Laboratorijska dijagnostika autoimunih bolesti – nove tehnologije, stare nedoumice

Laboratory diagnosis of autoimmune diseases – new technologies, old dilemmas

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Sažetak

Autoimuna bolest nastaje kada imuni sistem tijela počinje napadati vlastite antigene. Glavna značajka bolesti je proizvodnja visoko afinitetnih autoantitijela. Primjenom različitih tehnika razvijaju se specifični testovi za otkrivanje autoantitijela, kao što su imunodifuzija, imunoblot, imunofluorescencija, enzimski imunotestovi i od nedavno, protočna citometrija za testove temeljene na istovremenoj višestrukoj detekciji specifičnih antinuklearnih antitijela (engl. *flow cytometry for multiplex bead-based assays*). Zbog varijabilnosti u testovima, koja je dovela do nedoumica u tumačenju rezultata, neophodno je pokrenuti odgovarajuću standardizaciju tehnika i metoda. Za dijagnostičke testove, koji se temelje na reakciji antigen-autoantitijelo, ne postoje referentne metode, nema točno definirane vrijednosti standarda, a uzorci seruma su heterogeni te se razlikuju od standarda. Tvrtke koje proizvode testove za kliničku dijagnostiku, često primjenjuju poznate komercijalne testove kao referentnu metodu, no velike razlike u kalibraciji testa vode ka neodgovarajućem tumačenju rezultata testa. Laboratoriji bi se mogli naći u nedoumici zbog tehnika koje su relevantne i pouzdane u otkrivanju prisutnosti svih klinički značajnih autoantitijela. Istodobno, primijenjena metoda trebala bi biti visoko produktivna, djelotvorna, jednostavna za primjenu i financijski povoljna.

Standardizacija autoimunih testova, bilo da se radi o „starim" ili „novim" metodama, ključna je za postavljanje dijagnoze i praćenje širokog spektra autoimunih bolesti u kliničkoj praksi.

Ključne riječi: autoimune bolesti; autoantitijela; dijagnostika; tehnologija; standardizacija

Abstract

An autoimmune disease occurs when the body's immune system begins to attack its own antigens. A hallmark is the production of high-affinity autoantibodies. Different techniques have been used to develop specific tests for autoantibody detection including immunodiffusion, immunoblotting techniques, immunofluorescence, enzyme immunoassays and recently flow cytometry for multiplex bead-based assays. Due to a variability in assays that has led to confusion in results it is inevitable to conduct appropriate standardization for techniques and methods. For diagnostics tests based on an antigen-autoantibody reaction, there are no reference methods, no exact value of the standard and serum samples are heterogeneous and differ from the standard. Clinical diagnostic companies often use a well respected commercial test as a reference method and large differences in test calibration lead to inadequate interpretation of test results. The laboratory may have dilemmas about techniques that are relevant and reliable to detect all clinically significant autoantibodies. At the same time, the applied method should be a tool for high throughput, efficient, easy to use and inexpensive.

Standardization of autoantibody assay, whether „old" or „new", is critical to their use in the clinic to predict diagnosis and treat a very diverse group of autoimmune disorders.

Keywords: autoimmune diseases; autoantibodies; diagnostics; technologies; standardization

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Uvod

Do autoimune bolesti dolazi kada imuni sustav počne napadati svoje vlastite antigene. Općenito, te su bolesti povezane s humoralnim i stanično posredovanim imunim reakcijama na jedno ili više sastavnih dijelova vlastitog tijela (1). Kada se poremeti tolerancija na vlastite antigene

Introduction

An autoimmune disease occurs when the body's immune system begins to attack its own antigens. In general, these diseases are associated with humoral or cell-mediated immune reactions against one or more of the body's own constituents (1). When self-tolerance is disturbed as

kao rezultat upalnih patogena, promijenjenog receptora, radijacije ili genetske osnove, sve to vodi poremećenom funkcioniranju imunog sustava, što nadalje rezultira povišenom razinom autoantitijela u serumu. Dijagnostika autoimune bolesti ovisi o prepoznavanju kliničkih simptoma povezanih s tom bolesti i povezana je s otkrivanjem prisutnosti autoantitijela.

Kliničari konvencionalno klasificiraju autoimune bolesti kao sistemske ili organske.

Kod sistemskih autoimunih bolesti velik je broj autoantitijela visoko specifičan za određenu bolest: anti-dsDNA, anti-Sm, anti-ribosomalna P autoantitijela u sistemskom lupusu eritromatozu (SLE), anti-topoizomeraza I (Scl-70) u sklerodermi, autoantitijela na citrulinirane proteine (anti-CCP) kod reumatskog artritisa, anti-SS-A/Ro, anti-SS-B/La kod Sjögrenovog sindroma (SjS), anti-U1-RNP, anti-PM-Scl kod miješane bolesti vezivnog tkiva (engl. *mixed connective tissue disease*, MCTD) ili anti-Jo-1 kod polimiozitisa i dermatomiozitisa.

Organske autoimune bolesti povezane su s autoantitijelima specifičnima za glavni oboljeli organ, kao što su tireoglobulin (TGA) i enzim tireoidna peroksidaza (TPO) kod tiroiditisa, inzulin i autoantitijela na dekarboksilazu glutaminske kiseline kod šećerne bolesti tipa I. i anti-mitohondrijska autoantitijela kod primarne bilijarne ciroze. Autoantitijela mogu ukazivati na status aktivnosti bolesti ili predvidjeti buduće patogeno stanje.

Laboratoriji za dijagnostiku autoimunih bolesti analiziraju i mjere sve veći broj autoantitijela širokim spektrom tehnika i metoda (2).

Prva metoda otkrivanja antinuklearnih antitijela (engl. *antinuclear antibody*, ANA) bio je 1947. godine test LE stanica (engl. *Lupus erythematosus (*LE*) cell preparation test*), kojeg je prvi primjenio Hargraves, što je povezalo autoimune bolesti sa sistemskim lupusom eritematozusom (3). Tehnika imunofluorescencije za otkrivanje ANA, koja označava specifične podskupine na temelju nuklearne ili citoplazmatske komponente, predstavljena je 1957. (4). Narednih godina laboratorijski stručnjaci i dijagnostička industrija razvili su niz tehnika i različitih metoda za otkrivanje antitijela (5).

Prvi enzimski imunotest predstavljen je 1972. i otada su razvijeni različiti oblici enzimskih imunotestova, koji su bili jednostavne, visoko produktivne analize, koje su se nadalje mogle automatizirati i standardizirati (6,7).

Tradicionalne metode, temeljene na hemaglutinacijskim reakcijama, imunodifuziji i do određenog stupnja imunofluorescenciji, sve se više zamjenjuju manje zahtjevnim testovima temeljenim na tehnikama imunoblota ili enzimskom imunotestu (engl. *enzyme immunoassay*, EIA), koji je u širokoj primjeni za otkrivanje prisutnosti ili koncentracije pojedinačnih antitijela u biološkim tekućina-

a result of inflammatory pathogens, altered receptor, radiation or genetic background, it leads to a dysfunction of the immune system, which results in elevated autoantibody in the serum. The diagnosis of autoimmune diseases depends on the identification of disease-associated clinical symptoms and is associated with the detection of autoantibodies.

Clinicians conventionally classify autoimmune diseases as either systemic or organ-specific.

For systemic autoimmune diseases, varieties of autoantibodies are highly specific for certain diseases, including anti-dsDNA, anti-Sm, anti-ribosomal P autoantibodies in systemic lupus erythematosus (SLE), anti-topoisomerase I (Scl-70) in scleroderma, autoantibodies against citrulline-modified proteins (anti-CCP) in rheumatoid arthritis, anti-SS-A/Ro, anti-SS-B/La in Sjögren's syndrome (SjS), anti-U1-RNP, anti-PM-Scl in mixed connective tissue disease (MCTD) or anti-Jo-1 in polymyositis or dermatomyositis.

Organ-specific diseases are associated with autoantibodies specific to the main affected organ, like thyroglobulin (TGA) and thyroid peroxidase enzyme (TPO) in thyroiditis, insulin and glutamic acid decarboxilase autoantibodies in type 1 diabetes mellitus and anti-mitochondrial autoantibody in primary billiary cirrhosis. The autoantibodies may represent a status of disease activity or predict a future pathogenic condition.

Autoimmunity laboratories analyze and measure an increasing number of autoantibodies employing a broad spectrum of techniques and methods (2).

The first antinuclear antibody (ANA) detection method was the "LE cell" preparation test in 1947 by Hargraves, which linked autoimmunity to the systemic lupus erythematosus diseases (3). The immunofluorescence technique for detection ANA, which denotes specific subtypes based on the nuclear or cytoplasm component, was designed in 1957 (4). In the years following, laboratory scientists and the diagnostics industry have developed a variety of techniques and different methods for the detection of antibodies (5).

The first enzyme immunoassay method was introduced in 1972, and since that time several different forms of enzyme immunoassays have been developed, which were simple, high-throughput analyses and could be automated and standardized (6,7).

Traditional assays based on hemagglutination reactions, immunodiffusion and to some degree, immunoflurescence are increasingly being replaced by less demanding tests based on immunoblotting techniques or enzyme immunoassay (EIA), widely used for detecting the presence or concentration of individual autoantibodies in biological fluids. The newly developed multiplex immunoassay enables the simultaneous determination of different

ma. Novo razvijen višestruki imunotest, gdje je veliki broj antigena imobiliziran na čvrstoj podlozi te smješten ravno ili u prostoru na česticama, omogućuje istodobno otkrivanje različitih autoantitijela (8).

Čim se pojavi nova dijagnostička ili tehnička novost te se potvrdi kao korisna, dijagnostičke tvrtke počinju razvijati novu tehnologiju s ciljem njene komercijalizacije. Suprotno tome, razvoj standarda teče sporije i zahtjeva suglasnost tvrtki za kliničku dijagnostiku i medicinskih društava (9). Standardizacija tehnika i metoda je važna jer omogućuje usporedivost rezultata različitih istraživanja te poboljšava kliničko tumačenje nalaza. Standardizacija je moguća ako su standard i analiti identični, dok su biološki uzorci heterogeni pa se razlikuju se od standarda.

Standardizacija imunotestova za otkrivanje autoantitijela je složena, zbog činjenice da ne postoje čisti standard, referentne metode niti standardni referentni materijali (engl. *standard reference materials*, SRMs). Stručne organizacije koje okupljaju stručnjake koji se bave standardizacijom imunotestova, prepoznale su taj problem te započele razvijati serumske standarde u kojima je matriks sličan matriksu iz kliničkih uzoraka. Specifičnost imunotesta uglavnom ovisi o specifičnosti epitopa na antigenu koji određuje antitijelo. Standardizacija zahtjeva puno vremena i novčanih sredstava, tako da tvrtke za kliničku dijagnostiku obično posežu za često primjenjivanim i uspješnim komercijalnim testovima kao referentnim metodama. Velike razlike u kalibriranju testova, dovele su do neodgovarajućeg tumačenja rezultata. Imajući na umu probleme u standardizaciji kod imunologije, specifičnost autoantitijela i antigena, heterogenost biološkog materijala kao uzoraka analize i poznavajući prednosti i mane starih i novih tehnologija pri otkrivanju autoantitijela, laboratoriji bi se mogli naći u nedoumici pri otkrivanju prisustva svih klinički značajnih autoantitijela. Analize različitih sustava ispitivanja koje se rade u laboratorijima ili u sklopu nekog kliničkog istraživanja, nisu uvijek međusobno zamjenjive što štetiti medicini temeljenoj na dokazima (10).

Standardizacija „starih" ili „novih" testova za otkrivanje autoantitijela postaje prijeko potrebna u kliničkoj primjeni tih testova kod postavljanja dijagnoze i predlaganja liječenja za vrlo raznovrsnu skupinu autoimunih poremećaja.

Identifikacija ljudskih autoantitijela – odgovornost laboratorija

Laboratoriji koji se bave dijagnostikom autoimunih bolesti, primjenjuju imunotestove kao osnovnu tehniku otkrivanja autoantitijela, a ne antigena (2). Važni antigeni su dobro opisani te se primjenjuju u metodama za otkrivanje autoantitijela. Antigeni se mogu izolirati iz jezgre zečje prsne žlijezde ili ljudske slezene ili mogu biti rekom-

autoantibodies, in which a large number of antigens are immobilized on a solid carrier in spatial (planar) arrays or spectral (bead-based) arrays (8).

As soon as a new diagnostic or technical invention approaches and proves valuable, clinical diagnostic companies develop the new technology and commercialize it. By contrast, the development of standards is slower and requires consensus from the clinical diagnostics companies and the medical societies (9). Standardization of techniques and methods is important because it allows the comparison of results from different studies and improves the clinical interpretation of the findings. Standardization is possible if standard and analytes are identical, but biological samples are heterogeneous and differ from the standard.

Standardization of immunoassays methods for autoantibody detection is difficult due to the fact that pure standard, reference methods and standard reference materials (SRMs) are not available. Professional organizations of experts which are involved in immunoassays standardization accepted the problem and started the development of serum-based standards in which the matrix is similar to that in clinical samples. The specificity of an immunoassay is dependent mainly on the epitope specificity of the antigen that is used for determination of the autoantibody. Standardization is time-consuming and expensive, so clinical diagnostics companies often use a well respected commercial assay as a reference method. Large differences in assay calibration lead to an inadequate interpretation of the test results. By taking into consideration the standardization problems in immunology, the specificity of autoantibodies and antigens, the heterogeneity of biological material and by knowing the advantages and deficiencies of old and new technologies in the detection of autoantibodies, the laboratory could face a dilemma about detecting all clinically significant autoantibodies. Analyses of different test systems obtained in laboratories or in different clinical studies are not always interchangeable, which impairs evidence-based medicine (10).

Standardization of autoantibody assay ″old″ or ″new″ is critical to their use in the clinic to predict diagnosis and treat a very diverse group of autoimmune disorders.

Identification of human autoantibodies – responsibility of laboratory

Autoimmunity laboratories use immunoassays as the basic technique for the determination of autoantibodies and not the antigens (2). Important antigens have been well described and they are applied in methods that are used to detect autoantibodies. Antigens can be purified from nuclei of rabbit thymus or human spleen or recom-

binantni. Mnogi od važnih autoantigena dobro su karakterizirani, što je važno za razlikovanje metoda kojima se otkrivaju autoantitijela (10,11).

Otkrivanje autoantitijela na atigene SS-A/Ro, SS-B/La, Sm, RNP, Scl-70, PM-Scl i Jo-1 je klinički vrlo korisno kod sistemskih autoimunih bolesti.

Antigen SS-A/Ro je ribonukleoprotein molekularne mase 60 i 52 kDa. Autoantitijela prisutna u serumu bolesnika mogu biti usmjerena na obje sastavnice proteina.

Antigen SS-B/La je fosfoprotein molekularne mase 47 kDa povezan s mnoštvom malih molekula RNA unutar stanice.

Antigeni Sm i RNP predstavljaju skupinu heterogenih molekula koje se sastoje iz proteina koji su povezani s malim molekulama RNA. Autoantitijela Sm reagiraju s proteinom B/B' molekularne mase 28kDa i D proteinom mase 14 kDa. Autoantitijela RNP reagiraju najjače s proteinom mase 70kDa i kod pojedinih bolesnika s C proteinom mase 32 ili 20 kDa. Zbog zamršene prirode tih antigena, metode proizvodnje tih antigena za komercijalne testove vrlo su složene.

Antigen Scl-70 je protein molekularne mase 70 kDa, sadrži 765 aminokiselina i djeluje kao topoizomeraza. Rekombinantni Scl-70 je osjetljiv, no pokazuje visoku aktivnost enzima (4,6,7).

Antigen PM-Scl je sklop od 11-16 proteina s molekularnom masom koja varira od 20 do 111 kDa (4).

Antigen Jo-1 je identičan histidil-tRNA-sintetazi i prisutan je u citoplazmi. Veliki epitop nalazi se u amino-termalnom dijelu molekule proteina.

Postoji velik broj drugih antigena koji se primjenjuju za otkrivanje autoantitijela povezanih sa specifičnim sistemskim ili organskim autoimunim bolestima. Klinička korisnost rezultata ovisi o kvaliteti laboratorijskih testova. Idealan dijagnostički test je visoko specifičan i osjetljiv. Također može prepoznati sve bolesnike koji boluju od određene bolesti te ne daje pozitivne rezultate za ispitanike koji ne boluju od bolesti (11,12).

Laboratoriji bi se mogli naći u nedoumici po pitanju tehnika koje su važne i pouzdane u otkrivanju svih klinički značajnih autoantitijela. Istovremeno, primijenjena metoda mora biti visoko produktivna, djelotvorna, financijski povoljna i lako primjenjiva (13,14).

Na laboratorijima leži odgovornost procjene svake nove tehnike i nove metode korištenjem testnih seruma bolesnika. Sustav kontrole kvalitete u kliničko-imunološkim laboratorijima uključuje procese za osiguranje točnosti i ponovljivosti rezultata. Specifikacija testa za otkrivanje autoantitijela treba sadržavati podatke o roku valjanosti testa, njegovoj preciznosti, graničnim vrijednostima i njihovom značenju, osjetljivosti i specifičnosti testa iz kojih se mogu izračunati prediktivne vrijednosti i omjer vjerojatnosti (*engl. likelihood ratio*, LR) (15). Procesu kontrole

binant. Many of the important autoantigens have been well characterized, which is important for the distinctiveness of the methods by which means autoantibodies are detected (10,11).

The detection from autoantibodies to antigens SS-A/Ro, SS-B/La, Sm, RNP, Scl-70, PM-Scl and Jo-1 is clinically useful in systemic autoimmune diseases.

The SS-A/Ro antigen is a ribonucleoprotein with molecular weight of 60 and 52 kDa. The autoantibodies present in the patients serum may be directed against both of the protein components.

The SS-B/La antigen is a 47 kDa phosphoprotein associated with a variety of small RNAs within the cell.

The Sm and RNP antigens are a group of heterogeneous molecules consisting of proteins which associate with small RNAs. Sm autoantibodies react with the 28 kDa protein B/B', and the 14 kDa D protein. RNP autoantibodies react most strongly with a 70 kDa protein and in some patients with the 32 kDa or 20 kDa C proteins. Because of the complex nature of these antigens, the methods used to produce these antigens for commercial tests are very critical.

The Scl-70 antigen is a 70 kDa protein, contains 765 aminoacids and has a topoisomerase activity. The recombinant Scl-70 is sensitive and has a high enzyme activity (4,6,7).

The PM-Scl antigen is a complex of 11-16 proteins with molecular weights varying from 20 to 111 kDa (4).

The Jo-1 antigen is identical to histidil-tRNA synthetase and is present in the cytoplasm. A major epitope is in the amino-terminal portion of the protein molecule.

There are a number of other antigens which have been used in assays for the detection of autoantibodies associated with specific systemic or organ specific diseases. The clinical usefulness of the analysis' results depends on the quality of the laboratory tests. An ideal diagnostics test has both, high sensitivity and specificity. It also identifies all patients with diseases and is not positive in those who do not have diseases (11,12).

The laboratory may have dilemmas about techniques that are relevant and reliable to detect all clinically significant autoantibodies. At the same time, the applied method must be a tool for high throughput, efficient, easy to use and inexpensive (13,14).

It is responsibility of the laboratory to evaluate each new technique and methods with test sera from local patients. Quality management in clinical immunology laboratories include procedures used to ensure the accuracy and reproducibility of tests results and specification for autoantibody tests should include data on test validity, precision, cut-off value and their meaning, and test sensitivity and specificity, from which predictive values and likelihood ratio (LR) ratio can be generated. (15). This pro-

kvalitete treba pristupiti u uskoj međusobnoj suradnji dijagnostičkih laboratorija i kliničara, koji teže ka točnim dijagnozama i okrenuti su dobrobiti bolesnicima (16).

Tehnike i metode za otkrivanje antitijela

Testovi probira za otkrivanje autoantitijela provode se za mnoštvo sistemskih i organskih autoimunih bolesti. Zahtjevi za provođenjem tih testova znatno su porasli, najviše zbog povećanog poznavanja prirode autoantitijela. Istodobni razvoj novih metoda i analitičkih sustava u kliničkoj imunologiji uključuje neprekidno povećanje izdataka za testiranje antitijela (13). Neodgovarajuća primjena laboratorijskih testova jedan je od najčešćih problema kod autoimunih bolesti, koji vodi ka postavljanju netočnih dijagnoza iz kojih proizlaze i neodgovarajuća liječenja (2).

Imunofluorescencija

Tehnika indirektne imunofluorescencije (engl. *indirect immunofluorescence*, IIF), koja kao izvor antigena koristi razne dijelove tkiva ili ljudsku staničnu liniju tumora (HEp-2), imala je veliki utjecaj na dijagnostiku autoimunih bolesti u rutinskom laboratorijskom radu (17). Kod IIF, autoantitijela iz bolesnikovih seruma prepoznaju nedefinirane antigene i daju specifične obrasce koji se trebaju tumačiti prema njihovoj povezanosti s bolesti (Tablica 1.) (18).

Obrascima indirektne imunofluorescencije pozitivnih uzoraka može se procijeniti koje specifičnosti određenog antigena treba tražiti. Poznato je da stanice HEp-2, kojima se otkrivaju autoantitijela nemaju zadovoljavajuću sposobnost davanja pozitivnih rezultata IIF za antitije-

cess needs to be attended by close collaboration of diagnostic laboratories, experienced clinicians who strive for an accurate diagnosis and patients (16).

Techniques and methods used for the detection of antibodies

Screening tests for autoantibody detection are performed for a variety of systemic and organ specific autoimmune diseases. Requests for these tests have risen remarkably, mainly due to the increased understanding of the nature of autoantibodies. A simultaneous development of new methods and analytical systems in clinical immunology has involved a constantly increasing expenditure of economic resources for the assay of antibodies (13). Inadequate use of laboratory tests is one of the most frequent problems in autoimmunity, leading to incorrect diagnoses and inadequate treatment (2).

Immunofluorescence

The indirect immunofluorescence (IIF) technique, which uses various tissue sections or the human tumor cell line (HEp-2) as an antigenic source, has had major implications for the diagnosis of autoimmune diseases in a routine laboratory setting (17). In IIF, undefined antigens are recognized by autoantibodies from the patient´s sera and yield specific patterns which must be interpreted in their relation to disease association (Table 1) (18).

The IIF staining pattern of a positive sample can be used to evaluate which appropriate antigen specificities to look for. It is known that the HEp-2 cells used for the de-

Tablica 1. Obrasci fluorescencije i njihova veza sa specifičnim autoantitijela i bolesti

Table 1. Autoantibody patterns and their relation to specific autoantibodies and disease

AA patterns	Disease association	Target organelle or antigen
Speckled	SLE, SSc, SjS,MCTD, SCLE, NLS,	Sm, RNP,SS-A/Ro, SS-B/La, PCNA, RNAP II/III, Topo I
Homogeneous/difuse	SLE, DIL,SSc, AH	dsDNA, chromatin, Histone
Peripheral/rim	SLE, APS, AH	Nuclear envelope
Centromere	RP,lcSSc,	CENP-A, B, C
Mitotic apparatus	SLE, SjS	NuMA1, NuMA2 (HsEg5)
Nucleolar	dcSSc, SLE, PM, RP	PM/Scl, fibrillarin (U3-RNP), RNAP I/III, NOR-90 (hUBF)
Cytoplasmic	PM, DM, SLE, SjS, PBC, SSc	Ribosomes, mitochondria, Golgi complex, centrosomes, endosomes, GW bodies, SRP, Jo-1

AA – autoantibody; AH – autoimmune hepatitis; APS – anti-phospholipid syndrome; CENP – centromere protein; dcSS – diffuse cutaneous SSc; DIL – drug–induced lupus; DM – dermatomyositis; GW – glycine, tryptophan containing; hUBF; 1cSSc – limited cutaneous SSc; MCTD – mixed connective tissue disease; NLS – neonatal lupus syndrome; NOR – nucleolar organizer; NuMA – nuclear mitotic apparatus; PBC – primary biliary cirrhosis; PCNA – proliferating cell nuclear antigen; PM – polymyositis; RNAP – RNA polymerase; RNP – ribonucleoprotein; RP – Raynaud´s phenomenon; SCLE – subacute cutaneous lupus erythematosus; SjS – Sjögren syndrome; SLE – systemic lupus erythematosus; SRP – signal recognition particle; SSc – systemic sclerosis.

la na SS-A/Ro-52 i Jo-1 (histidil-tRN-sintetaza) (19). Mnogi uzorci iz seruma daju zrnaste ili homogene obrasce, koji se ne mogu odmah prepoznati kao jedan od poznatih obrazaca (17). Prisutnost raznih antigena na određenom dijelu tkiva, rezultira izvrsnom ukupnom osjetljivošću. Antinuklearna antitijela, koja imaju nedefinirane specifičnosti, mogu se naći u serumu bolesnika oboljelih od širokog spektra autoimunih bolesti, zaraznih bolesti, a ponekad i kod zdravih pojedinaca.

Manjak specifičnosti može rezultirati tumačenjem koje navodi na krivi zaključak što ukazuje na ograničenja tehnike IIF za potrebe probira (6,11). Mikroskopija kod IIF je osjetljiva metoda, pa ipak, kod nje postoje neka ograničenja kao što su varijacije supstrata, neautomatizirana izvedba, subjektivno tumačenje rezultata, niska ponovljivost i manjak standardizacije. IIF zahtjeva puno vremena, rezultira niskom produktivnošću i povećanim troškovima radne snage (20,30). Kako bi se prevladala ova ograničenja, nedavno su uvedeni potpuno automatizirani IIF sustavi za tumačenje s programima za prepoznavanje obrazaca. Standardizacija testiranja autoantitijela tehnikom IIF ostaje ključno i kritično pitanje u rutinskom radu laboratorija i u njihovoj međusobnoj suradnji, no to se stanje može poboljšati automatskim sustavom tumačenja (21).

Analiza autoantitijela tehnikom IIF i dalje ostaje glavnom metodom dijagnosticiranja, no neki stručnjaci tvrde da je ta tehnika zastarjela i da je u rutinskom laboratorijskom radu treba zamijeniti enzimskim imunotestovima ili višestrukim testovima (22).

Enzimski imunotest

Enzimski imunotest ELISA (engl. *enzyme-linked immunosorbent assay*, ELISA) s antigenima izoliranim iz jezgre ljudskih staničnih linija karcinoma (HEp-2) i jezgre visoko pročišćenih antigena ili iz rekombinantnih antigena je najperspektivniji test. Postoje razlike između različitih metoda enzimskih imunotestova, pogotovo što se pozitivnosti rezultata tiče (23-26). Izolacija autoantitijela iz prirodnih izvora, kao što je ljudsko tkivo ima veliko ograničenje u pogledu ponovljivosti testa i njegove čistoće. Mnogi su proteini prisutni samo u ograničenom broju te njihova čistoća zahtjeva odstranjivanje ostalih potencijalnih antigenskih ciljeva (17). Specifičnost testa ELISA za mjerenje koncentracije autoantitijela jako ovisi o kvaliteti primijenjenih antigena te je važno da antigen ima identičnu sekvencu, konformaciju i post-translacijske modifikacije kao i ljudski antigen (20,27).

Enzimski imunotest (*engl. enzyme immunoassay*, EIA) je danas u širokoj uporabi za otkrivanje specifičnih nuklearnih ili citoplazmatskih antigena različitih skupina kod organskih imunoloških poremećaja, kao što su Graveova bolest, primarna bilijarna ciroza, šećerna bolest tipa I. ili sistemskih imunoloških poremećaja koji zahvaćaju različite

tection of autoantibodies do not have a satisfactory ability to give positive IIF results for antibodies to SS-A/Ro-52 and Jo-1 (histidil-tRNA synthetase) (19). Many serum samples give speckled or grainy homogenous staining patterns which cannot be clearly identified as one of the known patterns (17). The presence of various antigenic targets on the tissue section results in an excellent overall sensitivity. Antinuclear antibodies which have undefined specificities can be seen in the serum from patients with a wide variety of autoimmune diseases, infectious diseases and in some healthy individuals.

The lack of specificity may result in misleading interpretation and demonstrate the limitations of the IIF technique for screening purposes (6,11). Indirect immunofluorescence (IIF) microscopy is a sensitive method; yet, it has some limitations like substrate variations, manual performance, subjective result interpretation, low reproducibility and a lack of standardization. IIF is time-consuming, resulting in a low throughput and increased personnel costs. (20,30). In order to overcome this limitation, fully automated IIF interpretation systems with pattern-recognition software have been introduced recently. Standardization of autoantibodies testing by IIF remains a critical issue in and between routine laboratories and may be improved by automated interpretation systems (21).

The analysis of autoantibodies by IIF remains the hallmark of diagnosis, but some investigators have claimed that this technique is becoming out-of-date and that it could/should be replaced by enzyme immunoassays or multiplexed assays in the routine laboratory diagnostics (22).

Enzyme immunoassay

The enzyme-linked immunosorbent assay (ELISA) based either on antigens prepared from human tumor cell line (HEp-2) nuclear extracts and highly purified nuclear antigens or from recombinant antigens, has been the most promising, but the main differences, in terms of positively results, among various enzyme immunoassay methods have been described (23-26). The isolation of autoantigens from natural sources such as human tissues has major limitations with respect to reproducibility and purity. Many proteins are present only in limited amounts and their purification requires the removal of other potentially antigenic targets (17). The specificity of ELISAs for autoantibody measurements is strongly dependent on the quality of antigens used, and it is important that an antigen should have exactly the same sequence, conformation and post-translational modifications as the human antigen (20,27).

EIA is now widely used for identifying specific autoantibodies to nuclear or cytoplasmic antigens of different group of organ-specific disorders, such as Grave´s disease, primary biliary cirrhosis, insulin-dependent diabetes

organe kao što su sistemska skleroza, Sjörgenov sindrom, miješana bolest veznog tkiva ili reumatoidni artritis (18,28).

Enzimski test HEp-2 ANA EIA je automatizirana metoda visoke ponovljivosti i interne kalibracije koja je temelj standardizacije. Međutim, procjena klinički dobro definiranih uzoraka na slučaju bolesnika oboljelih od skleroderme enzimskim testom HEp-2 ANA EIA, dala je niže pozitivne rezultate od onih dobivenih metodom ANA IIF (26,29). Mnoga su istraživanja, provedena u standardiziranim uvjetima, pokazala analitičku varijabilnost različitih sustava ispitivanja.

Višestruki imunotestovi

Višestruki imunotestovi podržavaju otkrivanje višestrukih autoantitijela u jednom pokušaju u isto vrijeme.

Metode koje se temelje na mikropostroju

Imunoblot (engl. *line-blot immunoassay*) je višestruki imunotest koji može paralelno analizirati različite tipove autoantitijela. Imunoblot s trakama (engl. *line assays*) koristi gotovo jedino rekombinantne antigene, koji su imobilizirani u ravnim linijama na najlonskoj test traci. Kad ih se inkubira sa serumom, autoantitijela iz uzorka vežu se na linije antigena na traci.

Vezana autoantitijela se vizualiziraju sustavom otkrivanja bojom, koji se temelji na aktivnosti alkalne fosfataze. Rezultati se tumače usporedbom intenziteta boje linija antigena s onim linijama koje predstavljaju graničnu vrijednost (*engl. cut-off lines*). Neke su publikacije pokazale da će manji postotak IIF ANA negativnih seruma postati pozitivan ispitamo li ga imunoblotom s trakama, posebice oni za anti-SS-A/Ro (10,31,32).

Tehnologija mikropostroja kod kojeg su antigeni smješteni ravno na dnu (engl. *planar*) razvijena je i primjenjuje se za simultano otkrivanje različitih autoantitijela korištenjem *sandwich* metode imunotesta. Različiti autoantigeni imobilizirani su na mikropostroju zajedno s kontrolnim proteinima. Nizovi se nakon toga inkubiraju serumom bolesnika te se vezana autoantitijela otkrivaju označenim sekundarnim antitijelom. Većina formata testova koji se temelje na mikropostroju koristi metode otkrivanja pomoću kemiluminescencije ili fluorescencije (33,34).

Test temeljen na česticama

Kao alternativa mikropostrojima, gdje su antigeni smješteni ravno na dnu posude, razvijena je protočna citometrija, za testove temeljene na istovremenoj višestrukoj detekciji specifičnih antinuklearnih antitijela (engl. *flow cytometry for multiplex bead-based assays*). Nedavno je za otkrivanje ANA predstavljen komercijalni fluorescentni test temeljen na mikrosferi. Potencijalno niski troškovi i ušteda u vremenu mogli bi biti razlozi njegove češće rutinske primjene u istraživanju i kliničkim laboratorijima (16). Sustav imunotesta s tehnologijom mikročestica (en-

mellitus or systemic affecting different organs like systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease or rheumatoid arthritis (18,28).

The HEp-2 antinuclear antibody EIA (HEp-2 ANA EIA) is an automated method with high reproducibility and internal calibration as a basis for standardization. However, the evaluation of clinically well-defined samples in case of scleroderma patients with the use of HEp-2 ANA EIA for example yielded a lower rate of positive results compared to ANA IIF (26,29). Many studies conducted under standardized conditions showed the analytical variability of different test systems.

Multiplexed immunoassays

Multiplexed immunoassays support the identification of multiple autoantibodies from a single determination in the same time.

Microarray based assays

The line-blot immunoassay is a multiplexed immunoassay which allows the parallel analysis of different types of autoantibodies. Line assays use recombinant antigens almost exclusively which are immobilized in straight lines on a nylon test strip. When incubated with serum, autoantibodies that are present in the sample bound to the autoantigen lines on the strip. Bound autoantibodies are visualized with a color-detection system that relies on alkaline phosphatase activity. Results are interpreted by comparing the color intensities of the antigen lines with those of the cut off lines. Some publications have demonstrated that a few percent of IIF ANA negative sera will be positive using assay line assay, especially for anti-SS-A/Ro (10,31,32).

The planar microarray technology was developed and applied for the simultaneous detection of different autoantibodies using the sandwich immunoassay format. Different autoantigens are immobilized on a microarray along with control proteins. The arrays are subsequently incubated with patient sera and bound autoantibodies are detected by a labeled secondary antibody. Most of the microarray assays formats applied chemiluminescence or fluorescence based detection methods (33,34).

Bead-based assay

As an alternative to planar microarrays, flow cytometry for the analysis of bead-based immunoassays has been developed (31). Recently a commercially available microsphere-based fluorescent assay has been introduced for the detection of ANA. The potential low cost and time saving may be a reason for the routine use of these assays in the research and clinical laboratories (16). Immunoassay systems with micro-bead technology and flow cytometry detection (xMAP technology) have been applied to

gl. *micro-bead*) i određivanje protočnom citometrijom (xMAP tehnologija) primjenjuje se za mjerenje koncentracije različitih autoantitijela. Sustav rabi polistrenske mikročestoce interno označene različitim omjerima dvaju različitih fluorokroma. Svaki fluorokrom može imati bilo koji od 10 mogućih stupnjeva intenziteta fluorescencije, stoga se stvara skupina od 100 spektarskih mjesta na česticama. Antigeni usmjereni na autoantitijela vezani su na mikročestice. Svaka od 100 mikročestica koja se može razlikovati po svojoj fluorescenciji, nosi specifični imobiliziran antigen za svako autoantitijela. Istodobno, zeleni laser ekscitira vanjski reporter da započne fluorescenciju kako bi uočio i kvantificirao specifične reakcije vezane za svako autoantitijela (35). Nekoliko tvrtki dobavlja komercijalne testove za simultano određivanje različitih autoantitijela protočnom citometrijom. Procjena testova različitih proizvođača za simultano kvantitativno određivanje u istom uzorku od specifičnosti 9 antinuklearnih autoantitijela (dsDNA, SS-A/Ro, SS-B/La, Sm Sm/RNP, Scl-70, Jo-1, ribosomi i centromera B) dala je dobre rezultate (36). Klinička procjena protočne citometrije za metode temeljene na istovremenoj višestrukoj detekciji specifičnih antinuklearnih antitijela, kojom se istovremeno otkrivaju antitijela na tireoidnu peroksidazu i tireoglobulin rezultirala je dobrim slaganjem s metodom ELISA (31).

Jedan problem kod otkrivanja autoantitijela kod bolesnika je manjak stvarne kvantitativne kalibracije zbog različitih afiniteta antitijela na antigene (37). Međutim, glavno pitanje ostaje jesu li kvantitativni podaci dobiveni metodama temeljenim na istovremenoj višestrukoj detekciji specifičnih antinuklearnih antitijela identični ili barem slični onima dobivenim primjenom drugih metoda (35). Osjetljivost, pouzdanost i točnost ovih testova slične su kao i kod ELISA procedura (16,38).

Proteomička tehnologija za ispitivanje i dijagnosticiranje autoimunih bolesti

Klinička proteomika nudi mogućnosti otkrivanja bioloških biljega novih bolesti u tjelesnim tekućinama, stanicama i tkivima. Pažnja kliničke proteomike usredotočena je na analitičku i kliničku validaciju i implementaciju novih dijagnostičkih i terapijskih biljega (39).

Mikropostroj predstavlja validiranu platformu za profiliranje koncentracije proteina i njihove posttranslacijske modifikacije. Proteomičke tehnologije s platformama mikropostroja antigena omogućuju širokopojasnu karakterizaciju imunih odgovora na strane i vlastite antigene koji bi mogli biti uključeni u razvoj i napredovanje autoimune bolesti. Mikropostroji s antigenima omogućuju opsežne analize autoantitijela usmjerenih na stotine i tisuće antigena, uključujući proteine, peptide, nukleinske kiseline, makromolekularne komplekse (33).

autoantibody measurement. The system uses polystyrene microspheres labeled internally with different ratios of two different fluorochromes. Each fluorochrome can have any of the 10 possible levels of fluorescence intensity, thereby creating a family of 100 spectrally addressed bead site. The antigens corresponding to autoantibodies are bound to the microspheres. Each of the 100 microbeads that can be differentiated by their fluorescence carries a specific immobilized antigen for a single autoantibody. At the same time, a green laser excites the external reporter fluorescence to quantify the specific reaction related to each autoantibody (35). Several companies supply commercial tests for simultaneous measurement of different autoantibodies by flow cytometry. The evaluation of a assay from different producers for the simultaneous quantitative determination in the same sample of nine antinuclear autoantibody specificities (dsDNA, SS-A/Ro, SS-B/La, Sm Sm/RNP, Scl-70, Jo-1, ribosome and centromere B) yielded good results (36). Clinical evaluation of a microsphere bead-based flow cytometry assays for simultaneous determinationof anti-thyroidperoxidase and anti-thyroglobulin antibodies showed good agreement with ELISA (31).

One problem in the detection of autoantibodies from patients is the lack of true quantitative calibration because of the different affinities of the antibodies to antigens (37). However, the major questions remain regarding whether the quantitative data obtained by multiplex bead-base assays are identical to, or at the least similar, to data obtained using other methods (35). Sensitivity, reliability and accuracy are similar to those observed with ELISA procedures (16,38).

Proteomic's technologies for the study and diagnosis of autoimmune diseases

Clinical proteomics offers opportunities to identify new disease biomarkers in body fluids, cells and tissues. The focus of clinical proteomics in on the analytical and clinical validation and implementation of novel diagnostic or therapy related markers. (39)

Protein microarrays represent a validated platform for profiling protein levels and their post-translational modifications. Proteomic technologies including antigen microarray platforms enable the large-scale characterization of immune responses against forigen and self antigens that may be involved in the development and progression of autoimmune diease. Antigen microarrays allow the comprehensive analysis of autoantibodies directed against hundreds to thousands of antigens, including proteins, peptides, nucleic acids, macromolecular complexes (33).

Monoklonalna ili poliklonalna antitijela mogu služiti kao molekularne probe. Nizovi se ispituju staničnom kulturom, nadtalogom, staničnim lizatom ili serumom. Ovisno o tome koja se molekularna proba primjenjuje, u uzorku se proteini ili antitijela vežu za ravno smješten niz. Sekundarno antitijelo označeno fluorescentnom bojom prepoznaje vezane molekule ili se to događa direktno ukoliko je uzorak označen fluorescentnom bojom. Različiti čitači mogu pročitati vrijednosti inkubiranih mikropostroja na antitijelima po principu ravnog vođenja (34).

Iako je postignut napredak u razumijevanju funkcije imunog sustava, razumijevanje i poznavanje narušene regulacije koja je u podlozi i specifičnosti autoimunog odgovora ostaje ograničeno. Alteracije u genima koji kontroliraju puteve regulirajući toleranciju na vlastite stanice, ključne su u patogenezi tih bolesti. Danas postoje tehnologije DNA mikropostroja koje pružaju mnoštvo informacija o patofiziologiji koja se nalazi u podlozi autoimunih bolesti (40).

Primjena proteomičkih tehnika u dijagnosticiranju autoimunih bolesti, pomoću kojih se može predvidjeti tijek bolesti, predložiti liječenje odgovarajućom terapijom i pratiti utjecaj terapije, ubuduće će promijeniti dijagnostički proces (41).

Razlike u metodama

Razvojem novih tehnologija javlja se potreba procjene i standardizacije u odgovarajućem rutinskom radu u kliničkom laboratoriju (16).

Mnoga su istraživanja, provedena u standardiziranim uvjetima, pokazala analitičku varijabilnost različitih sustava ispitivanja (29). Specifičnosti i osjetljivost autoantitijela na različite antigene važne su značajke za postavljanje dijagnoze, no varijabilnost rezultata ovisi o izvoru antigena, ponovljivosti rezultata testa, preciznosti i točnosti te kliničkoj manifestaciji bolesti (10,42-45). Neka su ispitivanja pokazala slaganja između metoda IIF ANA i EIA (6,23-25,29), dok su druga pokazala razlike u rezultatima (17,26,42,46). Odabir testa u velikoj mjeri ovisi o kliničkim okolnostima, a viša specifičnost i osjetljivost testa jako ovise o graničnoj vrijednosti (6).

Višestruke tehnologije za istraživanje profila autoantitijela predstavljaju nove tehnologije. Mogućnost istovremenog mjerenja nekoliko analita koji su u korelaciji (engl. *multiplexing*) može prevladati neka ograničenja konvencionalnih metoda. Međutim, podaci nisu u dobroj korelaciji s rezultatima dobivenim IIF testiranjem ili EIA te ukazuju na visoke razine lažno pozitivnih i lažno negativnih rezultata (14,47-52). Postoji nekoliko važnih razlika između testa temeljenog na istovremenom višestrukom otkrivanju specifičnih antinuklearnih antitijela i ELISA testova. Primjerice, test temeljen na istovremenom višestrukom otkriva-

Molecular probes can be monoclonal or polyclonal antibodies. Arrays are probed with cell culture, supernatant, cell lysate or serum. Depending on the molecule probe used, proteins or antibodies in the sample are bound to the planar array. The bound molecules are detected by a secondary antibody marked with fluorescent dye, or directly if the sample has been fluorescently labeled. The incubated chips can be read by a variety of scanners based on planar guide technology (34).

Although progress toward understanding the immune function has been made, the understanding of the underlying dysregulation and autoimmune response specificity remains limited. Alterations in genes that control pathways regulating self tolerance are critical in the pathogenesis of these diseases. The DNA microarray technologies are now available and providing a large number of information regarding the underlying pathophysiology of autoimmune diseases (40).

The application of proteomic techniques in diagnosing autoimmune diseases, predicting a disease course, treating with the proper therapy and monitoring the impact of the therapy will change the currently valid diagnostic procedure in the future (41).

Difference between methods

With the development of new technologies, there is a need to evaluate and standardize the technologies or diagnostic kits in an appropriate clinical laboratory setting (16).

Many studies conducted under standardized conditions showed the analytical variability of different test systems (29). Specificities and sensitivities of autoantibodies against different antigens are important for the diagnosis, but variability in results depend on the source of antigen, assays reproducibility, precision and accuracy and clinical manifestation of diseases. (10,42-45). Some studies showed agreement between IIF ANA and EIA (6,23-25,29), while others demonstrated differences in results (17,26,42,46). The choice of test is highly dependent on the clinical setting and higher sensitivity and specificity strongly depend on the cut-off value (6).

Multiplex technologies for the study of autoantibody profiles are the new technologies. The possibility of a simultaneous measurement of a number of correlated analytes (multiplexing) overcomes some limitations of conventional methods. However, the data do not correlate well with results obtained from IIF testing or EIA suggesting high rates of both false-positive and false-negative results (14,47-52). There are several substantial differences between multiplex bead-based assays and ELISAs. For example, the multiplex bead-based assay uses fluorescence as a reporter system where ELISAs use

nju specifičnih antinuklearnih antitijela rabi fluorescenciju kao sustav izvještavanja, dok ELISA koristi pojačavanje enzima u kolometrijskoj reakciji. Test temeljen na istovremenom višestrukom otkrivanju specifičnih antinuklearnih antitijela hvata ligande na mikročestice u otopini dok su kod metode ELISA antigeni smješteni na ravnoj ploči s 96 jažica. Metoda ELISA općenito ispituje jedno po jedno autoantitijelo, dok je test temeljen na istovremenom višestrukom otkrivanju specifičnih antinuklearnih antitijela višestruk i može simultano rješavati svaki problem koji se pojavi kao rezultat analiziranja višestrukih liganda, kao što su npr. unakrsne reaktivnosti (53).

Postoje mjerljive varijacije u rezultatima dobivenih dijagnostičkim kitovima za testove raznih proizvođača (18,19). Te su metode heterogene, pa različiti izvori antigena i različite granične vrijednosti mogu doprinijeti većoj varijabilnosti rezultata (5). Problematičnija je činjenica, da čak i ako se primjenjuje isti kit za otkrivanje antitijela određene reaktivnosti, može doći do varijacija rezultata između laboratorija (54).

Standardizacija rezultata testova ostaje nezadovoljavajuća

Rezultati dobiveni u laboratorijima ili drugim kliničkim istraživanjima, naglašavaju potrebu za drastičnom standardizacijom primjenjivanih procesa i važnost nezavisnih kalibratora ili međunarodnih standarda.

Kliničari i proizvođači testova stoje pred izazovom pružanja kvantitativnih i definitivnih mjerenja koncentracije autoantitijela, koja se zasnivaju na pouzdanim i ponovljivim testovima, koji mogu pružiti klinički korisne informacije s visokom specifičnošću i osjetljivošću. Prisutna raznolikost metodologija testova odražava složenost standardizacije testova (55).

Nekoliko je organizacija uključeno u razne aspekte standardizacije imunotestova. Na vrhu hijerarhije standardizacije nalazi se Međunarodna organizacija za standardizaciju (engl. *International Standardization Organization*, ISO). Svjetska zdravstvena organizacija (engl. *World Health Organization*, WHO) izdaje standarde za klinički važne antigene koji se određuju imunotestovima. Uz ta službena tijela, postoji nekoliko stručnih organizacija koje vode projekte standardizacije imunotestova, npr. Međunarodna federacija za kliničku kemiju (engl. *International Federation of Clinical Chemistry*, IFCC), Međunarodna unija čiste i primjenjene kemije (engl. *International Union of Pure and Applied Chemistry*, IUPAC), Udruga američkih patologa (engl. *College of American Pathologists*, CAP), Nacionalno povjerenstvo za standarde u kliničkoj kemiji (engl. *National Committee for Clinical Laboratory Standards*, NCCLS).

Povjerenstvo za standardizaciju autoantitijela (engl. *Autoantibody Standardization Committee,* ASC) osnovano

enzyme amplification of a colorimetric reaction. The multiplex bead-based assay captures ligands onto spherical beads in suspension while ELISAs generally rely upon flat surfaces in 96-well plates. The ELISA methods generally study one autoantibody at a time, while multiplex bead based assays are multiplexed and may be a subject to any perturbations that arise from analyzing multiple ligands simultaneously, such as cross-reactivities. (53).

There are measurable variations in the results obtained from assay kits obtained from different manufacturers (18,19). These methods are heterogeneous and different sources of antigens and different cut off values may contribute to a variability of results (5). More troubling is the observation that even if the same kits are used to detect antibodies of defined reactivity, there is an inter-laboratory variation of results (54).

The standardization of test results remains unsatisfactory

Results obtained in laboratories or in different clinical studies underline the need for a drastic standardization of the used procedures and the importance of independent calibrators or international standards.

Clinical scientists and assay manufacturers are challenged to provide quantitative and definitive autoantibody measurements based on reliable and reproducible assays which can provide clinically useful information with high specificity and sensitivity. The available diversity of test methodologies reflects the complexity of assays standardization (55).

Several organizations are involved in various aspects of immunoassay standardization. At the top of the standardization hierarchy is the International Standardization Organization (ISO). The World Health Organization (WHO) issues standards for clinically important antigens determined by immunoassay. In addition to these official bodies, several professional organizations have projects concerning immunoassay standardization, e. g. the International Federation of Clinical Chemistry (IFCC), International Union of Pure and Applied Chemistry (IUPAC), College of American Pathologists (CAP), National Committee for Clinical Laboratory Standards (NCCLS).

The Autoantibody Standardization Committee (ASC) was established in the early 1980s based on the recognized needs for reference human autoimmune sera in standardization (55). The European Autoimmunity Standardization Initiative (EASI) was formed to discuss how interaction between laboratories and clinical departments could be improved in practice, how algorithms in autoantibody testing could be harmonized and what an international concept of standardization of diagnostic strategies in this area should be considered (56).

je ranih 80-ih godina prošlog stoljeća, jer je prepoznalo potrebu za referentnim ljudskim autoimunim serumima u standardizaciji (55). Europska inicijativa za standardizaciju autoimuniteta (engl. *European Autoimmunity Standardization Initiative*, EASI) osnovana je kako bi se raspravilo kako se u praksi može poboljšati međudjelovanje između laboratorija i kliničkih odjela, kako se mogu harmonizirati algoritmi kod testiranja autoantitijela i koji bi se međunarodni koncept standardizacije dijagnostičkih strategija trebao uzeti u obzir (56).

Zaključak

Laboratoriji za dijagnostiku autoimunih bolesti primjenjuju imunotestove kao osnovnu tehniku za otkrivanje prisutnosti autoantitijela. Središnji i glavni princip postupka za sve dijagnostičke testove koji ispituju koncentraciju autoantitijela je hvatanje autoantitijela iz seruma primjenjujući imobilizirane autoantigene. Međutim, postoji velika varijabilnost između tih testova koja vodi ka razlikama u rezultatima, postoji varijabilni stupanj pouzdanosti njihove primjene, čak i mogućnost pogrešnog dijagnosticiranja bolesti kod bolesnika (57). Ne postoji univerzalno rješenje tih problema, no moguće je poboljšati stupanj standardizacije tehnika i metoda. Standardizacija je međunarodni problem i poželjno je da jedna međunarodna organizacija u suradnji sa svim organizacijama odgovornim za procjenu kvalitete testova bude odgovorna za koordinaciju između dijagnostičkih znanstvenika, kliničara i tvrtki za kliničku dijagnostiku. U praksi to znači da su laboratoriji odgovorni za rješavanje nedoumice oko prikladne primjene odgovarajućih metoda za otkrivanje autoantitijela u suradnji s laboratorijskim stručnjacima, kliničarima i proizvođačima.

Conclusion

Autoimmunity laboratories use immunoassays as the basic technique for the determination of autoantibodies. The central and main procedure principle for all autoantibody diagnostic assays is the capture of autoantibodies from serum using immobilized autoantigens. However, there is an enormous variability in these tests that has led to differences in results, a variable degree of confidence in their utility and even misdiagnosis of the patient´s disease (57). There is no universal solution to resolve these problems, but it is possible to improve the standardization level for techniques and methods. Standardization is an international problem and it is desirable that one international organization in collaboration with all organizations responsible for quality assessment of assays should be responsible for the coordination between diagnostic developers, clinicians and clinical diagnostics companies. In practice, the laboratories are responsible to solve the dilemma on suitable applications of respective methods for detecting autoantibodies in cooperation with the laboratory experts, clinicians and manufacturers.

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