coagulation). In 2005, 145 laboratories were registered in the program. Most laboratories (n=143) use automated coagulometers. Highly sensitive thromboplastins are used in 93% of laboratories (inter-laboratory CV <9%). There is great variability (CV 10%-20%) between diffeent groups of reagents for APTT; 70%-80% of laboratories use APTT-ratio. Fibrinogen is determinated by Clauss method in 50 of 52 laboratories. Inter-laboratory CV for normal and low concentration is <10% and 11%, respectively. Chromogenic method is used in 18 of 20 laboratories (CV 12.2%). The use of standardized methods for PT and fibrinogen and highly sensitive reagents for PT has reduced the inter-laboratory CV.

značajna varjabilnost (KV 10%-20%). Rezultate izražene u omjeru rabi 70%-80% laboratorija. Fibrinogen se određuje metodom po Claussu u 50 od ukupno 52 laboratorija. KV% za izrazito potološko područje je 11%, a za normalno <10%. ATIII se određuje preporučenom kromogenom metodom u 18 od 20 laboratorija (KV 12,2%). Rezultati za PV i fibrinogen potvrđuju da se primjenom visokoosjetljivih reagensa i preporučenih metoda smanjuje varijabilnost i omogućuje usporedivost laboratorijskih nalaza. Rezultati za APTV ukazuju na nužnost uvođenja APTV-omjera radi ujednačenog i usporedivog izražavanja rezultata.

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There is an urgent need to reach concordace and comparability in expressing APTT results.

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ZR1-4

Procjena rezultata u rutinskoj analizi mokraće

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Kvalitativna analiza mokraće kao opća medicinsko-biokemijska pretraga izvodi se na svim razinama zdravstvene skrbi. Nacionalni program vanjske procjene kvalitete rada je 1997.godine zbog toga proširen i na kvalitativnu analizu mokraće kroz suradnju Hrvatskoga društva medicinskih biokemičara i Zavoda za kliničku kemiju, KB Merkur, Zagreb, potvrđenog prema standardu ISO 9001:2000. U 2005./2006. godini su provedena po 2 ciklusa kemijske analize test trakom i morfološke analize mokraćnog sedimenta. Kemijska analiza mokraće test trakom izvodi se za glukozu,bilirubin, ketone relativnu volumnu masu, pH, eritrocite, proteine, urobilinogene, nitrite i leukocite u normalnom i patološkom području. Rabi se 8 -11 vrsta traka; 40% laboratorija očitavanja izvode instrumentalno. Rezultati se obrađuju prema vrsti trake i načinu očitavanja; 95%-98% rezultata je bilo prihvatljivih kod instrumentalnog, a 92%-95% kod vizualnog očitavanja. Unatoč različite osjetljivosti traka, kao i širokog raspona deklariranih vrijednosti kontrolnih uzoraka vidljivo je da instrumentalno očitavanje standardizira uvjete reakcije. Morfološka analiza mokraćnog sedimenta se izvodi pomoću 4 slike u boji sedimenta mokraće priređenih sukladno preporučenim metodama i harmonizaciji pretraga iz opće medicinske biokemije Hrvatske komore medicinskih biokemičara. U 2005. u 2 ciklusa prihvatljivi rezultati su bili za makrofage 18%, sluz 45%, granulirani cilindar 51%, bubrežni tubularni epitel 60%, hijalini cilindar 81%, gljivice 94%, pločasti epitel 98% i eritrocite 100%. U cilju bolje kvalitete slika

ZR1-4

Evaluation of results in qualitative urinalysis

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Qualitative urinalysis is a test performed at each level of medical care. Therefore, in 1997 the national external quality control program was extended to qualitative urinalysis through collaboration of the Croatian Society of Medical Biochemists and Department of Clinical Chemistry, Merkur University Hospital, Zagreb, certified to the ISO 9001:2000 standard. In 2005/2006, two surveys of chemical analysis using a test strip and morphological analysis of urinary sediment were performed. Chemical analysis is based on the determination of: bilirubin, ketones, relative volume mass, pH, erythrocytes, proteins, urobilinogen, nitrites and leukocytes in normal and abnormal ranges. Eight to eleven different test strips are in use; 40% of laboratories use instrumental measuring. Results were analyzed according to the type of test strip and way of reading, and showed 95%-98% and 92%-95% of results to fall within the acceptable range by instrumental and visual measuring, respectively. In spite of varying sensitivity of different test strips and a wide range of control values, instrumental measuring has standardized the conditions of reaction. Morphological analysis of urine sediment is based on 4 color pictures following the recommendations and harmonization of laboratory tests in general medical biochemstry by the Croatian Chamber of Medical Biochemists. In 2005, there were two surveys, and acceptable results were obtained for the following analytes: macrophages 18%, mucus 45%, granular cast 51%, renal tubular cells 60%, hyaline cast 81%, yeasts 94%,

uveden je prikaz na CD, jer se virtualnom analizom slike postiže bolja vidljivost prikazanog elementa.
Svaki laboratorij dobiva ukupne rezultate uz naznaku vlastitih rezultata. S obzirom na kliničku vrijednost analize sedimenta mokraće, naročito u hitnoj laboratorijskoj dijagnostici, smanjenje broja grješaka se postiže primjenom preporučenog postupka supravitalnog bojanja sedimenta mokraće, dok se dodatnom izobrazbom poboljšava prepoznavanje svih elemenata mokraćnog sedimenta u cilju stalnog poboljšanja kvalitete rutinske analize mokraće.

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squamous cells 98%, and erythrocytes 100%. In order to achieve better quality of pictures, presentation on CD has been introduced for better visibility because virtual picture analysis yields better element identification. Each laboratory receives overall results, its own results being specially marked. In the light of the clinical relevance of urinalysis, especially in laboratory diagnosis at emergency units, a reduced rate of errors has been achieved by using standardized procedures for urine sediment and by continuing education in sediment element identification in order to permanently improve the quality of routine urinalysis.

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ZR1-5

Procjena rezultata specijalističkih biokemijskih pretraga – pH i plinovi u krvi

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Program vanjske procjene kvalitete rada medicinsko biokemijskih laboratorija (MBL) koji provodi Hrvatsko društvo medicinskih biokemičara u suradnji sa Zavodom za kliničku kemiju KB Merkur, certificiranom prema standardu ISO 9001:2000, proširen je na analizu pH i plinova u krvi od 2003. godine, s obzirom na to da se ovaj dio specijalističke laboratorijske dijagnostike provodi u velikom broju MBL na svim razinama zdravstvene skrbi. Vanjska procjena kvalitete pH i plinova u krvi provodi se tri puta na godinu analizom jednog komercijalnog kontrolnog uzorka, koji je puferirana vodena otopina bikarbonata ekvilibrirana s točno poznatom razinom O_2, CO_2 i N_2. pH i plinovi u krvi određuju se u 42 MBL na acidobaznim analizatorima različitih proizvođača uključujući Bayer Diagnostics (68%), Nova Biomedical (12%), Instrumentation Laboratories (7%), Radiometer (5%). Rezultati pojedinih laboratorija za pH, pCO_2 i pO_2 grupiraju se prema metodama i procjenjuju u odnosu na ciljne vrijednosti koje označavaju ukupnu dozvoljenu analitičku pogrešku i/ili deklarirane vrijednosti proizvođača komercijalnog kontrolnog uzorka prema metodi određivanja. Procjena rezultata mjerenja pH pokazala je nisku varijabilnost (KV%) unutar metode koja je definirana proizvođačem acidobaznog analizatora (acidoza: 0,05%- 0,31%, normalno područje: 0,14%-0,24%, alkaloza: 0,04%-0,45%) i visoku razinu kvalitete uz 97%-100% prihvatljivih rezultata (±0,06 od srednje vrijednosti primijenjene metode). Rezultati za pCO_2 su pokazali da ciljeve

ZR1-5

Evaluation of specialist biochemistry test results – pH and blood gas analysis

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External quality assessment in medical biochemistry laboratories (MBL), which is performed by the Croatian Society of Medical Biochemists in collaboration with Department of Clinical Chemistry, Merkur University Hospital, certified to the ISO 9001:2000 standard, since 2003 has included acid base analysis because this part of specialist laboratory diagnosis is performed in a large number of MBLs at all levels of health care. External quality assessment in acid base analysis is performed three times a year by analysis of one commercial control sample of the buffered bicarbonate aqueous solution equilibrated with a predetermined level of O_2, CO_2 and N_2. Blood gas analyses are performed in 42 MBLs on instruments from different manufacturers including Bayer Diagnostics (68%), Nova Biomedical (12%), Instrumentation Laboratories (7%), and Radiometer (5%). Results from particular laboratories for pH, pCO_2 and pO_2 are grouped according to methods and assessed according to the quality specifications representing the goals for total analytical error and/or acceptable range according to the manufacturer's recommendatation. pH results showed a low between instrument variability, expressed as coefficient of variation (CV): 0.05%-0.31% in acidosis, 0.14%-0.24% at normal level, 0.04%-0.45% in alkalosis, and high level of quality: 97%-100% of results were satisfactory (±0.06 from the mean of the methods). Results for pCO_2 were within the quality criteria (±12%) for 93%-98% of MBLs. Between in-

analitičke kvalitete (±12%) zadovoljava 93%-98% MBL. Varijabilnost rezultata unutar metode kretala se od 1,2%-5,5% kod visoke, 4,0%-7,1% kod normalne i 0,4%-7,0% kod niske razine pCO_2. Rezultati mjerenja pO_2 u odnosu na zadane ciljeve analitičke kvalitete koji iznose ±1,6 kPa (pO_2 <13,3 kPa) i ±12% (pO_2 >13,3 kPa) bili su prihvatljivi u 88%-98% MBL. Varijabilnost rezultata pO_2 unutar metode kretala se od 1,5%-4,8% kod visoke, 1,5%-7,0% kod normalne i 5,3%-16,0% kod niske razine pO_2, što se može objasniti matriksom komercijalnih kontrolnih uzoraka koji su puferirane vodene otopine i slabije otapaju plinove od svježih humanih uzoraka. Dobiveni rezultati ukazuju na visoku razinu kvalitete rada MBL u ovom području laboratorijske dijagnostike, što je osobito važno jer se radi o pretragama najviše kategorije hitnosti.

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strument variability of the results ranged between 1.2% and 5.5% at high, 4.0% and 7.1% at normal, and 0.4% and 7.0% at low level of pCO_2. In 88%-98% of MBLs, results for pO_2 were within the quality criteria from ±1.6 kPa (pO_2 <13.3 kPa) and ±12% (pO_2 >13.3 kPa). Between instrument variability of the results was 1.5%-4.8% at high, 1.5%-7.0% at normal, and 5.3% -16.0% at low level of pO_2, which may be explained by the matrix of the control samples; aqueous based materials have poor buffer capacity and poor ability to dissolve gases compared with fresh whole blood. The results obtained pointed to the high quality of medical biochemistry laboratories in this part of laboratory diagnosis, which is very important because acid base analysis is among the analytes of the first category emergency.

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ZR1-6

Rezultati vanjske procjene kvalitete za HbA1c

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Laboratorijsko određivanje hemoglobina A1c (HbA1c) u Hrvatskoj prisutno je već gotovo 30 godina. Tijekom tog razdoblja uvodile su se i rigorozno evaluirale različite analitičke metode, u skladu s globalnim razvojem područja. Međutim, na nacionalnoj razini je izostala odgovarajuća potpora struke u smislu praćenja i unaprjeđenja kvalitete, te osiguranja uvjeta za primjenu međunarodnih standarda.
Rezultati istraživanja provedenog početkom 2005. g. pokazali su slabu dostupnost testa na području Hrvatske (radi se u samo 27 laboratorija), te šesterostruko manji broj određivanja u odnosu na preporučene potrebe za postojeću dijabetičnu populaciju. Metodologija je danas razmjerno ujednačena, s imunoturbidimetrijskim metodama zastupljenim u čak 92% laboratorija, ali postoji velik rasap u referentnim vrijednostima, koje su se kretale u rasponu od <5.7% do <7%. Također je zabrinjavajući podatak da se u 4 (15%) laboratorija rezultati izdaju u obliku ekvivalenata IFCC. Sudionici istraživanja iskazali su gotovo jednoglasno zanimanje za sudjelovanje u programu vanjske procjene kvalitete. Koncem 2005. pokrenut je nacionalni program vanjske procjene kvalitete HbA1c, i to kao modul 9 programa vanjske procjene kakvoće rada MBL koji se provodi

ZR1-6

Results of External Quality Assessment for HbA1c

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Laboratory determination of hemoglobin A1c (HbA1c) has been present in Croatia for almost 30 years. During this period, various analytical methods have been introduced and rigorously evaluated in keeping with global advancement in the field. However, appropriate professional support has failed at the national level in terms of monitoring and promoting quality and provision of conditions for the application of international standards. Results of a study conducted at the beginning of 2005 demonstrated poor test availability in Croatia (it is performed in only 27 laboratories), and six times lower number of determinations compared to the recommended requirements for the existing diabetic population. The methodology is presently rather uniform, with immunoturbidimetric methods applied in as many as 92% of laboratories, but there is a large dispersion of reference values that ranged from <5.7% to <7%. Also, it is rather inconvenient that in four (15%) laboratories results are reported in the form of IFCC equivalents. Study participants almost unanimously expressed their interest in taking part in the external quality control program which, however, had not yet been launched at the time of the survey. At the end of

u okviru HDMB. Prikazati će se rezultati preliminarnih triju ciklusa i raspraviti dometi i budući razvoj programa u svjetlu globalnog projekta harmonizacije HbA1c.

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2005, a national program for the external quality assessment for HbA1c was initiated as Modul 9 of the External Quality Assessment Program for Medical Biochemistry Laboratories, performed within the Croatian Society of Medical Biochemistry. Results of the three preliminary trials will be presented, and the scope as well as future development of the program with regard to the global HbA1c harmonization project will be discussed.

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ZR2 - Edukacija medicinskih biokemičara: Harmonizacija s temeljnim načelima Bolonjske deklaracije, ZR2-1

Nova koncepcija diplomskog Studija medicinske biokemije – magistar medicinske biokemije: stručnjak za 21. stoljeće

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Nova koncepcija diplomskog Studija medicinske biokemije posebnu važnost daje multidisciplinskom značaju struke, pa su bitni elementi postojećeg programa Studija posebno prošireni kliničkim znanjima kako bi se osigurali temelji za kvalitetnu primjenu zanstvenih spoznaja u kliničkom okruženju. Suvremena medicina sve veću pozornost poklanja razumijevanju dinamičkih promjena u fiziologiji i metabolizmu, osobito na molekularnoj razini, te zbog toga značajno ovisi o kvalitetnoj medicinsko biokemijskoj dijagnostici. Program je usklađen s preporukama koje su prihvaćene u većini europskih zemelja za rad u medicinsko biokemijskim laboratorijima, a predviđa jedan petogodišnji ciklus izobrazbe koji završava naslovom magistra medicinske biokemije. Multidisciplinarnost se postiže tako da se kroz studij stječu: 1. temeljna znanja (matematika, kemija, fizika, statistika, biokemija, biologija, molekularna biologija), 2. biomedicinska znanja (anatomija, fiziologija, patofiziologija, histologija i citologija, imunologija, genetika, mikrobiologija i parazitologija, farmakologija), 3. stručna medicinsko biokemijska znanja (klinička biokemija, hematologija s koagulacijom, klinička imunologija, transfuziologija, klinička citologija, mikrobiologija, analitička toksikologija, molekularna dijagnostika, laboratorijska dijagnostika hitnih stanja, dijagnostika uz bolesničku postelju, racionalna laboratorijska dijagnostika itd.) te 4. znanja i vještine iz komunikacijskih disciplina, laboratorijskog upravljanja, automatizacije, elektroničke

ZR2 - Education of medical biochemists: Compliance to fundamental principles of Bologna declaration, ZR2-1

A new concept of university degree in Medical Biochemistry – Master in Medical Biochemistry: competent expert for the 21st century

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The new concept of university degree in Medical Biochemistry is particularly emphasizing the multidisciplinary character of the profession building it upon the long-established Medical Biochemistry degree and by elaborating the application of scientific knowledge in the clinical context. Modern medicine places an increasing emphasis on the understanding of the dynamic human physiology and metabolism at the molecular level; therefore it depends on a reliable medical biochemistry diagnosis.
The curriculum is harmonized with the recommendations accepted in the majority of European countries, and consists of an integral five-year program providing the degree of Master in Medical Biochemistry. The multidisciplinary approach is achieved by introducing various disciplines into the curriculum: 1) fundamental natural sciences (mathematics, chemistry, physics, statistics, biochemistry, biology, molecular biology); 2) biomedical disciplines (anatomy, physiology, pathophysiology, histology, cytology, immunology, genetics, microbiology and parasitology, pharmacology, etc.); 3) professional medical biochemistry disciplines (clinical biochemistry, hematology, coagulation, clinical immunology, transfusion medicine, clinical cytology, analytical toxicology, molecular diagnosis, point of care diagnosis, evidence-based medicine, etc.); and knowledge, competences and skills in communication, laboratory management,

obrade podataka, organizacije i upravljanja medicinsko biokemijskim laboratorijem, te informatizacije laboratorijskog sustava. U ukupnom petogodišnjem studiju ima 28% temeljnih, 12% biomedicinskih, 45% stručnih i 15% izbornih predmeta. Program predviđa maksimalno povezivanje osnovnih i stručnih predmeta, a stručna praksa će se provoditi već od prve nastavne godine. Predmeti su ocijenjeni prema sustavu ECTS, tako da student može dio studija obaviti i na nekom drugom sveučilištu. Nastavni plan će se izvoditi u suradnji s kliničkim bazama Fakulteta, KBC Zagreb, KB Sestre milosrdnice i KB Dubrava. To se poglavito odnosi na stručnu praksu i veliki Integrirani kolegij laboratorijske dijagnostike koji će se u trajanju cijelog 9. semestra organizirati u kliničkim laboratorijima. Novi program studija omogućava diplomiranom stručnjaku da stekne cjelovito znanje iz svih aspekata medicinsko biokemijske znanosti koje je nužno za struku i kompetencije da organizira rad i primijeni suvremene tehnologije u laboratorijsku praksu, da kompetentno tumači laboratorijske nalaze, što ga čini bitnim članom stručnog medicinskog tima koji zbrinjava bolesnika ili članom tima koji razvija i istražuje nove znanstvene spoznaje. Suvremeno obrazovanje medicinskih biokemičara s dosta znanja iz citologije, mikrobiologije, imunologije i transfuziologije omogućuje mu da može uspješno zadovoljiti trendove u struci koji su usmjereni prema sveobuhvatnim laboratorijskim znanostima, kako je to predviđeno u medicinskoj biokemiji i laboratorijskoj medicini.

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automation, electronic data processing, organization and management of medical biochemistry laboratory, and information of laboratory systems. The relative proportion of the subjects is as follows: 28% of fundamental natural sciences, 12% of biomedical, 45% of professional and 15% of elective subjects.
The fundamental and professional subjects would be intensively correlated, and training in the hospital laboratory will start already from the first year. The ECTS credits will be assigned to each subject, and student mobility would be encouraged. There will be tight cooperation with the teaching hospitals connected with School of Pharmacy and Biochemistry, Zagreb University Hospital Center, Sestre milosrdnice University Hospital and Dubrava University Hospital, in particular for the course Integral Laboratory Diagnosis in the 9th semester and professional laboratory practice. At completion of the new Medical Biochemistry curriculum, the graduate would have a thorough knowledge of all aspects of medical biochemistry laboratory science relevant to the discipline and competences to organize work and apply current techniques in laboratory practice, to make interpretation of the diagnostic data and to function as a consultant in medical team, or to pursue a career in the fundamental and applied scientific research. The new concept comprises relevant knowledge in clinical cytology, microbiology, clinical immunology, transfusion medicine, analytical toxicology and molecular diagnosis, thus concurring with the current trends in Medical Biochemistry and Laboratory Medicine.

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ZR2-2

Poslijediplomski studiji na Farmaceutsko-biokemijskom fakultetu

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Doktorski studij Farmaceutsko-biokemijske znanosti je sveučilišni poslijediplomski znanstveni studij čijim se završetkom i obranom doktorske disertacije stječe akademski stupanj doktora znanosti, znanstveno područje Biomedicina i zdravstvo. Doktorski studij traje 3-4 godine za redovne i 6-8 godina za izvanredne studente, tijekom kojih je potrebno postići minimalno 180 ECTS (*European Credit Transfer System*) bodova. Doktorski studij organizira se u dva modula: Farmaceutske znanosti i Medicinsko-bi-

ZR2-2

Postgraduate studies at School of Pharmacy and Biochemistry

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Doctoral studies in Pharmaceutical-Biochemical Sciences are university postgraduate scientific studies which, upon completion and defense of doctoral dissertation, lead to the degree of Doctor of Science, scientific field Biomedicine and Health. Doctoral study takes 3-4 years for full-time and 6-8 years for part-time students, during which time a minimum of 180 ECTS credits have to be earned.
Doctoral study in Pharmaceutical-Biochemical Sciences at School of Pharmacy and Biochemistry, University of

okemijske znanosti. Namijenjen je farmaceutima, medicinskim biokemičarima te drugim stručnjacima iz znanstvenoga područja Biomedicine i zdravstva i područja Prirodnih znanosti. Program doktorskog studija načelno je usporediv s europskim programima (i šire) ili dijelovima programa doktorskih studija područja biomedicine i prirodnih znanosti, kao i različitih integriranih programa drugih doktorskih studija. Temelji se na kompetitivnim znanstvenim istraživanjima te obrazuje znanstvenike sa specifičnim stručnim znanjima neophodnima za prevenciju, otkrivanje, dijagnostiku i praćenje bolesti te oblikovanje i primjenu učinkovite terapije za specifičnu bolest. U tom smislu u skladu je s odgovarajućim nacionalnim prioritetima. Studij uključuje A) organiziranu nastavu (obvezne, modularne, metodološke i izborne predmete) i B) aktivno bavljenje znanstveno-istraživačkim radom, a završava polaganjem ispita, pozitivnom procjenom znanstvene aktivnosti, pozitivnom ocjenom i obranom doktorskog rada.

1. godina studija

A – temeljni predmeti (4 ECTS); modularni predmeti (6 ECTS); metodološki predmeti (4 ECTS); izborni predmeti (8 ECTS)

B – znanstvena aktivnost (38 ECTS)

2. godina studija

A – modularni predmeti (4 ECTS); izborni predmeti (10 ECTS);

B – znanstvena aktivnost (46 ECTS)

3. godina studija

B – znanstvena aktivnost (60 ECTS)

Farmaceutsko-biokemijski fakultet organizira i poslijediplomsku nastavu od godine dana u okviru 4 odobrena programa specijalizacija u sustavu zdravstva (Medicinska biokemija, Analitička toksikologija, Analitika i kontrola lijekova i Farmaceutska tehnologija). U tijeku je također izrada prijedloga programa za još 4 poslijediplomska specijalistička studija u trajanju od jedne godine.

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Zagreb, is organized in two modules: Pharmaceutical Sciences and Medical-Biochemical Sciences. The program is intended for pharmacists, medical biochemists and other professionals in the field of Biomedicine and Health and the field of Natural Sciences. The study *curriculum* proposed is, in principle, comparable with European *curricula* or parts of doctoral study *curricula* in the field of biomedicine and natural sciences as well as different integrated *curricula* of other doctoral studies. The study is based on competitive scientific research and educates scientists in acquiring specific competences indispensable for the prevention, detection, diagnosis and monitoring of diseases as well as for designing and application of efficient therapies. In this respect, the *curriculum* complies with the relevant national priorities. The study comprises: (A) organized instruction (basic, modular, methodological and elective courses), and (B) active engagement in scientific research; and is ended by taking an exam, favorable evaluation of research activities, passing grade and defense of doctoral dissertation.

1st year

A – basic courses (4 ECTS credits); modular courses (6 ECTS credits); methodological courses (ECTS credits); elective courses (8 ECTS credits)

B – research activity (38 ECTS credits)

2nd year

A – modular courses (4 ECTS credits); elective courses (10 ECTS credits)

B – research activity (46 ECTS credits)

3rd year

B – research activity (60 ECTS credits)

School of Pharmacy and Biochemistry also organizes one-year postgraduate courses within 4 approved programs of specialist training in the health system (Medical Biochemistry, Analytical Toxicology, Analytics and Drug Control, and Pharmaceutical Technology). Also, under way is making the proposal of the *curricula* for another four postgraduate specialist studies, each taking one year.

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ZR2-3

Međunarodna suradnja u izobrazbi medicinskih biokemičara

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U cilju prilagodbe visokim standardima obrazovanja u Europskoj Uniji, u skladu s Bolonjskim procesom i potrebama razvoja struke, Farmaceutsko-biokemijski fakultet je neprestano poduzimao niz mjera u reformi nastavnog procesa, koje su kroz uvođenje većeg broja izbornih predmeta, podučavanje i usvajanje znanja utemeljenog na rješavanju problema, te mobilnosti studenata omogućile bolje profiliranje studenata i njihovu bolju izobrazbu. Dobar uvod u ove promjene bilo je potpisivanje sporazuma Republike Hrvatske s drugim zemljama u centralnoj i istočnoj Europi radi promicanja suradnje na području visokoškolskog obrazovanja u okviru projekata CEEPUS (*Central European Exchange of Programs of University Studies*). Suradnja se u okviru visokoškolskog obrazovanja ostvaruje kao razmjena studenata i profesora između srodnih fakulteta koji su udruženi u projektu. Studenti registrirani na jednom od fakulteta u mreži mogu dobivati novčanu potporu za studiranje u inozemstvu ne samo na dodiplomskom nego i na poslijediplomskom ili doktorskom studiju, a vrijeme provedeno u studiranju vani, koje je srodno našem programu studija, priznaje se kao dio studiranja na matičnom fakultetu. Zavod za medicinsku biokemiju i hematologiju je od 1996. godine koordinator projekta CEEPUS s jedanaest partnera, a to su zavodi za kliničku biokemiju ili zavodi za biokemiju farmaceutskih i medicinskih fakulteta u zemljama srednje i istočne Europe. U okviru toga projekta studenti medicinske biokemije proveli su u inozemstvu od jednog do tri mjeseca slušajući predavanja, radeći eksperimentalno na temi diplomskog rada ili obavljajući obveznu ljetnu praksu kao dio nastavnih obveza. U okviru projekta CEEPUS Fakultet u suradnji sa studentskom udrugom svake godine organizira ljetne škole na odabranu temu koja se obrađuje s kliničkog, dijagnostičkog i terapijskog aspekta, uz sudjelovanje studenata medicinske biokemije, farmacije, medicine i stomatologije. Time se postiže bolje obrazovanje stručnjaka za sve zahtjevniju suvremenu praksu u sustavu zdravstvene zaštite.

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ZR2-3

International collaboration in the education of medical biochemists

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With respect to the current changes in the national system of high education, aimed at satisfying the high educational standards of the European Union in compliance with the Bologna process and the development of the profession, the Zagreb University School of Pharmacy and Biochemistry has undertaken a series of measures to reform the teaching process. Changes including the introduction of a larger number of elective subjects, problem-based teaching and learning (PBL), and obligatory mobility of the students will enable their better profiling and training for modern professional and research challenges. Good introduction in this manner was made in 1996, when Republic of Croatia signed agreement with other Central and Eastern European countries in promoting cooperation in the field of high education within the framework of the Central European Exchange Programme for University Studies (CEEPUS). Cooperation in the field of high education between the contracting parties has been realized through particular inter-university cooperation and mobility of students and teaching staff. Students registered at universities are eligible for support within the CEEPUS program, up to and including doctoral or postgraduate level, provided that the period of study or training at the host university, which is compatible with the *curriculum* at the student's home university, forms part of his or her university studies. Since 1996, Department of Medical Biochemistry and Hematology, School of Pharmacy and Biochemistry, has been coordinator of the CEEPUS project with eleven partner universities from Central and Eastern European countries. In the frame of this project, students of medical biochemistry used to spend one to three months abroad during their study period. Attending the lectures, working on diploma thesis or obligatory summer practice were the main types of instructions in the frame of the project for them. Each year, Zagreb School of Pharmacy and Biochemistry has organized summer school in the frame of the project, based on multidisciplinary approach to a selected topic, with international participation of professors and students of medical biochemistry, pharmacy, medicine and dental medicine. Achievements of this project have mainly been observed in better education of medical biochemists for the needs of the increasingly demanding modern practice within the health care system.

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ZR2-4

Kako uskladiti edukaciju s potrebama medicinsko biokemijske struke?

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Brz napredak medicinskih znanosti te razvoj novih dijagnostičkih postupaka uvjetovao je da se medicinski biokemičari tome napretku moraju trajno prilagođavati, a nove spoznaje ugrađivati u temeljna znanja stečena u dodiplomskoj izobrazbi. U tijeku studija Medicinske biokemije na Farmaceutsko biokemijskom fakultetu medicinski biokemičar stječe temelje za razna područja: biokemijska, hematološka, molekularno biološka i kemijska istraživanja u biološkom materijalu u svrhu utvrđivanja uzroka bolesti, održavanja zdravlja, prevencije bolesti i praćenja uspjeha liječenja. Daljnje usavršavanje medicinskog biokemičara trajan je proces, koji se odvija na poslijediplomskom studiju i specijalizaciji, te sudjelovanjem na stručnim predavanjima, tečajevima trajne izobrazbe, kongresima iz područja medicinske biokemije, ali i iz područja medicine (ovisno o kategoriji zdravstvene ustanove u kojoj je medicinski biokemičar zaposlen, subspecijalizacija u području kojim se zdravstvena ustanova bavi). U ovoj će se radionici raspravljati o osposobljenosti medicinskog biokemičara za današnje potrebe struke.

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ZR2-4

How to harmonize education and requirements of the medical biochemistry profession?

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Since modern clinical chemistry is changing rapidly as new medical knowledge and technologies become available, a competent medical biochemist must be able to synthesize and implement both graduate and postgraduate skills and knowledge. The study of Medical Biochemistry at School of Pharmacy and Biochemistry, University of Zagreb, is designed to provide a medical biochemist with the basic skills needed for a career in clinical biochemistry (biochemistry, hematologiy, molecular and chemistry investigations) within the frame of health service: detection of the cause of disease, health maintenance, disease prevention, and therapy monitoring. Medical biochemist requires continuing education to maintain his professional skills. Much of this is done by postgraduate study and specialization; and through professional meetings, courses of continuing education in the field of both clinical chemistry and medicine (depending on the health care institution where the medical biochemist is employed, "in house" subspecialization). In this workshop, participants will discuss the qualifications of medical biochemist for contemporary professional requirements.

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ZR3 - D-dimeri: Definicija, primjena u klinici, standardizacija i harmonizacija metoda, ZR3-1

Definicija i pregled dostupnih metodologija za kvantitativno određivanje D-dimera

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Prema definiciji naziv D-dimer se odnosi na završni, odnosno najmanji razgradni proizvod umreženog fibrina približne molekularne mase od 190 kDa, koji nastaje fibrinolitičkim djelovanjem plazmina. Sastoji se od 2 D-domene susjednih Y-lanaca fibrinskog polimera međusobno povezanih kovalentnom vezom koja nastaje djelovanjem aktiviranog faktora zgrušavanja XIII (XIIIa) te predstavlja neoepitop specifičan za fibrin. U svim testovima za određivanje D-dimera rabe se monoklonska protutijela specifična za epitope koji se nalaze na fibrinskom fragmentu D-dimer, ali ne i na fibrinogenskom fragmentu D, drugim razgradnim proizvodima fibrinogena ili nativnom fibrinogenu. Od 1983. godine kad je opisano prvo monoklonsko protutijelo koje reagira sa specifičnim neoepitopom D-dimer, priređena su različita monoklonska protutijela te brojni specifični imunokemijski testovi za kvantitativno određivanje D-dimera. Danas je komercijalno dostupno više od 30 testova za određivanje D-dimera u kojima se rabi više od 20 različitih monoklonskih protutijela specifičnih za D-dimer. S obzirom na metodologiju, testovi za određivanje D-dimera mogu se podijeliti na: enzimimunokemijske testove na krutom nosaču (ELISA), automatiziranu fluorescentnu metodu ELISA za pojedinačne uzorke (ELFA), imunofiltraciju (membranska ELISA), lateks imunoturbidimetrijske testove (LPIA) i imunofluorimetrijske testove. Većina ovih metodologija je automatizirana, a kao uzorak se rabi plazma ili puna krv. Testovi se međusobno razlikuju, a to se osobito odnosi na preporučeni referentni interval i graničnu vrijednost za isključivanje tromboze. Uz to, razlikuju se prema monoklonskom protutijelu i vrsti kalibratora koji se rabi u testu, te jedinicama u kojima se izražavaju rezultati (mg/L ili jedinice ekvivalentne fibrinogenu – mg FEU/L). U praksi fragment D-dimer predstavlja samo mali dio ukupnih D-dimera koji se određuje u testovima. Naime, protutijela reagiraju s razgradnim proizvodima fibrina različite molekularne mase, koji uključuju fragment D-dimer kao i visokomolekularne te niskomolekularne razgradne proizvode fibrina te intaktni fibrinski ugrušak. Zbog razlike u specifičnosti protutijela testovi se uvelike razlikuju s obzirom na osjetljivost prema razgradnim proizvodima fibrina, kao i prema utje-

ZR3 - D-Dimers: Definition, clinical application, standardization and harmonization of methods, ZR3-1

Overview and brief description of available methodologies for quantitative determination of D-dimers

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By strict definition, D-dimer fragment represents a stable, terminal product generated by plasmin degradation of cross-linked fibrin, with an approximate molecular weight of 190 kD. It consists of 2 covalently bound D-domains on adjacent fibrin Y chains within a fibrin polymer. All assays for the determination of D-dimer antigen are based on monoclonal antibodies that react with conformational epitopes generated by factor XIIIa-induced crosslinking on fibrin fragment D-dimer that are not present on fibrinogen fragment D, other fibrinogen degradation products or native fibrinogen. Since 1983, when a study on the first monoclonal antibody reactive with D-dimer specific neoepitope was published, various monoclonal antibodies have been prepared and a number of quantitative assays have been developed. Nowadays, more than 30 D-dimer assays, based on more than 20 different D-dimer specific monoclonal antibodies, are present on the market. According to methodology, assays can be divided into: microtiter plate ELISA assays, automated single-sample ELISA system (enzyme-linked fluorescence assay-ELFA), immunofiltration assays (membrane ELISAs), latex-enhanced photometric immunoassays (LPIA) and immunofluorometric assays. These principles have been incorporated in a variety of automated techniques that use plasma or whole blood as sample material. There are discrepancies in the comparability of various assays, particularly in terms of reference ranges and cut-off values used for the exclusion of thrombosis. The potential sources of variation among D-dimer assays include the use of a variety of different monoclonal antibodies and different commercial calibrators. Furthermore, results are reported in mg/L or as fibrinogen equivalent units (FEU). Generally, D-dimer represents only a small part of the total D-dimer antigen measured by current D-dimer antigen assays. Assays detect an array of fibrin compounds of different molecular weights, including fibrin fragment D-dimer as well as higher molecular weight fibrin degradation products, fibrin X-oligomers and intact fibrin clots. Due to differences in epitope specificity and assay performance, assays differ concerning their preference to high or low molecular weight fibrin derivatives and cross-reactivity

caju razgradnih proizvoda fibrinogena. Niti jedan test za određivanje D-dimera nije idealan. Idealan bi test morao biti jednostavan za izvođenje, brz, jeftin, kvantitativan, s velikim mjernim rasponom te dokazan u odgovarajućim kliničkim studijama.

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with non-crosslinked fibrinogen and fibrin compounds. No assay is superior to the others. A perfect D-dimer assay should be simple, inexpensive, fast, quantitative, with a large measurement range, and confirmed in appropriate clinical studies.

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ZR3-2

D-dimeri: različite metode, različiti rezultati. Iskustvo s programom vanjske procjene kvalitete

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D-dimer se sve više rabi u dijagnostici venske tromboembolije (VTE). Stoga se javila potreba za dobro kontroliranim testovima za D-dimer koji će se moći uspješno primjenjivati i izvan pažljivo kontroliranih kliničkih ispitivanja. Potrebni su potpuno kvantitativni testovi, a polu-kvantitativni testovi na osnovi lateksa na stakalcu nisu prikladni za ovu namjenu, jer su najniže razine D-dimera koje se mogu otkriti normalno više od razina D-dimera prisutnih u nekim slučajevima s potvrđenom VTE. U preglednoj studiji u okviru UK National External Quality Assessment Scheme (NEQAS, web stranica www.ukneqasbc.org) kasne 2005. godine raspodijeljen je uzorak pripremljen kao skup plazma dobivenih od osoba s povišenim D-dimerom. Medijani rezultata dobivenih različitim tehnikama (uz najmanje 10 korisnika) prikazani su na tablici, a pokazuju broj centara koji su vratili rezultate prema metodama koje rabe (za metode koje se primjenjuju u najmanje 10 centara), kao i medijan rezultata za svaku skupinu, raspon i prijelomne vrijednosti (*cut-off*) primijenjene za isključivanje DVT prema primijenjenoj tehnici.

ZR3-2

D-dimers: different methods, different results. The experience within an external quality assessment programme

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D-dimer is increasingly used in the diagnosis of venous thromboembolism (VTE). There is therefore a requirement for well controlled D-dimer tests which can be successfully employed outside of carefully controlled clinical trial settings. Fully quantitative assays are required and the slide based semi-quantitative latex based assays are unsuitable for this purpose because the lowest levels of D-dimer which can be detected are normally higher than the D-dimer levels present in some cases with confirmed VTE. In a UK National External Quality Assessment Scheme (NEQAS, website www.ukneqasbc.org) survey in late 2005, a sample prepared as a pool of plasmas from subjects with elevated D-dimer was distributed. The median results by different techniques (with at least 10 users) are presented below, showing the number of centres returning results according to the method used (for methods used by at least 10 centres) as well as the median of each group results, the range and the cut-offs used for DVT exclusion according to the technique in use.

Metoda / Method	**n**	**Medijan rezultata /** Median result	**KV /** CV	**Raspon /** Range	**Medijan cut-off za DVT /** Median cut-off for DVT	**Raspon cut-off /** Range of cut-offs
VIDAS (FEU)	43	990 ng/mL	12%	512–1150	500	300–1000
MDA (FEU)	24	801 ng/mL	17%	490–1040	500	300–500
Stago (FEU)	30	1030 ng/mL	8%	840–1240	500	400–2000
DB DDimer +	32	176 ng/mL	32%	96–360	192	90–500
Inst Lab	106	445 ng/mL	20%	209–700	250	130–700
MDA	17	409 ng/mL	19%	290–530	275	190–500
Trinity/ Biopool	85	288 ng/mL	13%	135–936	190	100–1000

Naši podatci pokazuju hitnu potrebu za standardizacijom testiranja i izdavanja nalaza. Kad se D-dimer rabi za isključivanje VTE, primijenjena prijelomna vrijednost (*cut-off*) mora u obzir uzeti tehniku koja se rabi za određivanje.

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Our data show that there is an urgent need for standardisation of testing and reporting. When D-dimer is used to exclude VTE, the cut-off employed must take account of the technique used.

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ZR3-3

Kliničko značenje određivanja D-dimera u likvoru

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D-dimeri su specifični razgradni proizvodi umreženog fibrina. Povišene vrijednosti znak su ubrzane fibrinolitičke aktivnosti i mogu poslužiti kao specifični indeks hiperkoagulabilnosti i pojačane fibrinolize. Ranija istraživanja su pokazala da su vrijednosti D-dimera u normalnom likvoru vrlo niske ili negativne. U stanjima hipoksije ili ishemije, kada dolazi do oštećenja krvno-moždane barijere, molekule D-dimera mogu ući iz periferne cirkulacije u likvor, što rezultira povišenjem njihovih vrijednosti u likvoru. Pokazalo se, međutim, da su u stanjima akutnog subarahnoidnog krvarenja vrijednosti D-dimera u likvoru značajno više nego u krvi i opadaju s vremenom. Stoga bi određivanje D-dimera u likvoru moglo biti korisno u diferencijalnoj dijagnostici intrakranijskih krvarenja kod bolesnika s negativnim nalazom CT. Također, istraživanja su pokazala da se povišene vrijednosti D-dimera u likvoru mogu naći i kod bolesnika s malignim bolestima kod kojih postoje klinički znakovi infiltracije središnjega živčanog sustava (SŽS) malignim stanicama. Cilj ovoga rada je bio ispitati vrijednost određivanja D-dimera u likvoru kao potencijalnog biljega metastatskog procesa unutar SŽS. Isto tako, željelo se ispitati može li test poslužiti za razlikovanje patološkog intrakranijskog krvarenja od artificijelnog krvarenja. Ukupno su analizirana 62 uzorka likvora, od toga 33 uzorka bolesnika s hematološkim malignim bolestima, 5 uzoraka likvora bolesnika s metastazama solidnih tumora, 11 uzoraka likvora sa sumnjom na na krvarenje, te 13 normalnih likvora. D-dimeri su određivani automatiziranom enzim-fluorimetrijskom metodom (ELFA) na uređaju mini Vidas proizvođača bioMerieux. U svim uzorcima normalnih likvora vrijednosti D-dimera su bile <0,05 mg/L. Povišene vrijednosti D-dimera su dokazane kod 7 uzoraka likvora hematoloških bolesnika, kao i kod svih bolesnika s metastazama solidnih tumora. Od 11 uzoraka likvora koji

ZR3-3

Diagnostic value of cerebrospinal fluid D-dimer assay

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D-dimer is a specific breakdown fragment of cross-linked fibrin, reflecting the secondary acceleration of fibrinolytic activity, and can be used as a specific index of hypercoagulability and hyperfibrinolysis. Recent investigations showed very low or negative values of D-dimers in the normal cerebrospinal fluid (CSF). Due to hypoxia and ischemia, when the function of the blood-brain barrier was damaged, these molecules were elevated in CSF. Also, the CSF D-dimer level has been reported to be significantly higher than its blood level in the acute stage of subarachnoid hemorrhage, decreasing with time. Therefore, D-dimer determination could be useful in the differential diagnosis of intracranial hemorrhage in patients with negative CT scan. Also, some investigators showed that the CSF D-dimer levels were signifficantly higher in neoplastic diseases with clinical evidence of the central nervous system (CNS) involvement. The aim of our study was to assess the diagnostic value of CSF D-dimer test as a potential marker of CNS involvement with neoplastic cells and carcinoma. We also wanted to assess the diagnostic value of CSF D-dimer test as a rapid method to distinguish intracranial hemorrhage from traumatic tap. D-dimer assay was performed on 63 CSF samples: 33 samples from patients with malignant hematologic diseases, 5 samples from patients with carcinoma with CNS involvement, 11 hemorrhagic samples, and 13 control samples. D-dimers were measured by the automated immunoassay system using ELFA (Enzyme Linked Fluorescent Assay) technology on a mini Vidas (bioMerieux). The levels of D-dimers were below 0.05 mg/L in all control samples. Higher levels of D-dimers were detected in 7 samples of patients with malignant hematologic diseases, and also in all 5 samples from patients with carcinoma with CNS involvement. The levels of D-dimers were higher in 9 hemorrhagic samples.

su bili krvavi, 9 ih je imalo povišene vrijednosti D-dimera. Rezultati su pokazali da određivanje D-dimera u likvoru bolesnika sa sumnjom na maligni proces može biti koristan pokazatelj infiltracije malignih stanica unutar SŽS. Na relativno malom broju uzoraka krvavih likvora test se nije pokazao dovoljno specifičnim za razlikovanje artificijelnog od patološkog krvarenja, no za donošenje konačnih zaključaka test treba ispitati na znatno većem broju bolesnika.

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Our results suggest that the measurement of D-dimers in CSF may be useful in the diagnosis of CNS involvement with neoplastic cells. Our study failed to differentiate pathologic hemorrhage from traumatic lumbar puncture, but these results could be due to the small number of samples. Additional investigations should be performed in a greater number of samples.

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ZR3-4

Dinamičko praćenje D-dimera različitim metodama za kvantitativno određivanje

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Osnovni problem u danas dostupnim testovima za kvantitativno određivanje koncentracije D-dimera predstavlja različita specifičnost svakog pojedinog testa prema razgradnim produktima umreženog fibrina i fibrinogena. Određivanjem koncentracije D-dimera kvantitativim testovima različite metodologije ispitivana je međuovisnost dobivenih koncentracija D-dimera i ukupnih razgradnih produkata fibrinogena i fibrina (TDP). Koncentracije D-dimera izmjerene su u plazmama 40 bolesnika pomoću NycoCard D-Dimer, IL Test D-Dimer, D-Dimer PLUS i STA Liatest D-DI, a koncentracije TDP-a pomoću testa Fibrinostika TDP. Rezultati su se razlikovali u 16/40 (40%) uzoraka koji su podijeljeni u 4 skupine prema rezultatima dobivenih NycoCard testom: I. <0,3 mg/L; II. 0,3-0,6 mg/L; III. 0,7-1,2 mg/L; IV. >1,2 mg/L. Usporedivi rezultati su dobiveni u uzorcima skupine IV. Većina rezultata je bila usporediva u skupini III, osim kod 4 negativna uzorka: 1 sa STA Liatest D-DI i 3 s IL Test D-Dimer. Najveće razlike su opažene u skupini II (9/12 uzoraka), oko granične vrijednosti za NycoCard. U 3/5 uzoraka s normalnim NycoCard vrijednostima (skupina I) dobiveni su pozitivni rezultati: 1 sa STA Liatest D-DI i 2 s D-Dimer PLUS testom. Usporednim određivanjem koncentracije D-dimera Nycocard testom i Vidas D-dimer testom dobivene su razlike u 10/48 uzoraka,a usporedbom IL Test D-dimer i Vidas D-dimer u 18/102 uzorka.Najveći broj rezultata odstupao je kod Nycocard vrijednosti <=0.3 mg/L (7/10) odnosno kod granične vrijednosti za IL test (16/102). U pojedinim uzorcima su Nycocard testom odnosno IL testom izmjerene izrazito visoke koncentracije D-dimera dok su Vidas testom dobivene

ZR3-4

Dynamic D-dimer monitoring by different methods for quantitative determination

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The main problems in nowadays commercially available tests for quantitative D-dimer assays are that they display variable specifity toward cross-linked fibrin derivatives as well as fibrinogen degradation products.D-dimer determination with quantitative D-dimer assays based on different methodology enabled us to investigate interdependence of D-dimer values and a correlation with total fibrinogen/fibrin degradation products (TDP). D-dimer concentrations were measured in plasma of 40 patients using NycoCard D-dimer,IL Test D-dimer,D-dimer PLUS,STA Liatest D-DI ,and fibrinogen/fibrin degradation products were measured with Fibrinostika TDP. The samples were separeted into four groups according to NycoCard results: I.‹0.3 mg/L;II.0.3-0.6 mg/L;III. 0.7-1.2 mg/L;IV.›1.2 mg/L.All assays gave comparable results in group IV. Majority of the results were comparable in group III,except for 4 samples that were negative:1 with STA Liatest D-DI and 3 with IL Test D-Dimer.The greatest discrepancy between results was found in group II (9/12 samples),above the NycoCard cut-off value. At 3/5 samples with normal NycoCard values (group I) we identified 3 positive results :1 with STA Liatest D-DI and 2 with D-dimer PLUS assay.Results obtained by comparison of D-dimer concentration with NycoCard D-dimer and Vidas D-dimer indicated differences in 10/48 samples and comparison of IL test D-dimer and Vidas D-dimer gave discrepant results in 18/102 samples. The most discrepant results were at NycoCard value <=0.3 mg/L (7/10) and above cut-off value for IL Test D-dimer (16/102).In some samples we measured a significantly higher values with NycoCard and IL Test

normalne ili tek neznatno povišene vrijednosti.U jednom od tih uzoraka dokazana je visoka koncentracija reumatoidnog faktora pa je i to jedan od čimbenika koji treba uzeti u obzir pri interpretaciji nalaza . Razlike dobivenih rezultata većinom su ipak posljedica uporabe različitih kalibratora i monoklonskih protutijela specifičnih za pojedini test, što upućuje da je neophodno bolesnika pratiti uvijek istim testom.

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D-dimer beside normal or almost normal D-dimer levels with D-dimer Exclusion assay.In one of those samples we proved high values of rheumathoid factor which is one of the factors that should be taken into consideration with interpretation of results.The differences of the obtained results are mostly due the usage of different calibrators and different monoclonal antibodies which suggest that the follow-up of patients should be always performed with the same assay.

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ZR4 – Novi hematološki parametri, ZR4-3

Napredak u hematologiji: nedostatak željeza u svjetlu novih biokemijskih biljega i indeksa RBC (% Hypo, CHr) – Terapijski dijagram Thomas-Plot i njegova uloga u dijagnostici i terapiji

Kosanke Wolfgang

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Nedostatak željeza (latentan ili funkcijski) kao i pojavna anemija uslijed nedostatka željeza najčešći su učinci primarne ili sekundarne nedostatne opskrbe željezom u okviru eritropoeze, kao i anemija kroničnih bolesti (ACD). Ispravna i dobro utemeljena diferencijalna dijagnoza postojećeg nedostatka željeza moguća je uz pomoć parametara kompletne krvne slike (RBC, Hgb, MCV, MCHC, MCH) te feritina. Suvremeni hematološki sustavi zasnovani na jednostaničnoj analizi pružaju dodatne morfološke podatke o kvantitativnoj konfiguraciji populacije crvenih krvnih stanica, što je osobito znakovito za nedostatak željeza. Omjer mikrocitičnih i hipokromnih eritrocita (M:H) od <0,9 opisuje postojeći nedostatak željeza uz visoku statističku značajnost, dok je hipokromija normalno dvostruko viša od mikrocitoze (usp. ovdje također i talasemija, M:H >0,9). To je suprotno oblicima funkcijskog nedostatka željeza u kombinaciji s akutnom ili kroničnom upalom, te s porastom proteina akutne faze (CRP) ili pak s ranim stadijem terapije eritropoetinom (r-hu EPO). Ovi oblici funkcijskog nedostatka željeza mogu se otkriti uz pomoć novih hematoloških parametara i pojasniti u smislu diferencijalne dijagnostike, omogućavajući tako "dublju analizu malfunkcije metabolizma željeza". Parametri kompletne krvne slike su, blago rečeno, "prespori" za rano otkrivanje nedostatne opskrbe željezom tijekom eritropoeze, jer se njezine jasne promjene post-

ZR4 – New hematological parameters, ZR4-3

Advances in hematology: iron deficiency states in the light of new biochemical markers and RBC indices (% Hypo, CHr) – Thomas-Plot Therapeutic Diagram and its relevance in diagnosis and therapy

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Iron deficiency (latent or functional) as well as a manifest iron deficiency anemia are the most frequently appearing impacts of primary and secondary iron supply shortage within erythropoiesis and also in anemias of chronic diseases (ACD). A correct and well-founded differential diagnosis of an existing iron deficiency is possible with the aid of CBC parameters (RBC, Hgb, MCV, MCHC, MCH) and with ferritin. Modern hematology systems based on single cell analysis yield additional morphological information about the quantitative configuration of the red blood cell population, which is especially characteristic for iron deficiency. A ratio of microcytic and hypochromic erythrocytes (M:H) of <0.9 describes an existing iron deficiency with high statistical significance, and hypochromia is normally twice as high as microcytosis (cp. here also thalassemia, M:H >0.9). This contrasts with the forms of functional iron deficiency in combination with acute or chronic inflammation and with an increase of acute phase proteins (CRP) or in the early stage of erythropoietin therapy (r-hu EPO). These forms of functional iron deficiency can be detected with the aid of new hematology parameters and can be clarified in a the differential diagnostic way, thus enabling a "profound analysis of iron metabolism malfunctions". The parameters of CBC are, "to say the least, too slow" for the early detection of deficiency in iron supply during erythropoiesis because

aju očite tek nakon više tjedana. Funkcijski nedostatak željeza može se otkriti ranije uz pomoć hipokromnih RBC kad se prijelomna vrijednost premaši za >5%, prije negoli nastupi značajno sniženje indeksa RBC kao što su MCV, MCHC ili MCH. Nadalje, postotak hipokromnih RBC opisuje "povijest eritropoeze" tijekom proteklih 70 dana, i to bez ograničavajućeg utjecaja bioritma ili metabolizma željeza ili koeficijenta varijacije (CV) dotične metode na konačne zaključke. Funkcijski nedostatak željeza je teško dijagnosticirati kad je istodobno prisutna upala i anemija udružena s kroničnim bolestima, jer reakcija aktune faze remeti zasićenost feritinom i transferinom. Kvocijent rezultata sTfR* i dekadni logaritam feritina, indeks feritina, koristan je pokazatelj opskrbe željezom tijekom eritropoeze. Indeks feritina, biljeg opskrbe željezom i sadržaj hemoglobina u retikulocitima (CHr), pokazatelj potreba za željezom, čine osnovu za tzv. Dijagnostički dijagram (Thomas-Plot) za definiranje nekih stanja metabolizma željeza, poglavito u bolesnika s upalnom reakcijom ili bez nje. Odlučujući parametar je ovdje C-reaktivni protein (CRP) > ili <5,0 mg/L.

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their explicit changes only become apparent after weeks. Functional iron deficiency can be detected earlier with the aid of hypochromic RBCs when the cut-off is exceeded by >5%, before the RBC indices such as MCV, MCHC or MCH have significantly decreased. Furthermore, the percentage of hypochromic RBCs describes the „history of erythropoiesis" during the elapsed 70 days, without either the biorhythm or iron metabolism or coefficient of variation (CV) of the method having a limiting impact on the conclusions. Functional iron deficiency is difficult to diagnose in conjunction with inflammation and anemia stemming from chronic diseases because ferritin and transferrin saturation are impaired by the acute phase reaction. The quotient of sTfR* result and the decade logarithm of ferritin, the ferritin index, is a useful indicator of iron supply during erythropoiesis. The ferritin index, a marker of iron supply and the hemoglobin content of reticulocytes (CHr), an indicator of iron requirements, generate the basis for the so-called Diagnostic Diagram (Thomas-Plot) to define several states of iron metabolism, especially in patients with or without inflammatory reaction. The decisive parameter here is C-reactive protein (CRP) > or <5.0 mg/L.

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ZR4-1

Procjena novih parametara u odnosu na standarde

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Godinama su automatizirani hematološki uređaji pružali podatke o crvenim krvnim stanicama (RBC): MCV, RDW, MCH. Danas pak neki instrumenti mogu točnije diferencirati I različite bijele krvne stanice (WBC), i to ne samo u slučaju normalnih stanica, nego također u mnogim nenormalnim kliničkim situacijama. Neke tehnologije poput VCS® (Volume-Conductivity-Scatter) mogu razlikovati WBC bez značajnijih morfoloških promjena u ponašanju stanica (gotovo nativno stanje), što omogućava automatiziranu morfološku analizu WBC i primjenu tih podataka u probiru na različite nenormalnosti WBC, kao u slučaju RBC. Danas izazov predstavlja primjena ovih podataka u svrhu što boljeg otkrivanja/označavanja nenormalnih stanica, kao što su nezreli granulociti, blasti, reaktivni i maligni limfociti, plazma stanice, neutrofilna displazija (hipogranularnost) itd. Čini se kako je, nakon mnogo godina, došlo vrijeme bijelih krvnih stanica.

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ZR4-1

New clinical applications with WBC automated morphological analysis

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For many years, automated hematology instruments were used to provide morphological information on red blood cells (RBC): MCV, RDW, MCH. Today, some instruments are capable of a more accurate differentiation among different white blood cells (WBC) not only when the cells are normal but also in many of abnormal clinical situations. Some technologies such as VCS® (Volume-Conductivity-Scatter) can differentiate WBCs without significant morphological changes in WBC behavior (near native state), thus permitting automated morphological analysis of WBC and use of this information in the screening of different WBC abnormalities, just as for RBCs. The challenge today is to use this information for better detection/flagging of abnormal cells, such as immature granulocytes, blasts, reactive and malignant lymphocytes, plasma cells, neutrophil dysplasia (hypogranularity), etc. It seems that now, after many years, the time has come for WBCs.

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IR-1 – Industrijom sponzorirana radionica
ROCHE, IR1-1

Analitička procjena imunokemijskog analizatora Modular-E170 tvrtke Roche Diagnostics

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Analitička procjena imunokemijskog analizatora MODULAR E – 170 provedena je prema preporukama ECCLS – a za određivanje koncentracije Ca-125, CEA, Ca-19-9, α-fetoprotein i PSA.
Procjena je obuhvatila nepreciznost u seriji i iz dana u dan, netočnost, te usporedna određivanja. Sva ispitivanja napravljena su za obje ćelije MODULARA E – 170.
Nepreciznost u seriji određena je na 10 uzoraka istog seruma pacijenta sa normalnim vrijednostima i na 10 uzoraka istog seruma pacijenta sa visokim vrijednostima. Za sve ispitivane analite dobiveni su zadovoljavajući koeficijenti varijacije. Prosječno za Ca-125 koeficijent varijacije bio je 1,1%, za CEA 0,86%, za Ca-19-9 iznosio je 0,9%, za α-fetoprotein 1,1% i za PSA 0,43%.
Nepreciznost iz dana u dan određivana je u 2 kontrolna seruma (niski i visoki nivo) kroz 10 dana. Prosječno za Ca-125 nepreciznost je iznosila 4,4%, za CEA 3,7%, za Ca-19-9 3,1 % za α-fetoprotein 2,9% i za PSA 4,1% što nam pokazuje da su koeficijenti varijacije iz dana u dan za sve analite zadovoljavajući. Netočnost mjerenja izračunata je kao % odstupanja (R%) srednje izmjerene vrijednosti od srednje deklarirane vrijednosti kontrolnih seruma. U izračunu su korištene srednje izmjerene vrijednosti dobivene pri određivanju nepreciznosti iz dana u dan. Vrijednost za "R" prihvatljiva je za sve analite u oba kontrolna seruma i na obje ćelije i prosječno je iznosila 1,03% za Ca-125 za CEA 4,8%, za Ca-19-9 bila je 6,9%, za α-fetoprotein 2,5 % i za PSA 1,8%.
Linearnost je za sve ispitivane parametre bila unutar dozvoljene analitičke pogreške.
Rezultati usporednih ispitivanja analizirani su u odnosu na rezultate dobivene na Architectu tvrtke Abbott te su pokazali visok stupanj korelacije.
Prikazani rezultati provedene analitičke procjene imunokemijskog analizatora MODULAR E – 170 za određivanje koncentracije navedenih tumorskih biljega pokazuju da se aparat i reagensi mogu koristiti u laboratoriju.

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IR-1 – Industry sponsored workshop
ROCHE, IR1-1

Analytical estimation of Roche immunochemistry analyzer Modular E-170

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We estimate analytical performance of Roche immunochemistry analyzer according to ECCLS recommendations by determination of tumor marker concentrations of CA-125, CEA, CA 19-9, a-fetoprotein and PSA. Imprecision within-run, imprecision between-run and accuracy were determined for both Modular E-170 photometric cells. Imprecision within-run was analyzed on ten replicates of same serum samples for two different concentrations (normal and high) and mean CVs were 1,1% for CA-125, 0,86% for CEA, 0,9% for CA 19-9, for a-fetoprotein 1,1% and 0,43% for PSA.
Imprecision between-run (for two control samples measured ten days) showed a little bit higher but acceptable CVs: 4,4 % for Ca-125, 3,7% for CEA, 3,1 % for CA 19-9, for a-fetoprotein 2,9% and for PSA it was 4,1%.
Inaccuracy was tested using two different control materials (normal and high) for 10 days. Mean biases from target values were acceptable: 1,0 3% for CA-125, for CEA it was 4,8%, 6,9% for CA 19-9, for a-fetoprotein 2,5 % and 1,8% for PSA.
Linearity was also determined and was satisfactory for all tests. Comparison with Abbott Architect results were assessed and results of the comparison study showed no statistical difference according to the Passing & Bablok regression analysis. Results of the study indicate that determination of tumor marker concentrations on Roche Modular E-170 provides precise and accurate results and are convenient for use in routine laboratory.

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IR1-2

Roche/Hitachi Modular – Analytics E170

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Ovaj potpuno automatizirani instrument za imunokemijska određivanja dio je MODULAR – ANALYTICS sustava te može funkcionirati kao njegov sastavni dio, ali i kao samostalni analizator, vrlo prihvatljiv za rutinski rad. Nakon vrlo dobrog iskustva na ELECSYS-u 2010, Modular – Analytics E170 ponudio nam je rješenje za brži protok uzoraka i veći broj parametara "on board" u našem laboratoriju. "Family" koncept reagenasa otvorio nam je mogućnost usporedbe rezultata na oba instrumenta, a Elecsys 2010 stavio u funkciju "back up" uređaja (R. V. su u oba slučaja identične, štoviše koriste se identični reagensi).
Dnevni protok u našem laboratoriju je 700 do 800 različitih testova (reproduktivni hormoni, hormoni štitnjače, tumorski biljezi, koštani biljezi, kortizol, PTH, feritin, B-12, ukupni IgE). Uređaj je umrežen na LIS. Rađena je skraćena evaluacija na 200 različitih uzoraka/10 različitih testova i dobiveni rezultati su u skladu s deklariranim C. V.
Prednosti uređaja:

- Kapacitet 170 testova/sat, uz mogućnost povećanja (dogradnja modula)
- Mogućnost kontinuiranog dodavanja uzoraka i potrošnog materijala bez zaustavljanja aparata
- Automatski rerun i dilucija
- Vrlo dobra "lot to lot" varijabilnost i stabilnost kalibracijskih krivulja

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IR1-2

Roche/Hitachi Modular – Analytics E170

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This entirely automatized instrument for immunological tests is a part of MODULAR - ANALYTICS system and is capable of working as one of its parts, but also as an individual analyser, well established for routine use. After indeed positive experience with ELECSYS 2010, Modular – Analytics E 170 offers a solution for the higher samples throughput and greater number of "on board" parameters in our lab. "Family concept" in the heterogenous immunology reagent enables us to obtain comparable results on both instruments, while it sets up Elecsys as a "back up" device (reference values are identical in both cases, moreover the same reagents are used).
Daily throughput in our lab is 700 – 800 different tests (reproductive hormons, thyroid hormons, tumor markers, bone markers, cortizol, PTH, ferritin, B-12, total IgE). The analyser is integrated in LIS. Shortened evaluation was made with 200 heterogenous samples/10 heterogenous tests. The results obtained were compatible with declarated C.V.
Advantages of E 170:

- Test throughput: 170 tests/hour, with the increase possibility (on-site modul extendability)
- Continuous reloading of samples and disposable materials during routine operation possible without interruptions
- Automatical rerun and dilution
- Very good «lot to lot» variability and stability of calibration curves

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IR1-3

Biljezi koštane pregradnje na imunokemijskom analizatoru Modular Analytics <E> tvrtke Roche Diagnostics

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Osteoporoza je metabolička koštana bolest karakterizirana smanjenom mineralnom gustoćom, promjenama

IR1-3

Bone turnover markers on immunochemistry analyzer Modular analytics <E> by Roche Diagnostics

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Osteoporosis is metabolic bone disease characterized with decreased mineral density, michroarhitetural de-

mikroarhitekture i smanjenim biomehaničkim svojstvima kosti koji mogu imati za posljedicu prijelome i deformitete. Najviše pogađa starije ljude, a žene u postmenopauzi, predstavljaju najrizičniju skupinu.
Trenutni standard u dijagnozi osteoporoze je mineralna gustoća kostiju (BMD). Međutim, ona nije prediktor gustoće kostiju u budućnosti, pokazalo se i da smanjenje rizika loma često ne korelira s odgovarajućim BMD porastom.
Biokemijski biljezi koštane pregradnje su molekule koje izravno proizlaze iz strukture i funkcije koštanog tkiva. Iako nisu specifični za određenu bolest, njihovo je uvođenje u kliničku praksu značajno poboljšalo dijagnostički potecijal jer se pokazalo da dobro koreliraju sa učestalošću prijeloma, pa primjenjeni zajedno s DXA, značajno olakšavaju odluku o tome kada početi liječenje. Dijelimo ih na:

1. biljege koštane izgradnje (ALP, BAP, osteocalcin, P1NP, P1CP)
2. biljege koštane razgradnje (β-CrossLaps, NTX, DPD, PYD).

International Osteoporosis Foundation (IOF) preporuča uporabu koštanih markera u predviđanju prijeloma i deformiteta kostiju, te u praćenju terapije. Prije davanja terapije preporuča se inicijalno određivanje β-crosslapsa i P1NP.
Oba markera se nalaze u programu imunokemijskog analizatora MODULAR ANALYTICS <E> firme ROCHE DIAGNOSTICS. To su potpuno automatizirani testovi za određivanje u serumu. Vrijednosti β-crosslapsa mjerene Elecsys® β-CrossLaps setom vrlo dobro koreliraju s određivanjem u urinu. Osim toga β-crosslaps i P1NP antigeni su vrlo stabilni na 4°C pa su uzorci stabilni i do dva dana na sobnoj tempetaturi.

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terioration of bone tissue and decreased biomehanical properties of bone, which can lead to a bone fractures and deformations. Elderly are most troubeld, and postmenopauzal women are in greatest risk.
Bone mineral densitometry (BMD) is the current standard for diagnosis of osteoporosis. But, BMD is not a predictor of future bone density and a reduction in the fracture risk does not always correlate with corresponding BMD increase.
Biochemical markers of bone turnover are related primarily to bone structure and function. Although not related specifically to any disease, they enhanced significitanly diagnostic potential in clinical praxis. In combination with DEX, they are of great help to physician in decision when to start terapy. They are divided into:

1. bone formation markers (ALP, BAP, Osteocalcin, P1NP, P1CP)
2. bone resorption markers (β-CrossLaps, NTX, DPD, PYD).

International Osteoporosis Foundation (IOF) recommends bone markers for use in therapy monitoring and prediction of fragility fractures in their guidelines. For the initial assessment before treatment selection P1NP and β-CrossLaps should be measured.
Both assays are fully automated serum assays available on MODULAR ANALYTICS <E> SYSTEM from ROCHE DIAGNOSTICS. Serum values measured with Elecsys® β-CrossLaps very vell correlate with urine samples. Beside that β-CrossLaps and P1NP antigens are very stabile at 4°C so samples can be stored at room temperature for up to 2 days.

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IR2 – Industrijom sponzorirana radionica 2 – MDLAB, IR2-1

Rutinske koagulacijske analize na koagulacijskom analizatoru ACL TOP

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ACL TOP (Instrumentation Laboratory, Italija) je potpuno automatizirani koagulacijski analizator za istodobno mjerenje rutinskih i specijalnih analiza uporabom koagulacijskih, kromogenih i imunokemijskih metoda. U ovom smo radu ispitivali svojstva analizatora u izvođenju rutin-

IR2 – Industry sponsored workshop 2 – MDLAB, IR2-1

ACL TOP performance in routine coagulation testing

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ACL TOP (Instrumentation Laboratory, Italy) is a fully automated coagulation analyzer, designed for simultaneous measurement of routine and special coagulation parameters by using clotting, chromogenic and immunological methods. In the present study we evaluated its

skih koagulacijskih analiza: protrombinskog vremena (PV, RecombiPlasTin), aktiviranoga parcijalnog tromboplastinskog vremena (APTV, SynthASil i APTT-SP) i aktivnosti fibrinogena (FIB, Fibrinogen-C XL) ispitivanjem nepreciznosti u seriji, nepreciznosti iz dana u dan i nepreciznosti kalibracijske krivulje za PV i FIB. Napravljena je i korelacija s Behring Coagulation System (BCS; Dade Behring, Njemačka) za sva 3 testa na najmanje 100 uzoraka plazme sa širokim rasponima vrijednosti uporabom slijedećih reagensa: Innovin za PV, ActinFS za APTV, Multibren U za FIB. Za nepreciznost u seriji dobiveni su koeficijenti varijacije (CV) od 1% (PV INR u terapijskom rasponu) do 7,7% (FIB u patološkom području), a za nepreciznost iz dana u dan od 2,7% (APTV u Low Abnormal Control) do 7,7% (PV u Normal Control). Dobiveni su slijedeći CV za nepreciznost standardne krivulje: PV 1,3-2,3%; FIB 4,8-7,0%. Ispitivanjem korelacije dobiveni su zadovoljavajući koeficijenti korelacije: r=0,936 za PV%, 0,944 za PV INR, 0,863 za APTV sa SynthASilom, 0,922 za APTV s reagensom APTT-SP, 0,960 za FIB, dok su prema analizi prema Blandu i Altmanu dobivene određene razlike između vrijednosti dobivene na BCS i ACL TOP. Srednja razlika za PV za sve ispitivane uzorke bila je 0,19, dok je za uzorke u terapijskom rasponu (INR-BCS=2,00-3,50) bila 0,25, a za uzorke s INR-BCS >3,50 iznosila je 1,05, što može dovesti do različitih odluka o doziranju oralnih antikoagulanata. Srednja razlika za APTV bila je manja za SynthASil (1,3) nego za APTT-SP (-4,0). Nadalje, prema referentnom intervalu preporučenom od proizvođača za SynthASil, od 63 normalna rezultata za APTV dobivena na BCS samo 31 (49%) normalan rezultat je dobiven na ACL TOP, a od 39 patoloških rezultata na BCS dobivena su 24 (66%) patološka rezultata na ACL TOP. Srednja razlika za FIB bila je -0,44 za sve ispitivane uzorke, dok je u skupini normalnih rezultata bila nešto viša (-0,7). Prema navedenim rezultatima i nakon usklađivanja s dokumentom Hrvatske komore medicinskih biokemičara o harmonizaciji laboratorijskih nalaza u koagulaciji, ACL TOP može biti pogodan analizator za koagulacijski laboratorij srednje veličine.

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performance in routine coagulation testing: prothrombin time (PT, RecombiPlasTin), activated partial thromboplastin time (aPTT, SynthASil and APTT-SP) and fibrinogen activity (FIB, Fibrinogen-C XL), by determining within-run and between-run imprecision and imprecision of the standard curve for PT and FIB. Additionally, the correlation to Behring Coagulation System (BCS; Dade Behring, Germany) was performed with the following reagents: Innovin for PT, Actin FS for aPTT and Multifibren U for FIB, by using at least 100 plasma samples in normal and pathological range for all tested parameters. Within-run coefficients of variation (CVs) ranged from 1.0% (PT INR in the therapeutic range) to 7.7% (FIB in the pathological range), and between-run from 2.7% (aPTT in the Low Abnormal Control) to 7.7% (PT in the Normal Control). The obtained CVs for standard curve imprecision were 1.3-2.3% for PT, and 4.8-7.0% for FIB. In the correlation study satisfactory correlation coefficients were obtained: r=0.936 for PT%, 0.944 for PT INR, 0.863 for aPTT with SynthASil, 0.922 for aPTT with APTT-SP, 0.960 for FIB, while according to Bland and Altman analysis we found some differences between the values obtained on BCS and ACL TOP. The mean difference for PT INR was 0.19 for all tested samples, being higher for samples in the therapeutic range (INR-BCS=2.00-3.50) and for samples with INR-BCS >3.50 (0.25 and 1.05, respectively); these differences may lead to different anticoagulant dosage decisions. The mean difference for aPTT was lower with SynthASil (1.3) than with APTT-SP (-4.0). Additionally, according to the reference interval recommended by the manufacturer for SynthASil, only 49% (31/63) of normal aPTT results on BCS were normal on ACL TOP, and 66% (24/39) of pathological results on BCS were pathological on ACL TOP. The mean difference for FIB was -0.44 for all tested samples, being greater in the group with normal FIB activity (-0.70). According to the above results and after adjustment to the document of the Croatian Chamber of Medical Biochemists on harmonization of coagulation laboratory reports, ACL TOP could be a suitable analyzer for mid-size coagulation laboratories.

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The 6th FESCC Continuous Postgraduate Course in Clinical Chemistry

NEW TRENDS IN DIAGNOSIS, MONITORING AND MANAGEMENT OF METABOLIC SYNDROME

Dubrovnik, November 11-12, 2006, Croatia

Organized by:

FESCC
Croatian Society of Medical Biochemists
Slovenian Association for Clinical Chemistry
Inter-University centre Dubrovnik

Course program…

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DIAGNOSTIC EXACTNESS OF BIOCHEMICAL MARKERS — **SUNDAY: 8^{00}-12^{00}**

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9. Pro-inflammatory and thrombotic factors
10. Approach to the treatment of MS

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Elizabeta Topić, Dragan Primorac, Stipan Janković

Medicinskobiokemijska dijagnostika u kliničkoj praksi

Ovaj suvremeni i sveobuhvatni udžbenik prikazuje primjenu medicinske biokemijske dijagnostike u kliničkoj praksi i time premošćuje edukacijsku prazninu između medicinske biokemijske dijagnostike i kliničkih disciplina. Udžbenik je podijeljen na dva glavna dijela: Uvodni dio obuhvaća značajke medicinske biokemije kao dijela znanstvene skrbi, osvrt na biološke varijacije biokemijskih i hematoloških sastojaka krvi te utjecaj različitih čimbenika na interpretaciju laboratorijskog nalaza.
Posebni dio uključuje medicinskobiokemijsku dijagnostiku hitnih stanja, bolesti srca i krvnih žila, gastrointestinalnoga sustava, urološkoga sustava, endokrinološkoga sustava, hematoloških bolesti i bolesti zgrušavanja, neuroloških bolesti i ostalih medicinskih područja. Knjiga je namijenjena studentima, odnosno dodiplomskoj nastavi iz medicinske biokemije i poslijediplomskoj nastavi različitih predmeta iz područja biomedicinskih znanosti te specijalizantima različitih medicinskih struka (interna medicina, pedijatrija, neurologija), a posebice medicinske biokemije.

Elizabeta Topić, Dragan Primorac, Stipan Janković

Medical biochemistry diagnosis in clinical practice

This modern and comprehensive textbook presents the use of medical biochemistry diagnosis in clinical practice, thereby bridging the educational gap between medical biochemistry diagnosis and clinical disciplines. The textbook is divided into two main parts. The introductory section presents the characteristics of medical biochemistry as part of the health care system, and provides a review of biological variation in biochemical and hematological blood components, along with an account of the effect of different factors on the result interpretation.
A special section is dedicated to medical biochemistry diagnosis of emergency states, cardiovascular diseases and diseases of the gastrointestinal, urinary and endocrine systems, hematological and coagulation disorders, neurological diseases, etc. The book is intended for undergraduate students of medical biochemistry, postgraduate students in various fields of biomedical sciences, and residents in different medical professions (internal medicine, pediatrics, and neurology), medical biochemistry in particular.

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Ivana Čepelak, Slavica Dodig, Boris Labar, Božidar Štraus

Medicinsko-biokemijske smjernice

Naglasak u ovom udžbeniku stavljen je na racionalni odabir laboratorijskih pretraga u kliničkom odlučivanju i na njihovu dijagnostičku pouzdanost. Gradivo je podijeljeno na 4 poglavlja. U prvom se poglavlju (*Uvod*) opisuju osnovne karakteristike laboratorijskih pretraga i njihovo korištenje u kliničkom odlučivanju te načela racionalnog odabira laboratorijskih pretraga. U drugom poglavlju, *Probiranje*, opisana su načela općeg i ciljanog probiranja. U drugom dijelu knjige, možda najvažnijem, obrađene su bolesti i patološka stanja pojedinih organa i organskih sustava. Opisane su najučestalije bolesti, navedene su dijagnostičke smjernice za njihovu dijagnozu te interpretacija laboratorijskih nalaza. Zadnje poglavlje, *Prilozi*, sadrži korisne tablice s podatcima o referentnim rasponima, kritičnim vrijednostima, terapijskim i toksičnim koncentracijama lijekova, indikacijama za laboratorijske pretrage, stabilnosti te biološkim i interferirajućim čimbenicima i kritičnim razlikama.

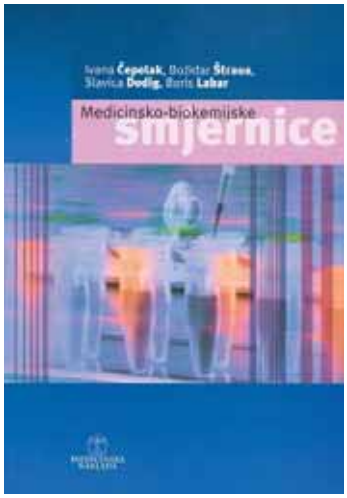
Ukratko, ovaj udžbenik sadrži najnovija saznanja iz područja kliničke biokemije i laboratorijske hematologije. Namijenjen je studentima medicine, medicinske biokemije i farmacije, općenito liječnicima i stručnjacima koji dolaze u kontakt s laboratorijskom dijagnostikom.

Ivana Čepelak, Slavica Dodig, Boris Labar, Božidar Štraus

Medical biochemistry guidelines

This textbook is focused on the rational selection of laboratory tests in clinical decision making and their diagnostic reliability. The material is divided into four chapters. Chapter one (Introduction) describes basic characteristics of laboratory tests and their use in clinical decision making, and presents the principles of rational choice of laboratory tests. Chapter two (Screening) is dedicated to the principles of general and target screening. The second and probably the most important part of the book brings a thorough account of the diseases and pathological states of particular organs and organ systems, describing the most common diseases, respective diagnostic guidelines and interpretation of test results. The last chapter (Appendices) contains useful tables providing data on reference intervals, critical values, therapeutic and toxic drug concentrations, indications for laboratory testing, stability, and biological and interfering factors and critical variations.

In brief, the textbook offers the latest state-of-the-art in the field of clinical biochemistry and laboratory hematology. It is intended for undergraduate students of medicine, medical biochemistry and pharmacy, physicians and all professionals engaged in laboratory diagnosis.



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Upute za autore

Biochemia Medica je znanstveni i stručni časopis Hrvatskog društva medicinskih biokemičara, namijenjen članovima Društva kao i stručnjacima svih ostalih područja biomedicine čiji su stručni i znanstveni interesi medicinska biokemija i laboratorijska dijagnostika. Časopis je dvojezičan s člancima na hrvatskom i engleskom jeziku. Članci se ravnopravno procjenjuju, neovisno o državi iz koje potječu autori, ukoliko su zadovoljeni osnovni kriteriji:

1. Članak je napisan u skladu s ***Uputama za autore*** časopisa *Biochemia Medica*.
2. Članak se tematski uklapa u časopis, tj. zadire u neko od slijedećih područja: opća medicinska biokemija, hematologija i koagulacija, molekularna dijagnostika, imunologija, analitička toksikologija, neurobiokemija, endokrinologija, analitičke metode, organizacija laboratorija, informatizacija i automatizacija, kontrola kvalitete i laboratorijska medicina temeljena na dokazima.

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The Journal is bilingual, with papers both in Croatian and English. All submitted manuscripts are given equal consideration regardless to what country they originate as long as the main criteria are met:

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Acknowledgments

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, statistical analysis or a department chair who provided only general support. Financial and material support should also be acknowledged.

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