Usporedba dviju imunoanaliza za tumorske biljege CA19-9, CEA i AFP

Comparison of two immunoassays for CA19-9, CEA and AFP tumor markers

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Sažetak

Uvod: Monoklonska antitijela koriste se za otkrivanje antigena u serumu koji su povezani sa specifičnim zloćudnim oboljenjima. Ti su tumorski biljezi najkorisniji za praćenje odgovora na terapiju i otkrivanje ranog recidiva, međutim, rezultati dobiveni različitima analizama razlikuju se te promjena metode tijekom praćenja može biti uzrokom problema. Cilj ove studije bio je provesti analitičku evaluaciju usporedivosti analiza za tumorske biljega CA19-9, CEA i AFP na dva različita automatizirana kemijska analizatora.

Materijali i metode: Koncentracije CA19-9, CEA i AFP su određene na analizatorima Vitros ECi (Ortho Clinical Diagnostics, Johnson & Johnson, Buckinghamshire, V. Britanija) i Cobas e 411 (Hitachi High Technologies Corporation, Tokyo, Japan). Korelacija među metodama ispitana je na 38 uzoraka seruma za CA19-9 i AFP te 39 uzoraka seruma za CEA.

Rezultati: Vrijednosti komercijalnih kontrolnih uzoraka bile su unutar raspona navedenih od proizvođača za sve tumorske biljege uključene u istraživanje na oba analizatora. Najveće odstupanje od deklariranih kontrolnih vrijednosti nađene su za CA19-9 na analizatoru Vitros ECi te za AFP na analizatoru Cobas e411. Visoka je korelacija utvrđena među metodama za sva tri ispitana tumorska biljega ($r = 0,978$ za CA19-9; $r = 0,995$ za CEA, te $r = 0,999$ za AFP). Nagib i odsječak na osi Y iznosili su 1, odnosno 0, samo za usporedbenu analizu AFP.

Zaključak: Rezultati istraživanja pokazali su najbolje podudaranje vrijednosti za AFP dobivene na dva ispitana analizatora za imunoanalize. Bez obzira na snažne korelacije, regresija po Passing-Babloku ukazala je na nižu usporedivost dviju imunoanaliza za CEA i CA19-9.

S obzirom da su rezultati studije potvrdili zapažanja iz svakodnevnog rutinskog rada, za svakoga je pacijenta od najveće važnosti da ga se prati primjenom iste imunoanalize i reagensa na istom analizatoru.

Ključne riječi: tumorski biljezi; ugljikohidratni antigen 19-9; alfa-fetoprotein; karcinoembrionalni antigen; imunoanaliza

Abstract

Introduction: Monoclonal antibodies are used to detect serum antigens associated with specific malignancies. These tumor markers are most useful for monitoring response to therapy and detecting early relapse, but results obtained by different assays vary, and a change of method during follow-up may cause problems. The aim of our study was to perform the analytical evaluation of the inter-assay comparability for tumor markers CA19-9, CEA and AFP on two different automated chemistry analyzers.

Materials and methods: CA19-9, CEA and AFP concentrations, using Vitros ECi (Ortho Clinical Diagnostics, Johnson and Johnson, Buckinghamshire, UK), and Cobas e 411 (Hitachi High Technologies Corporation, Tokyo, Japan) immunoassay analyzers, were determined. Between-method correlation was studied in 38 serum samples for CA19-9 and AFP and 39 serum samples for CEA.

Results: The values of commercial controls were within the range declared by the manufacturer for all tumor markers included in the study on both analyzers. The highest deviation from the declared control values was found for CA19-9 on Vitros ECi and for AFP on Cobas e411 analyzer. High correlation was found between methods for all of three tumor markers studied ($R = 0.978$ for CA19-9; $R = 0.995$ for CEA and $R = 0.999$ for AFP). Passing-Bablok regression slope and y-axis intercept included 1 and 0 only for AFP comparative assays.

Conclusion: Study results showed the best compliance of values for AFP obtained on two studied immunoassay analyzers. Regardless of high correlations, Passing-Bablok regression indicated lower comparability between two immunoassays for CEA and CA19-9.

As the study results confirmed the observations from daily routine, it is of utmost importance for individual patients to be monitored using the same immunoassay and reagents on the same analyzer.

Keywords: tumor markers; carbohydrate antigen 19-9; alpha-fetoprotein; carcinoembryonic antigen; immunoassay

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Uvod

Tumorski se biljezi koriste za procjenu rizika karcinoma, rano otkrivanje bolesti, probir, dijagnozu, prognozu i predviđanje uspješnosti terapije te otkrivanje recidiva ili praćenje napredovanja bolesti. Ne postoji, međutim, tumorski biljeg koji bi bio dovoljno specifičan i osjetljiv za probiranje zdrave populacije. Monoklonska se antitijela koriste za određivanje specifičnog antigena u serumu, kojeg stvaraju tumorske stanice ili stanice koje induciraju tumorske stanice domaćina (1).

Karcinoembrionalni antigen (CEA) je po prvi put opisan prije više od tri desetljeća kad je njegova prisutnost dokazana u fetalnom crijevnom tkivu te u tumorima probavnog sustava. CEA je nadalje otkriven u krvotoku bolesnika i prepoznat kao serumski biljeg kolorektalnog karcinoma. Iako se taj tumorski biljeg ne preporuča kao pretraga probiranja na kolorektalni karcinom, prijeoperacijska koncentracija CEA u serumu je korisna za dijagnozu i prognozu recidiva te preživljenja bolesnika s kolorektalnim karcinomom. Koncentracije CEA u serumu rastu s napredovanjem bolesti (2,3).

Ugljikohidratni antigen 19-9 (CA19-9) je najvredniji kao serumski biljeg karcinoma gušterače i žuči, no njegove se povišene koncentracije pojavljuju i u nekoliko drugih zloćudnih oboljenja probavnog sustava (npr. karcinoma želuca, jetre, dojke, pluća, te u kolorektalnim i ginekološkim karcinomima). Povišene se koncentracije mogu, međutim, pojaviti i u benignim bolestima. Koncentracije CA19-9 u serumu su povišene u 70-90% bolesnika s karcinomom gušterače te odražavaju opterećenje tumorom, a visoke koncentracije povezane su s nepovoljnim ishodom. Poslijeterapijsko praćenje koncentracija biljega pruža informaciju o odgovoru na terapiju i o recidivu (3-5).

Alfa-fetoprotein (AFP) je biljeg hepatocelularnog karcinoma. Koristi se za dijagnozu, prognozu, te otkrivanje recidiva bolesti, praćenje terapije, kao i probir populacije s visokim rizikom razvoja hepatocelularnoga zloćudnog oboljenja (1,6). Izmjerena koncentracija tumorskih biljega ovisi o njihovoj biološkoj i analitičkoj varijaciji (7,8). Uporaba reagensa različitih proizvođača može rezultirati različitim rezultatima pretraga za isti uzorak, čak i ako je metoda određivanja ista (uključujući uporabu standardiziranih antitijela). To može dovesti do pogrešnog tumačenja rezultata. Zbog tih se razloga, kod određivanja koncentracije tumorskih biljega moraju ispuniti zahtjevi kvalitete, a uz rezultate treba navesti mjernu metodu. Ako je metodu potrebno promijeniti, preporuča se istodobno mjerenje korištenjem obiju metoda (4,9,10).

Cilj ovog istraživanja bio je provesti analitičku evaluaciju usporedivosti analiza za tumorske biljege CA19-9, CEA i AFP na dva različita automatizirana kemijska analizatora. Rezultati su dobiveni na analizatorima Vitros ECi (Ortho Clinical Diagnostics, Johnson & Johnson, Buckinghamshi-

Introduction

Tumor markers are used for the cancer risk estimation, early detection of the disease, screening, diagnosis, prognosis, prediction of therapy success and detecting the recurrence or monitoring progression of the disease. However, there is no tumor marker sufficiently specific and sensitive for healthy population screening. Monoclonal antibodies are used for determination of specific serum antigen, produced by the tumor cells or cells induced by host tumor cells (1).

Carcinoembryonic antigen (CEA) was first described more than three decades ago, when its presence was demonstrated in fetal gut tissue and in tumors from gastrointestinal tract. Subsequently, CEA was detected in the circulation of patients and recognized as a serum marker for colorectal cancer. This tumor marker has not been advocated as a screening test for colorectal cancer; however a preoperative CEA serum level is useful for diagnosis and prognosis of recurrence and survival in colorectal cancer patients. The levels of CEA increased with increasing tumor stage (2,3).

Carbohydrate antigen (CA) 19-9 is most valuable as a serum marker for pancreatic and biliary cancer, but increased concentrations occur in several other GI malignancies (e.g. gastric, colorectal, liver cancer and also in breast, lung, and gynaecological cancers). However, elevated levels may also occur in benign diseases. Serum CA 19-9 concentrations are elevated in 70-90% of patients with pancreatic cancer; the concentrations reflect tumor burden and high concentrations are associated with adverse outcome. Post-therapeutic monitoring of marker levels provides information on treatment response and recurrence (3-5).

Alfa-fetoptotein (AFP) is a hepatocellular carcinoma marker. It is used for the diagnosis, prognosis, detecting recurrence of the disease, monitoring of therapy as well as for high-risk population screening for development of hepatocellular malignancy (1,6). The concentration of tumor markers depends on the biological and analytical variation (7,8). Using the reagents from different manufacturers can result in a different test result in the same sample, even if the method of determination is the same (including the use of standardized antibodies). That can lead to the wrong interpretation of the results. Due to these reasons, when determining the concentration of tumor markers, quality requirements must be fulfilled, and the method of determination must be reported with the results. If the method of determination needs to be changed, it is recommended to perform simultaneous determination, using both methods (4,9,10).

The aim of our study was to perform the analytical evaluation of the inter-assay comparability for tumor markers CA19-9, CEA and AFP on two different automated chemistry analyzers. Results were obtained on the analyzers Vitros ECi (Ortho Clinical Diagnostics, Johnson and Joh-

re, V. Britanija) i Cobas e 411 (Hitachi High Technologies Corporation, Tokyo, Japan).

Materijali i metode

Materijali

Usporedba metoda provedena je koristeći rutinske uzorke pacijenata koji su analizirani radi standardne dijagnostičke obrade u bolnici; ispitano je 38 uzoraka seruma na CA19-9 i AFP te 39 uzoraka na CEA. Uzorci su potjecali od hospitaliziranih bolesnika s kliničkom sumnjom ili potvrđenom dijagnozom gastrointestinalnih karcinoma koji su primljeni u Klinički zavod za nuklearnu medicinu Kliničke bolnice Dubrava u razdoblju od 21. 3. do 31. 3. 2009. godine. Svaki je serum podijeljen u dva alikvota odmah nakon centrifugiranja: jedan za određivanje koncentracije biljega na analizatoru Vitros ECi u Kliničkom zavodu za nuklearnu medicinu Kliničke bolnice Dubrava, a drugi za mjerenje koncentracije biljega na analizatoru Cobas e 411 u Kliničkom zavodu za laboratorijsku dijagnostiku Kliničke bolnice Dubrava. Serumi su dobiveni nakon centrifugiranja uzoraka krvi 10 minuta na 1006 x g u centrifugi Hettich Rotina 35 R (Hettich, Tuttlingen, Njemačka). Osim analiziranja uzoraka na analizatoru Vitros ECi, dobivene su vrijednosti kontrolnih uzoraka za CEA i AFP nakon imunoanalize BioRad, Lypochek Immunoassay Plus (Bio-Rad Laboratories, Marnes-la-Coquette, France; kontrolni broj serije 40200 - razine 1, 2 i 3). Za CA19-9 korištene su kontrole Vitros Immunodiagnostic Product Oncology Controls (Ortho Clinical Diagnostics, High Wycombe, V. Britanija, kontrolni broj serije 220). Uz analizu uzoraka na analizatoru Cobas e 411 dobivene su vrijednosti kontrolnih uzoraka PreciControl Tumor Marker (Roche Diagnostics GmbH, Mannheim, Njemačka; razine 1 i 2; kontrolni broj serije 150568).

Metode

Koncentracije tumorskih biljega u serumu i kontrolnih uzoraka određene su na analizatoru Vitros ECi kemiluminiscencijskom analizom (imunometrijskom), a na analizatoru Cobas e 411 elektrokemiluminiscencijskom analizom prema uputama proizvođača.

Kontrolni su uzorci ispitani svakog dana tijekom 10 dana koliko je trajala studija.

Analitička netočnost prikazana je kao sistemska pogreška (%), a nepreciznost iz dana u dan kao koeficijent varijacije CV (%).

Zahtjevi kvalitete (kriteriji prihvatljivosti) za sve analize postavljeni su prema Westgardovim pravilima (na temelju bioloških varijacija).

Referentni intervali koje navodi proizvođač iznosili su: 0-31,3 kIU/L za CA19-9, 0-6,5 µg/L za CEA, te 0-7 µg/L za AFP na analizatoru Cobas e 411, te 0-37 kIU/L zar CA19-9, 0-5 µg/L za CEA te 0-7,51 µg/L za AFP na analizatoru Vitros ECi.

nson, Buckinghamshire, UK) and the Cobas e 411 (Hitachi High Technologies Corporation, Tokyo, Japan).

Materials and methods

Materials

Methods comparison was conducted using routine patient samples analyzed for the purpose of the standard diagnostic work-up in our hospital. 38 sera samples for CA19-9 and AFP, and 39 sera samples for CEA. The tested samples were from hospitalized patients with clinical suspicion or confirmed diagnosis of gastrointestinal carcinomas admitted to the Department of Nuclear Medicine, Dubrava University Hospital in the period of March 21st to March 31st 2009. Each serum was divided into two aliquots immediately after centrifugation – one to determine the concentration of markers on the Vitros ECi analyzer at the Department of Nuclear Medicine, Dubrava University Hospital, and the other to determine the concentration of the markers on Cobas e 411 analyzer at Clinical Department for Laboratory Diagnostics, Dubrava University Hospital. Sera were obtained after centrifuging at 1006 x g for 10 minutes in Hettich Rotina 35 R (Hettich, Tuttlingen, Germany) centrifuge. In addition to analyzing the samples on Vitros ECi analyzer, values of BioRad, Lypochek Immunoassay Plus (Bio-Rad Laboratories, Marnes-la-Coquette, France; Control lot 40200 - Level 1, 2 and 3) control samples were obtained for CEA and AFP. For CA19-9, Vitros Immunodiagnostic Product Oncology Controls (Ortho Clinical Diagnostics, High Wycombe, UK; Control lot 220) were obtained. In addition to analyzing the samples on Cobas e 411 analyzer, values of control samples PreciControl Tumor Marker (Roche Diagnostics GmbH, Mannheim, Germany; Level 1 and 2; Control lot 150568) were obtained.

Methods

Concentrations of tumor markers in sera and control samples were determined on the Vitros Eci analyzer with chemiluminiscence assay (imunometric) and on the Cobas e 411 analyzer with electrochemiluminiscence assay according to the manufacturer instructions.

Control samples were tested every day during the 10 days study period.

Analytical inaccuracy was shown as bias (%) and day-to-day imprecision as coefeicient of variation CV (%).

Quality requirements for the tests were stated according to Westgard rules (derived from biological variations).

Expected ranges provided by the manufacturer were as follows: 0-31.3 kIU/L for CA 19-9, 0-6.5 µg/L for CEA and 0-7 µg/L for AFP for Cobas e 411 analyzer and 0-37 kIU/L for CA 19-9, 0-5 µg/L for CEA and 0-7.51 µg/L for AFP for Vitros ECi analyzer.

Statistička analiza

Za kontrolne uzorke izračunata je srednja vrijednost, minimalna i maksimalna vrijednost, kao i koeficijent varijacije te sistemska pogreška.

Za ispitivane uzorke bolesnika izračunat je medijan, minimalna i maksimalna vrijednost te 95%-tni interval pouzdanosti.

Određena je korelacija između rezultata za CA19-9, CEA i AFP te izračunat Spearmanov koeficijent korelacije. Razina značajnosti postavljena je na $P < 0,01$. Regresija po Passing-Babloku korištena je za usporedbu metoda za svaki tumorski biljeg, uključujući i Cusumov test linearnosti. Statistička analiza provedena je pomoću programske podrške MedCalc 10.1.2.0 (MedCalc, Mariakerke, Belgium).

Rezultati

Izmjerene vrijednosti komercijalnih kontrolnih uzoraka bile su unutar raspona kojega preporuča proizvođač. Rezultati nepreciznosti iz dana u dan te sistemske pogreške bili su unutar raspona poželjnih specifikacija dobivenih prema biološkim varijacijama. Navedene i izmjerene vrijednosti kontrolnih uzoraka kao i sistemska pogreška i CV% nepreciznosti iz dana u dan prikazane su u tablicama 1. i 2.

Statistical analysis

Mean, minimal and maximal measured values, as well as the coefficient of variation and bias, were calculated for control samples.

Median value, 95% CI, minimal and maximal value were calculated for tested samples.

Correlation of CA19-9, CEA and AFP results was preformed and Spearman correlation coefficient was calculated. The level of significance was set at $P < 0.01$. Passing-Bablok regression was used for method comparison for each tumor marker, including the Cusum test for linearity. Statistical analysis was performed using MedCalc 10.1.2.0 software (MedCalc, Mariakerke, Belgium).

Results

The measured values of commercial control samples were within the range recommended by the manufacturer. Results of the day-to-day imprecision and bias were in the range of desirable specificiations derived from biological variations. Declared and measured values of control samples, as well as their bias and CV% from day-to-day imprecison are presented in the Tables 1 and 2.

Tablica 1. Usporedba izmjerenih i zadanih vrijednosti komercijalnih kontrolnih uzorkaka za CEA, AFP i CA19-9 na analizatoru Vitros ECi

Table 1. Comparison of the declared values of commercial control samples for CEA AFP and CA 19-9 on Vitros ECi analyzer with the obtained values

Vitros ECi				Bio-Rad Level 2 Control LOT 40202 N = 10				Bio-Rad Level 3 Control LOT 40203 N = 10			
	Measured value mean (range)	CV %	Bias%	Declared value mean (range)	Measured value mean (range)	CV %	Bias%	Declared value mean (range)	Measured value mean (range)	CV%	Bias%
CEA (µg/L)	1.5 (1.4-1.7)	3.8	6.2	21 (16-26)	21.7 (20.6-23.2)	8.0	3.3	42 (32-52)	44.3 (42.3-46.3)	4.0	5.5
AFP (µg/L)	17.8 (16.8-19.1)	3.7	4.8	89.4 (69.7-109)	83.9 (58.9-89.4)	6.0	6.0	159 (124-194)	162 (151-171)	2.8	2.0
	Product Oncology Controls Level 1 LOT 220 N = 10			Product Oncology Controls Level 2 LOT 220 N = 10				Product Oncology Controls Level 3 LOT 220 N = 10			
CA19-9 (kIU/L)	Declared value mean (range)	Measured value mean (range)	CV %	Bias%	Declared value mean (range)	Measured value mean (range)	CV %	Bias%	Declared value mean (range)	Measured value mean (range)	CV %	Bias%
	18.1 (14.6-21.6)	19.2 (18.4-20.5)	6.2	6.1	84.6 (68.1-101)	91.8 (86.1-107)	4.1	8.5	287 (231-343)	291.8 (278-306)	3.2	1.7

Tablica 2. Usporedba izmjerenih i zadanih vrijednosti komercijalnih kontrolnih uzorkaka za CEA, AFP i CA19-9 na analizatoru Cobas e 411 s

Table 2. Comparison of the declared values of commercial control samples for CEA, AFP and CA19-9 on Cobas e411 analyzer with the declared values

Cobas e411	PreciControl TM 1 LOT 150568 N = 10				PreciControl TM 2 LOT 150568 N=10			
	Declared value mean (range)	Measured value mean (range)	CV %	Bias%	Declared value mean (range)	Measured value mean (range)	CV %	Bias%
CEA (µg/L)	5.9 (4.7-7.2)	5.9 (5.2-7.1)	3.1	0	55.6 (43.9-67.3)	56.1 (51.1-65.6)	3.1	0.9
AFP (µg/L)	11.1 (8.77-13.4)	10.7 (8.9-12.9)	4.7	3.6	87.5 (74.1-100.0)	87.3 (77.9-90.3)	3.5	5.7
CA 19-9 (kIU/L)	20.4 (14.9-25.9)	20.4 (16.0-23.7)	5.5	0	88.8 (70.2-107)	88.4 (73.8-99.7)	4.5	0.5

Najveće je odstupanje od navedenih kontrolnih vrijednosti nađeno za CA19-9 na analizatoru Vitros ECi, te za AFP na analizatoru Cobas e 411. Ukupna opisna statistika prikazana je u Tablici 3. Koeficijenti korelacije za koncentracije tumorskih biljega dobiveni iz uzoraka ispitanih na uređajima Vitros ECi i Cobas e411 iznosili su: 0,978 za CA19-9, zatim 0,995 za CEA, te 0,999 za AFP ($P < 0,001$).

Cusumov test linearnosti koji je proveden u sklopu regresije po Passing-Babloku pokazao je da nije bilo značajnog odstupanja od linearnosti ($P > 0,01$). Rezultati regresije po Passing-Babloku za CEA, AFP i CA19-9 prikazani su na slikama 1., 2. i 3. te u tablici 4.

The highest deviation from the declared control values was found for CA19-9 on Vitros ECi and for AFP on Cobas e 411 analyzer. Overall descriptive statistics is presented in Table 3. Correlation coefficients of tumor markers concentrations obtained in the tested samples on Vitros ECi and Cobas e 411 were as follows: 0.978 for CA 19-9, 0.995 for CEA and 0,999 for AFP ($P < 0.001$).

The Cusum linearity test performed on Passing-Bablok regression showed that there was no significant deviation from linearity ($P > 0.01$). Passing-Bablok regression results for CEA, AFP and CA 19-9 are shown in Figures 1, 2 and 3 and in Table 4.

Tablica 3. Opisna statistika dobivenih vrijednosti uzoraka bolesnika za sve ispitane tumorske biljege

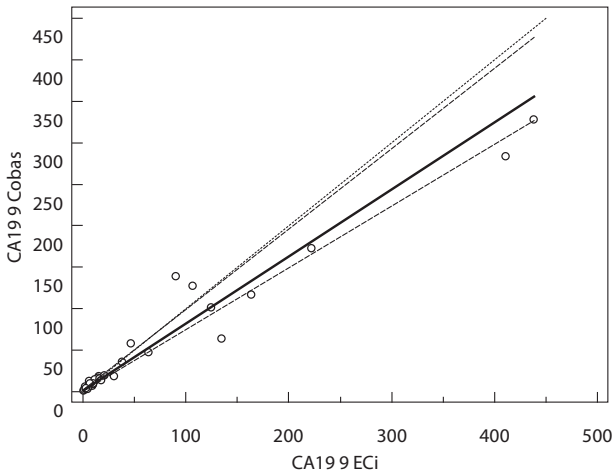
Table 3. Descriptive statistics of the obtained patients' values for all tumor markers

	CEA (µg/L)		CA19-9 (kIU/L)		AFP (µg/L)	
	Vitros ECi	Cobas e411	Vitros ECi	Cobas e411	Vitros ECi	Cobas e411
N	39	39	38	38	38	38
Median	2.00	2.20	11.15	13.02	2.75	3.10
95 CI%	1.05-4.56	1.81-3.50	6.44-23.95	7.50-18.70	1.67-4.16	1.92-4.42
Max	170.00	134.20	438.00	327.60	520.00	520.00
Min	0.46	0.46	1.10	1.00	0.60	0.61

Tablica 4. Podaci o regresiji prema Passing-Babloku – nagib i odsječak s 95%-tnim intervalom pouzdanosti (95% CI)

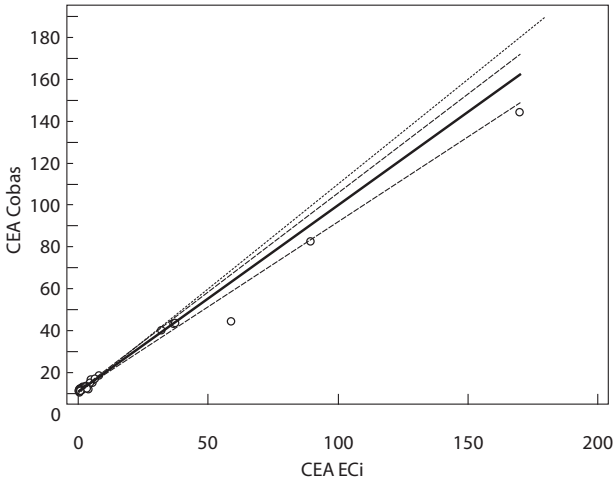
Table 4. Passing-Bablok regression data – slope and intercept with 95% CI

Tumor marker	Slope (95% CI)	Intercept (95%CI)
CEA (N = 39)	0.8870 (0.8021-0.9431)	0.7820 (0.7006-1.0283)
AFP (N = 38)	0.9996 (0.9275-1.1405)	0.1847 (-0.2633-0.5847)
CA19-9 (N = 38)	0.8102 (0.745-0.9708)	1.1832 (0.1464-1.8818)



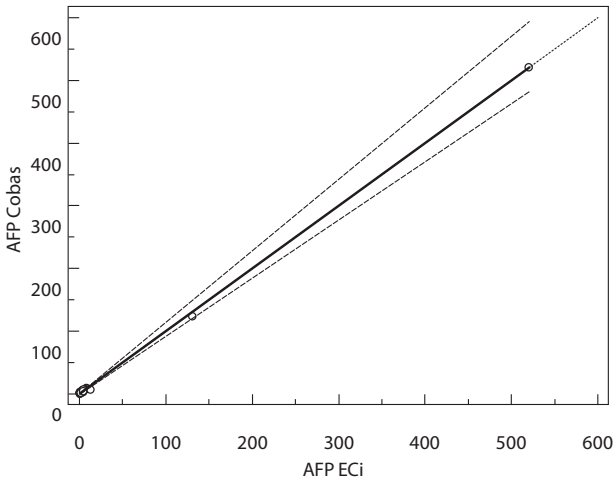
Slika 1. Regresija prema Passing-Babloku za CA19-9 određena na Vitros ECi i Cobas e 411

Figure 1. Passing-Bablok regression plot for CA 19-9 determined on Vitros ECi and Cobas e 411



Slika 2. Regresija prema Passing-Babloku za CEA određena na Vitros ECi i Cobas e 411

Figure 2. Passing-Bablok regression plot for CEA determined on Vitros ECi and Cobas e 411



Slika 3. Regresijska krivulja prema Passing-Babloku za AFP određena na Vitros ECi i Cobas e 411

Figure 3. Passing-Bablok regression plot for AFP determined on Vitros ECi and Cobas e 411

Rasprava

Cilj ovog istraživanja bio je provjeriti naše iskustvo stečeno u dnevnoj rutini o usporedivosti koncentracija tumorskih biljega dobivenih različitim metodama na različitim analizatorima. Koncentracije svih triju biljega u kontrolnim uzorcima dobivenima na oba analizatora (Vitros ECi i Cobas e 411) podudarale su s obzirom na ciljne vrijednosti koje su naveli proizvođači, no međusobno su se razlikovale. Najveće odstupanje od navedenih kontrolnih vrijednosti nađeno je za CA19-9 na analizatoru Vitros ECi te za AFP na analizatoru Cobas e411. Zanimljiva je činjenica da je najveće odstupanje od navedenih vrijednosti kod analizatora Vitros ECi bilo u normalnom te neznatno povišenom rasponu vrijednosti kontrolnih uzoraka (razine 1 i 2). To, međutim, ne uključuje CEA za koji je najveće odstupanje utvrđeno u normalnom i visokom rasponu kontrolnih uzoraka (razine 1 i 3). Važno je naglasiti da su koeficijenti korelacije za sva tri biljega pokazali visoku korelaciju njihovih specifičnih koncentracija dobivenih na oba analizatora. Podaci dobiveni korištenjem regresije po Passing-Babloku pokazali su da koncentracije CEA i CA19-9 nisu bile podudarne niti su slijedile istu linearnost (nagib i odsječak na osi Y nisu uključivali 1 ili 0), dok su koncentracije AFP dobivene na oba analizatora bile podudarne i imale istu linearnost (nagib i odsječak na osi Y uključivali su 1 ili 0). Na temelju tih podataka možemo zaključiti da nije bilo razmjerne razlike između rezultata za CEA i CA19-9. Zbog svega navedenog, usporedivost koncentracija tumorskih biljega na dva različita analizatora nije zadovoljavajuća. Koncentracije AFP dobivene na dva analizatora zadovoljile su prioritete regresije prema Passing-Babloku; nagib je bio vrlo blizu 1, a odsječak na osi Y bio je vrlo mali. Bez obzira na visoke korelacije očigledno je da postoje razlike između vrijednosti dobivenih različitim metodama na različitim analizatorima koje se ne mogu zanemariti. Za CEA i AFP dostupne su međunarodne norme SZO (CEA-IRP 73/601; AFP IRP 72/225) (4,9) koje bi trebale osigurati standardizirano i točno umjeravanje; ipak, još uvijek postoje jasne razlike među rezultatima dobivenima na dva ispitana analizatora. Prethodne studije u ovom području ukazuju da pretraga CA19-9 pokazuje značajne razlike između metoda te da se rezultati dobiveni jednom analitičkom tehnikom ne mogu prenijeti na drugu (4,11,12). Razlike između vrijednosti CEA i AFP mogu se objasniti uporabom antitijela s različitim specifičnostima. Što se tiče CA19-9, u objema metodama koristi se isto antitijelo (1116-NS-19-9), što ukazuje da su razlike među rezultatima za CA19-9 dobivene na dva različita analizatora posljedice umjeravanja i osmišljavanja metode (npr. uporabe različitih biljega). Na temelju svih prikazanih rezultata možemo zaključiti da postoje značajne razlike u koncentracijama tumorskih biljega među pojedinačnim pacijentima koje su utvrđene na različitim analizatorima i različitim meto-

Discussion

The aim of the study was to verify our daily routine experience with comparability of tumor markers concentrations obtained with different methods on different analyzers. Concentrations of all three markers in control samples obtained on both analyzers (Vitros ECi and Cobas e 411) performed well in terms of target values declared by the manufacturer, but differed between each other. The highest deviation from the declared control values was found for CA19-9 on Vitros ECi and for AFP on Cobas e 411 analyzer. An interesting fact is that the largest deviation from the declared values for Vitros ECi analyzer was in the normal and slightly elevated range of values of the control sera (Level 1 and 2). That does not include CEA that showed the largest deviation in the normal and high range of the control sera (Level 1 and 3). It is important to emphasize that the correlation coefficients for all three markers showed a high correlation of their specific concentration obtained on both analyzers. Data obtained using Passing-Bablok regression showed concentrations of CEA and CA 19-9 not to be aligned nor to follow the same linearity (slope and y-axis intercept did not include 1 or 0), while the concentrations of AFP obtained on two analyzers were aligned and followed the same linearity (slope and y-axis intercept included 1 or 0). From these data we can conclude that there is no proportional difference between the results of CEA and CA19-9. Therefore, comparability of the concentration of tumor markers on two different analyzers is not satisfactory. Concentrations of AFP obtained on two analyzers met the preferences of Passing-Bablok regression; the slope was very close to 1, and y-axis intercept was very small. Regardless of high correlations, it is evident that there are differences in values obtained with different methods on different analyzers, and that cannot be ignored. WHO international standards for CEA and AFP are available (CEA-IRP 73/601; AFP IRP 72/225) (4,9) and that should ensure standardized and accurate calibration, but nonetheless there are clear differences in the results obtained on two studied analyzers. Previous studies in this field indicate that CA 19-9 assay shows significant differences between methods and that results cannot be extrapolated from one analytical tehnique to another (4,11,12). Differences in values for CEA and AFP could be explained with the usage of antibodies with different specificities. For of CA19-9, both methods use the same antibody (1116-NS-19-9), which suggests that differences in CA19-9 results obtained on two different analyzers are the consequences of the calibration and method design (e.g. use of different markers). From all the presented results we can conclude that there are significant differences in the results of tumor markers concentrations for individual patients tested on different analyzers and with different methods. Conside-

dama. S obzirom da je broj ispitanih uzoraka vrlo mali da bi se mogli donijeti bilo kakvi čvrsti zaključci, to bi se ograničenje trebalo uzeti u obzir kod tumačenja naših rezultata. Još jedno ograničenje ovog istraživanja je nedostatak osnovnih podataka o pacijentima.

Važno je pratiti vrijednosti tumorskih biljega kod svakog bolesnika primjenom istih reagensa na istom analizatoru, unatoč njihovoj usporedivosti i visokoj korelaciji (13-16). Najveći se problem pojavljuje kod dugoročnog praćenja, jer u tijeku jedne godine bolesnik može promijeniti bolnicu ili pak laboratorij može uvesti novu metodu mjerenja tumorskih biljega. U idealnom slučaju rezultati dobiveni različitim metodama mogu biti potpuno usporedivi no, kao što pokazuje ishod ove studije, to u praksi nije zabilježeno. U slučajevima kad ta usporedivost nije moguća te kad se mijenjaju metode i analizatori potrebno je definirati novu polaznu koncentraciju tumorskih biljega za praćenje svakog bolesnika.

ring the number of studied samples is very small to make any strong conclusions, this limitation should be taken into consideration when interpreting our results. Another limitation of the study is lack of background information for the patients.

It is important to monitor the values of tumor markers of each patient with the same reagents on the same analyzer despite their comparability and high correlations (13-16). The biggest problem is the long-term monitoring, because in a course of one year the patient can change hospital or the laboratory can introduce a new method of determination of tumor markers. Ideally, the results obtained by different methods should be fully comparable, but as the outcome of this study shows, that is not noted in practice. In cases where the latter is not possible, when changing the methods and analyzers, it is necessary to define a new baseline concentration of tumor markers for monitoring each patient.

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