PLENARNE SEKCIJE

PLENARY SESSIONS

Sekcija 1 Session 1

SLOBODNI RADIKALI U CIRKULACIJI: DETEKCIJA I KLINIČKI ZNAČAJ

FREE RADICALS IN CIRCULATION: DETECTION AND CLINICAL SIGNIFICANCE

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Plenarne sekcije
Plenary sessions

KOMPLEKSNOST METABOLIZMA SLOBODNIH RADIKALA U HUMANIM ERITROCITIMA

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Produkcija slobodnih radikala u eritrocitima uglavnom se odnosi na nastajanje superoksid anjon radikala ($O_2^{\cdot -}$) putem autooksidacije oksihemoglobina u methemoglobin. Ljudski eritrociti izloženi su prooksidacionom delovanju vodonik-peroksida nastalog dismutacijom $O_2^{\cdot -}$ ili iz cirkulacije, kao i azot oksidu (NO) iz cirkulacije. Od direktnih reakcija slobodnih radikala, reakcija $O_2^{\cdot -}$ i NO uz nastajanje peroksinitrita je reakcija sa primarno štetnim posledicama po eritrocite. U eritrocitima se nalaze enzimi zaštite od oksidacionih oštećenja, kao što su superoksid dismutaza (SOD, EC 1.15.1.1), katalaza (CAT, EC 1.11.1.6), glutation peroksidaza (GSHPx, EC 1.11.1.9) i glutation reduktaza (GR, EC 1.6.4.2) kao i komponente male molekulske mase (glutation, vitamini E i C). Njihovim sadejstvom se kanališu reakcije slobodnih radikala tako da direktna oštećenja biomakromolekula budu što manja. Međutim, kako nema *de novo* sinteze enzima u maturiranim eritrocitima, kapacitet ovih sistema je ograničen, jer slobodnoradikalske vrste i direktno inhibiraju neke od enzima. Promene na enzimima i njihova inhibicija slobodnim radikalima utiču na kapacitet zaštite od oksidacionih oštećenja i relativni udeo pojedinih komponenti u ukupnom antioksidativnom potencijalu. To se može pratiti i preko promena aktivnosti pojedinačnih komponenti, ali i međusobnih odnosa između komponenti antioksidativne odbrane diskriminacionim statističkim metodama, koje ukazuju na sveukupnost i kompleksnost odnosa antioksidativnih komponenti u eritrocitima i njihov sistemski značaj.

COMPLEXITY OF FREE RADICAL METABOLISM IN HUMAN ERYTHROCYTES

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The auto-oxidation of oxyhaemoglobin to methaemoglobin generating superoxide anion radical ($O_2^{\cdot -}$) represents the main source of free radicals in the erythrocytes. Hydrogen peroxide is produced by $O_2^{\cdot -}$ dismutation or originates from the circulation. Human erythrocytes are also exposed to the prooxidative actions of nitric oxide (NO) from circulation. Free radicals that may induce reactions with direct dangerous consequences to erythrocytes are also preceded by the reaction of $O_2^{\cdot -}$ and NO producing peroxynitrite. In physiological settings, erythrocytes show a self-sustaining activity of antioxidative defence (AD) enzymes, such as: superoxide dismutase (SOD, EC 1.11.16), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSHPx, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2), as well as low molecular weight antioxidants: glutathione and vitamins E and C. Their coordinate actions protect the erythrocyte's biomacromolecules from free radical-mediated damage. Since there is no *de novo* synthesis of AD enzymes in mature erythrocytes, their defence capacity is limited. Free radicals influence antioxidative enzymes capacities and relative share of particular components in the whole antioxidative system. Therefore, by measuring changes in the activity of individual AD components, as well as their interrelations by statistical canonical discriminant methods, valuable data about the complexity, overall relations and coordinated actions in the AD system in erythrocytes and its relevance for systemic effects can be acquired.

ELEKTRONSKA PARAMAGNETNA REZONANCA – MOĆNO ORUĐE MEDICINSKE BIOHEMIJE U OTKRIVANJU MEHANIZAMA OBOLJENJA I MOGUĆIH TRETMANA

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U patofiziološkim uslovima povezanim sa oksidativnim stresom, primenjivanje određenih antioksidativnih materija može biti od koristi za ljudsko zdravlje. Elektronska paramagnetna rezonantna (EPR) spektroskopija predstavlja tehniku koja pruža jedinstveni uvid u biohemijske redoks procese, zahvaljujući svom kapacitetu da: (i) razlikuje i kvantifikuje različite reaktivne vrste, kao što su hidroksil radikal, superoksid, ugljenični radikali, vodonični atom, azot monoksid, askorbil radikal, melanin i druge; (ii) odredi antioksidativne kapacitete različitih jedinjenje, ekstrakata i namirnica; (iii) pruži informacije o drugim važnim parametrima bioloških sistema. Kombinacija EPR spektroskopije i tradicionalnih biohemijskih metoda predstavlja efikasno oruđe u ispitivanju mehanizama oboljenja i moguće antioksidativne terapije, pružajući kompletniji uvid u redoks procese u ljudskom organizmu.

ELECTRON PARAMAGNETIC RESONANCE – A POWERFUL TOOL OF MEDICAL BIOCHEMISTRY IN DISCOVERING MECHANISMS OF DISEASE AND TREATMENT PROSPECTS

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In pathophysiological conditions related to oxidative stress, the application of selected antioxidants could have beneficial effects on human health. Electron paramagnetic resonance (EPR) spectroscopy is a technique that provides unique insight into the redox biochemistry, due to its ability to: (i) distinguish and quantify different reactive species, such as hydroxyl radical, superoxide, carbon centered radicals, hydrogen atom, nitric oxide, ascorbyl radical, melanin, and others; (ii) evaluate the antioxidative capacity of various compounds, extracts and foods; (iii) provide information on other important parameters of biological systems. A combination of EPR spectroscopy and traditional biochemical methods represents an efficient tool in the studies of disease mechanisms and antioxidative therapy prospects, providing a more complete view into the redox processes in the human organism.

DIJAGNOSTIČKI I TERAPIJSKI ZNAČAJ PARAMETARA OKSIDATIVNOG STRESA KOD DECE

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Farmakoterapija oboljenja kod dece predstavlja veliki izazov s obzirom da najveći broj lekova koji se svakodnevno koristi nije pedijatrijski evalurian. Efikasnost terapije zavisi u velikoj meri od poznavanja patofizioloških procesa u organizmu dece različitih uzrasta te istraživanja u tom pravcu predstavljaju imperativ. Narušen balans u produkciji slobodnih kiseoničkih/azotnih vrsta i parametara antioksidantne zaštite značajan je patofiziološki činilac brojnih oboljenja (npr. srčana insuficijencija, plućna hipertenzija, astma, neonatalna sepsa, karcinom i dr.) u različitim dečijim uzrastnim periodima. Reaktivne kiseonične/azotne vrste imaju

DIAGNOSTIC AND THERAPEUTIC SIGNIFICANCE OF THE OXIDATIVE STRESS PARAMETERS IN CHILDREN

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Pharmacotherapy of pediatric diseases represents a major challenge considering that the majority of medicines in everyday practice have not been pediatrically evaluated. The efficacy of therapy depends to a large extent on the knowledge of pathophysiological processes in the children organism at different ages. Therefore, research in that direction is of the utmost importance. An imbalance in the production of free oxygen/nitrogen species and parameters of antioxidative protection is a significant factor in many diseases (e.g. heart failure, pulmonary hypertension, asthma, neonatal sepsis, cancer etc.) in

funkciju signalnih molekula u normalnim fiziološkim procesima. Njihova povećana produkcija može izazvati oštećenja koja mogu narušiti normalne fiziološke procese u ćeliji i u krajnjem ishodu izazvati ćelijsku smrt. U ovom radu dat je pregledni prikaz dosadašnjih ispitivanja parametara oksidativnog stresa kod dece različite starosne dobi za pojedina oboljenja. Takođe, razmotrili smo sve potencijalne dijagnostičke i terapijske mogućnosti parametara oksidativnog stesa u dečijem uzrastu.

children of different age groups. Reactive oxygen/nitrogen species serve as cell signaling molecules for normal biologic processes. An increase in their generation can cause damages which can disrupt normal physiological cellular processes and eventually cause cell death. This review outlines the previous assessments of oxidative stress parameters in children of different ages for some diseases. Also, the potential diagnostic and therapeutic possibilities for the oxydative stress parameters in children have been considered.

Sekcija 2 Session 2

DIJAGNOSTIKA TIROIDNE BOLESTI | THYROID DISEASE DIGNOSTICS

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Plenary sessions

DIJAGNOSTIKA TIROIDNE BOLESTI: PRINCIPI I PROBLEMI

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Konceptualno, poremećaji štitaste žlezde se mogu svrstati u četiri grupe: 1. poremećaji morfologije štitaste žlezde, 2. poremećaji tiroidne funkcije, 3. prisustvo tiroidne autoimunosti i 4. dijagnoza i praćenje karcinoma štitaste žlezde. Naravno, ove grupe se često preklapaju. Za dijagnostiku poremećaja morfologije štitaste žlezde najbitniji je ultrazvučni pregled. Za dijagnozu poremećaja tiroidne funkcije neophodno je određivanje TSH i tiroidnih hormona. Prisustvo tiroidne autoimunosti potvrđuje se merenjem antitela na tiroidno specifične antigene. Za dijagnozu, praćenje i prognozu autoimunih bolesti štitaste žlezde koriste se antitela na tiroidnu peroksidazu (TPO), tireoglobulin (TG) i antitela na TSH receptore. Određivanje tireoglobulina u serumu nema značaj u dijagnostici karcinoma štitne žlezde, ali se koristi u praćenju bolesnika lečenih od diferentovanog karcinoma tiroide. Medularni tiroidni karcinom (MTK) sekretuje kalcitonin i karcinoembrioni antigen (CEA), ali je kalcitonin specifičan za MTK. Kod obolelih od MTK neophodno je genetsko testiranje a u pozitivnim slučajevima potrebno je i gensko testiranje srodnika.

DIAGNOSIS OF THYROID DISEASE: PRINCIPLES AND PROBLEMS

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Conceptually, thyroid disorders can be classified into four groups, namely: 1. disorders of thyroid morphology, 2. disorders of thyroid function, 3. presence of thyroid autoimmunity, and 4. diagnosis and follow-up of thyroid carcinoma. Of course, these groups are non-exclusive, and often there is overlap between the groups. Ultrasound exam is a standard for the diagnosis of the disorders of thyroid morphology. To diagnose disorders of thyroid function TSH and thyroid hormones should be measured. Presence of thyroid autoimmunity is confirmed by measuring antibodies against thyroid-specific antigens. Thyroid peroxidase (TPO), thyroglobulin (Tg) and TSH receptors antibodies are used in the diagnosis, follow-up and prognosis of autoimmune thyroid disorders. The measurement of serum thyroglobulin has no role in the diagnosis of thyroid cancer, but it is used in the follow-up of patients treated for differentiated thyroid carcinoma of the follicular epithelium. Medullary thyroid cancer (MTC) produces calcitonin and carcinoembryonic antigen (CEA), but calcitonin is specific for MTC. In subjects with MTC, genetic testing should be done, and in positive cases family screening is necessary.

ZNAČAJ ODREĐIVANJA HORMONA I PROTEINA U MATERIJALU DOBIJENOM ASPIRACIJSKOM PUNKCIJOM TANKOM IGLOM

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Više od pola veka iskustva sa aspiracijskom punkcijom nodoznih promena u štitastoj žlezdi utvrdilo je ovaj postupak kao zlatni standard u ispitivanju tiroidne

IMPORTANCE OF HORMONES AND PROTEINS DETERMINATION IN THE MATERIAL OBTAINED BY FINE-NEEDLE ASPIRATION

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More than a half century of experience with aspiration punch of nodal changes in the thyroid gland has confirmed this procedure as a golden standard in the

nodozne bolesti. Iako su osetljivost, specifičnost, pouzdanost i reproducibilnost dokazano visoke, ovaj postupak ipak u skoro petini slučajeva ne može jednoznačno da odgovori da li je ispitivana promena benigne ili maligne prirode. Mnogobrojni pokušaji da se postupak popravi doveli su do značajnog poboljšanja vrednosti njime dobijenih nalaza. Pored rafiniranja tehnika citopatoloških pretraga, dokazivanje ili određivanje hormona, proteina i drugih supstanci u materijalu dobijenom aspiracijom tankom iglom danas predstavlja najveći doprinos u poboljšanju dijagnostičke vrednosti postupka. Ovi belezi se danas u najvećem broju centara prate u aspiratima nodoznih promena u štitastoj žlezdi ali i okolnih limfnih nodusa kako bi se sa većom sigurnošću ocenile vrsta promene, obim i stepen proširenosti, što je od značaja u pripremi terapijskih postupaka ali i za ocenu rezidualne bolesti posle primenjenog lečenja.

examination of thyroid nodal disease. Although sensitivity, specificity, reliability and reproducibility are incontestably high, this procedure cannot give a simple answer on whether the change examined is benign or malignant. Numerous attempts to improve the procedure resulted in considerably advanced findings. Besides refining the cytopathologic examination techniques, confirmation or determination of hormones, proteins and other substances in the material obtained by fine-needle aspiration are actually the greatest contribution to improvement the of procedure's diagnostic value. These markers are actually followed, in most medical centers, in aspirates of thyroid nodal changes but also surrounding lymph nodes in order to evaluate with greater certainty the type, volume and spread; this is important to establish treatment procedures and to evaluate the residual disease after accomplishing the treatment.

GENETIKA KARCINOMA ŠTITASTE ŽLEZDE: DIJAGNOSTIČKE I KLINIČKE IMPLIKACIJE

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Apstrakt nije dostavljen.

GENETICS OF THYROID CANCER: DIAGNOSTIC AND CLINICAL IMPLICATIONS

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Summary not submited.

MERENJE KONCENTRACIJE TIREOGLOBULINA KOD PACIJENATA SA DIFERENTOVANIM KARCINOMIMA ŠTITASTE ŽLEZDE

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Tiroidni karcinomi su najčešći maligni endokrini tumori. Tireoglobulin (Tg), specifični protein štitaste žlezde, najvažniji je tumorski marker u tireoidnoj onkologiji. Kod pacijenata sa diferentovanim karcinomima tireoideje, nakon operativnog lečenja, koncentracija Tg određuje se radi otkrivanja rezidualnog tumorskog tkiva ili postojanja lokalnih, odnosno udaljenih metastaza. Na koncentraciju Tg u serumu utiču: masa prisutnog tireoidnog tkiva (benignog ili malignog), intenzitet stimulacije receptora za tireostimulišući hormon (TSH) i sposobnost tumorskih

MEASURING THYROGLOBULIN CONCENTRATIONS IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

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Thyroid carcinomas are the most common malignant endocrine tumors. Thyroglobulin (Tg), a specific thyroid protein, is the most important tumor marker in thyroid oncology. After total thyroidectomy or radioiodine therapy, detectable or increasing serum Tg levels in patients with differentiated thyroid carcinoma indicate persistence of active thyroid tissue or cancer recurrence. Serum Tg concentration primarily reflects three variables: the mass of differentiated thyroid tissue present; the degree of thyrotropin receptor stimulation and the intrinsic ability of the tumor to synthesize and

ćelija da sintetišu i luče Tg. Savremene metode, imunometrijske (IMA) i radioimunološke (RIA), kojima se određuje koncentracija Tg u serumu ispitanika, imaju određena ograničenja koja mogu da umanje klinički značaj dobijenih rezultata. Usled metodoloških razlika, koncentracije Tg u istim uzorcima seruma, izmerene različitim testovima, mogu se razlikovati. Faktori koji mogu prouzrokovati razlike u izmerenim koncentracijama Tg su brojni: različiti referentni materijali, razlike u specifičnosti primarnih i sekundarnih antitela za antigenske determinante Tg, različit afinitet vezivanja tih antitela za epitope Tg, i interferencija serumskih faktora. Princip testa, kao i eventualno prisustvo TgAt u serumima ispitanika, može uticati na izmerenu koncentraciju Tg. Svako odstupanje izmerenih koncentracija Tg od stvarnih vrednosti može imati ozbiljne posledice: lažno niske vrednosti Tg mogu odložiti neophodni tretman pacijenata, dok lažno povećane vrednosti Tg mogu prouzrokovati nepotrebni stres, ili čak tretman pacijenata. I pored ograničenih mogućnosti savremenih metoda, određivanje koncentracije Tg u serumu pacijenata operisanih od diferentovanog tiroidnog karcinoma je koristan test za otkrivanje pogoršanja bolesti i za praćenje efekata terapije.

secrete Tg. Measurement of serum Tg by current immunometric (IMA) and radioimmunological (RIA) assays encounters some methodological problems which can diminish its clinical importance. Discrepancy between the results for Tg using different methods may be caused by: different reference materials, specific properties of the primary and secondary antibodies for antigenic determinants on Tg and diverse binding affinities of these epitopes, together with interference by serum factors (usually antibodies to Tg (TgAb)) with the primary and secondary Tg antibodies from the diagnostic set. In the presence of endogenous TgAb, Tg values measured by immunoradiometric assay (IRMA) and similar assays are usually lower than the real concentrations, while in RIA apparently lower or higher results can be obtained. Falsely low values may lead to delay in necessary treatment, while an inappropriately high Tg value can cause patient anxiety and unnecessary scans. Despite current methodological limitations, serum Tg measurement is a useful test for determining worsening disease and monitoring the effects of therapy in patients who have undergone surgery for differentiated thyroid carcinoma.

Sekcija 3 Session 3

NOVI BIOHEMIJSKI MARKERI | NEW BIOCHEMICAL MARKERS

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Plenary sessions

KLINIČKA VREDNOST LIPOPOLISAHARID-VEZUJUĆEG PROTEINA (LBP) I INFEKCIJI I SEPSI

D. Schmidt

Medical Marketing & Scientific Support
Siemens Healthcare Diagnostics GmbH,
Eschborn, Germany

Apstrakt nije dostavljen.

CLINICAL VALUE OF LIPOPOLYSACCHARIDE-BINDING PROTEIN (LBP) IN INFECTION AND SEPSIS

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Summary not submited.

ESR TEST: STARI TEST SA NOVOM NAMENOM

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Brzina sedimentacije eritrocita (ESR) ostaje jedan od najčešće korišćenih laboratorijskih testova. Kliničku primenu i korisnost ovaj test ima u praćenju inflamatornih bolesti, naročito reumatoidnog artritisa, temporalnog arteritisa i reumatske polimijalgije. Referentni metod za merenje ESR predložen od strane Međunarodnog komiteta za standardizaciju u hematologiju koristi punu krv sa antikoagulansom EDTA za izvođenje testa pomoću metode koju je 1921. opisao Westergren. Trenutno zanimanje za metodologiju fokusirano je na razvoj automatizovanih zatvorenih sistema koji omogućavaju određivanje brzine sedimentacije uz odabrane radne metode, koje koriste jedan uzorak za više hematoloških testova i unapređuju biohazardne aspekte postupka testiranja. Usled toga, standardizacija postaje neophodna. Rezultati ESR moraju biti pouzdani uprkos povećanom broju različitih metoda i varijabli za testiranje. Danas su dostupni kontrolni materijali i šeme za osiguranje spoljašnjeg kvaliteta i treba ih koristiti. Dakle, inovativne tehnike mogu dalje opravdati korisnost ESR u kliničkoj praksi, ali pored toga moraju garantovati sledivost rezultata u poređenju sa referentnom metodom kako bi se obezbedila uporedivost rezultata između više kliničkih laboratorija.

THE ESR TEST: AN OLD TEST WITH NEW CONTENTS

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The erythrocyte sedimentation rate (ESR) remains one of the most widely used laboratory tests. Its clinical usefulness and interpretation are in the monitoring of inflammatory diseases, in particular rheumatoid arthritis, temporal arteritis and polymyalgia rheumatica. At present, the reference method for measuring the ESR proposed by the International Committee for Standardization in Haematology (ICSH) utilizes EDTA-anticoagulated-undiluted blood to perform the test using the method described by Westergren in 1921. Current interest in the methodology focuses on the development of an automated closed system that allows the determination of the sedimentation rate with selected working methods, using a single sample for more than one haematological test, improving the biohazardous aspects of the testing procedures. As a consequence, standardization becomes necessary. ESR results should be reliable, despite the increased number of different methods and testing variables. Control materials and External Quality Assurance Schemes are now available, and should be used. In conclusion, innovative techniques may improve the appropriateness and usefulness of ESR in clinical practice, but in addition, they need to guarantee the traceability of results in comparison to the reference method in order to ensure comparability of results among different clinical laboratories.

PRAĆENJE VENSKOG TROMBOEMBOLIZMA (VTE) POMOĆU D-DIMERA – ŠTA LABORATORIJA I KLINIČARI TREBA DA ZNAJU?

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D-dimer je proizvod razgradnje fibrina koji se u serumu može otkriti pomoću više različitih testova. Zahvaljujući visokoj osetljivosti na prisustvo tromba i neinvazivnom karakteru, merenje D-dimera se često pri menjuje kao test prve dijagnostičke linije da bi se isključila bolest kod pacijenata sa sumnjom na duboku vensku trombozu (DVT) ili plućnu emboliju (PE). S druge strane, specifičnost testa za D-dimer je niska, što sprečava njegovu samostalnu upotrebu prilikom donošenje dijagnoze (čak i sumnje na) VTE. Dostupni testovi za D-dimer su heterogeni i nisu svi prošli isti nivo kliničke validacije. Svaki test za D-dimer mora biti integrisan u sveobuhvatne, validirane sekvencijalne dijagnostičke strategije koje obuhvataju kliničku verovatnoću procene i imaging tehnike, kao što je ultrasonografija venske kompresije u donjim ekstremitetima kod sumnje na DVT ili multislajs kompjuterizovana tomografija kod sumnje na PE. Rezultate testa za D-dimer potrebno je tumačiti u kombinaciji sa procenom kliničke izvesnosti: negativni test dozvoljava isključivanje VTE kod pacijenata sa malom kliničkom izvesnošću za testove aglutinacije pune krvi i kod pacijenata sa nevelikom kliničkom izvesnošću za najosetljivije testove (ELISA ili neki imunoturbidimetrijski testovi). Korisnost testa za D-dimer je naročito velika u ambulantnim kliničkim uslovima, jer dozvoljava isključivanje VTE bez daljeg testiranja kod približno jedne trećine sumnjivih pacijenata. Neka stanja povezana sa povišenim nivoima D-dimera ograničavaju korisnost testa u kliničkoj praksi: trudnoća, primljeni i pacijenti posle hirurške intervencije, pacijenti sa istorijom prethodne VTE, maligniteti, stariji pacijenti. Međutim, čak i kada su takva stanja prisutna, osetljivost D-dimera ostaje nepromenjena a test je često i dalje finansijski isplativ. Dalji klinički razvoj testa za Ddimer uključuje validaciju novih *cut-off* vrednosti kod starijih pacijenata i predviđanje rekurentne VTE posle prve tromboembolijske epizode.

D-DIMER IN THE MANAGEMENT OF VENOUS THROMBOEMBOLISM (VTE) – WHAT LABORATORIANS AND CLINICIANS SHOULD KNOW?

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D-Dimer is a degradation product of cross-linked fibrin. It can be detected in serum using a variety of different assays. Thanks to its high sensitivity to the presence of a thrombus and to its non-invasiveness, D-Dimer measurement is widely used as a first-line test to rule out the disease in patients with suspected deep vein thrombosis (DVT) or pulmonary embolism (PE). On the other hand, D-Dimer test specificity is low, precluding its use alone for ruling in (and even for suspecting) the diagnosis of VTE. Available D-Dimer assays are quite heterogeneous, and not all of them received the same level of clinical validation. Each D-Dimer assay must be integrated in comprehensive, validated sequential diagnostic strategies that include clinical probability assessment and imaging techniques such as lower limb venous compression ultrasonography for suspected DVT or multi-slice computed tomography for suspected PE. D-Dimer test result needs to be interpreted in combination with clinical probability assessment: a negative test allows ruling out VTE in low-clinical probability patients for whole blood agglutination assays and in non-high clinical probability patients for the most sensitive tests (ELISA or some immunoturbidimetric assays). The usefulness of D-Dimer test is particularly high in outpatient settings, allowing ruling out VTE in approximately one third of suspected patients without further testing. Some conditions are associated with increased d-dimer levels and limit D-Dimer test usefulness in clinical practice: pregnancy, admitted and post-surgical patients, patients with a history of previous VTE, malignancy, elderly patients. However, even when these conditions are present, D-dimer sensitivity is not altered, and the test is often still cost-effective. New clinical developments for D-Dimer test include validation of new cut-off values in elderly patients, and prediction of recurrent VTE after a first thromboembolic episode.

»POINT-OF-CARE« TESTIRANJE D-DIMERA

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Određivanje D-dimera omogućava efikasno isključivanje venskog tromboembolizima (VTE). Laboratorijsko testiranje D-dimera pretežno se izvodi u centralizovanim laboratorijama u okviru bolnica, iako se većina pacijenata kod kojih se sumnja na VTE javlja na pregled u ustanove primarne zdravstvene zaštite. Takođe, skraćenje ukupnog trajanja laboratorijskog testiranja bi znatno poboljšalo efikasnost u urgentnim centrima. Stoga bi uvođenje brzog »Point of Care (POC)« D-dimer testa koji se lako izvodi, dovelo do poboljšanja dijagnostike VTE u primarnoj zdravstvenoj zaštiti kao i u urgentnim centrima, ali takođe i u dijagnostici drugih teških kliničkih stanja (diseminovana intravaskularna koagulacija (DIK), aneurizma aorte) u kojima je D-dimer povišen. Većina dostupnih POC D-dimer testova ispunjava kriterijume za brzo i sigurno isključivanje VTE-a. U našem ispitivanju tri testa (Stratus, Pathfast i Cardiac) pokazala su karakteristike slične D-dimer testu koji je u rutinskoj upotrebi u našoj laboratoriji (Tinaquant).

»POINT-OF-CARE« D-DIMER TESTING

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D-dimer testing is efficient in the exclusion of venous thromboembolism (VTE). D-dimer laboratory assays are predominantly performed in centralised laboratories in intra-hospital settings although most patients with suspected VTE are presented in primary care. On the other hand decreasing turnaround time for laboratory testing may significantly improve efficacy in emergency departments. Therefore an introduction of a rapid, easy to perform point of care (POC) assay for the identification of D-dimer may offer improvement in diagnostics flow of VTE both in primary care and emergency departments while it could also improve our diagnostic possibilities in some other severe clinical conditions (e.g. disseminated intravascular coagulation (DIC) and aortic aneurism (AA)) associated with increased D-dimer. Several POC D-dimer assays have been evaluated and majority of them have met the criteria for rapid and safe exclusion of VTE. In our hands three assays (Stratus, Pathfast and Cardiac) have the laboratory performance profile comparable with our routine D-dimer laboratory assay (Tinaqaunt).

OSETLJIVI TESTOVI ZA SRČANI TROPONIN: MIT I MAGIJA ILI PRAKTIČAN NAPREDAK?

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Srčani troponini (cTn) smatraju se »zlatnim standardom« među biomarkerima za dijagnostikovanje akutnog koronarnog sindroma (ACS), patološkog spektra koji obuhvata srčanu ishemiju, anginu, infarkt miokarda i konačno prestanak rada srca. Sve veći broj dokaza koji idu u prilog dijagnostičkoj i prognostičkoj upotrebi cTn u ACS doveo je do opšteg ponovnog definisanja akutnog infarkta miokarda (AMI). Dijagnoza AMI uključuje detekciju povišenog cTn (ili CK-MB) – najmanje jednom u 24 časa od srčane epizode izmeren je nivo > gornjeg 99. procenta referentne populacije – uz dokaze o ishemiji miokarda. Izrađeno je nekoliko veoma osetljivih imunoeseja s navodno superiornom

SENSITIVE CARDIAC TROPONIN ASSAYS: MYTH AND MAGIC OR A PRACTICAL WAY FORWARD?

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Cardiac troponins (cTn) are considered to be the 'gold standard' biomarkers for the diagnosis of acute coronary syndrome (ACS) a pathological spectrum which includes cardiac ischemia, angina, myocardial infarction and ultimately cardiac failure. The growing evidence base for the diagnostic and prognostic use of cTn in ACS has resulted in a universal redefinition of acute myocardial infarction (AMI). A diagnosis of AMI includes the detection of an elevated cTn (or CK-MB) with at least one measurement within 24 hours of the cardiac episode being >upper 99th percentile of a reference population, in conjunction with evidence of myocardial ischemia. A number of high sensitivity

nepreciznošću i 99. procentilnom vrednošću koji se može definisati. U kliničkom smislum, oni imaju dvojaku važnost. Prvo, postoji težnja da se vrednosti promene u cele brojeve, menjanjem jedinice koja unosi zabunu. Drugo, gotovo normalna Gaussova raspodela osetljivog cTn kod zdravih subjekata povećaće učestalost pozitivnog cTn u populaciji bez ACS. Problem je kako utvrditi da li su ti blago povišeni nivoi cTn od kliničkog značaja. Ono što je sigurno jeste da AMI ostaje klinička a ne biohemijska dijagnoza i da se tumačenje koncentracije cTn mora izvoditi u skladu s kliničkim kontekstom.

DIJAGNOSTIČKE I PROGNOSTIČKE INFORMACIJE DOBIJENE ODREĐIVANJEM VISOKO OSETLJIVOG SRČANOG TROPONINA T

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Srčani troponini (cTns) preporučuju se kao biomarkeri za dijagnozu akutnog infarkta miokarda, procenu rizika i prognoze, kao i za određivanje antitrombotske terapije i strategije revaskularizacije kod pacijenata sa akutnim koronarnim sindromima. Implementacija visokoosetljivih testova za cTn u kliničkoj praksi je povećala broj pacijenata kod kojih je dijagnostikovan infarkt miokarda. Pored toga, povećan je i broj pacijenata sa povišenim nivoima cTn koji se ne mogu pripisati akutnom ishemijskom oštećenju, što je primećeno kod pacijenata sa hroničnim srčanim bolestima i drugim neishemijskim srčanim oštećenjima ili kod pacijenata sa oštećenom renalnom funkcijom. Nova definicija infarkta miokarda podržava tumačenje povišenih cTn izmerenih pomoću visokoosetljivih testova za cTn kod pacijenata kod kojih se sumnja na akutni koronarni sindrom. Ovde se prikazuju kliničke studije sa nedavno uvedenim visokoosetljivim testom za cTnT (TnT hs) uz uopšteni osvrt na iskustva sa visokoosetljivim cTn testovima.

immunoassays with claims of superior imprecision and a definable 99th percentile have been produced. Clinically, these have two important impacts. First, there is a drive to change the values into whole numbers by the application of a unit change which carries the scope for confusion. Secondly, the near-normal Gaussian distribution of sensitive cTn in healthy subjects will increase the frequency of cTn positivity in the non-ACS population. The problem is to decipher if such minor elevations in cTn are of clinical concern. What is certain is that AMI remains a clinical not a biochemical diagnosis and the interpretation of cTn concentrations should be made according to the clinical context.

DIAGNOSTIC AND PROGNOSTIC INFORMATION PROVIDED BY A HIGH SENSITIVITY ASSAY FOR CARDIAC TROPONIN T

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Cardiac troponins (cTns) are the preferred biomarkers for the diagnosis of acute myocardial infarction, assessment of risk and prognosis, and for determination of antithrombotic and revascularization strategy in patients with acute coronary syndromes. The implementation of high sensitivity cTn assays into the clinical routine has increased the number of patients diagnosed with myocardial infarction. In addition, the number of patients with elevated cTn levels that cannot be explained by acute ischemic injury was increased, which is observed in patients with chronic heart disease and other nonischemic cardiac injury or in patients with impaired renal function. The new definition of myocardial infarction provides support for the interpretation of elevated cTn measured with high sensitivity cTn assays in patients with suspected acute coronary syndrome. This review will summarize clinical studies with the recently introduced high sensitivity cTnT assay (TnT hs) with reference to recent experience with high sensitivity cTn assays in general.

Sekcija 4 Session 4

BIOHEMIJSKI MARKERI BUBREŽNIH OBOLJENJA | BIOCHEMICAL MARKERS OF THE KIDNEY DISEASES

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Plenarne sekcije
Plenary sessions

ODREĐIVANJE BIOMARKERA U SERUMU I MOKRAĆI I NJIHOV ZNAČAJ U DIJAGNOSTICI BOLESTI BUBREGA

V. Ležaić

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Zbog neprekidnog porasta bolesnika koji se leče nekom od metoda za zamenu rada bubrega i visokog pratećeg kardiovaskularnog morbiditeta i mortaliteta, hronične bolesti bubrega (HBB) predstavljaju zdravstveni problem širom sveta. Jedini pravilan pristup ovom problemu je prevencija i rano lečenje HBB. S druge strane, uprkos napretku u lečenju, akutno oštećenje bubrega (AOB) i dalje je praćeno visokim morbiditetom i mortalitetom bolesnika. Nedostatak ranih pokazatelja za AOB i nedovoljno brzo započinjanje lečenja predstavljaju važne uzroke. Zbog svega navedenog postoji potreba za uvođenjem ranih pokazatelja oštećenja bubrega, tzv. biomarkera (proteini i drugi molekuli u serumu i mokraći), koji će pomoći u dijagnostici i prognozi bolesti i praćenju toka bolesti u slučajevima primene lekova za usporavanje progresije bolesti bubrega. Pored određivanja kreatinina u serumu, pokazatelji AOB se mogu otkriti u plazmi (neutrophil gelatinase-associated lipocalin-NGAL i cistatin C) i u mokraći (NGAL, kidney injury molecule-1= KIM-1, interleukin-18, cistatin C, alfa1-mikroglobulin, fetuin-A, Gro-alfa, i meprin). Pokazatelji HBB su slični biomarkeri u serumu i urinu (NGAL, asimetrični dimetilarginin, protein koji se vezuje za masne kiseline). Povišena koncentracija TGF-β1 u plazmi i urinu mogla bi da bude odgovorna za razvoj fibroze u tubulointersticijumu tokom HBB, što ukazuje i na moguće terapijsko dejstvo. Takođe, da bi se razlikovala infekcija donjih izvodnih puteva i pijelonefritisa uvedeni su kao dobri pokazatelji interleukin-6 i prokalcitonin. Bilo bi korisno odrediti senzitivnost i specifičnost navedenih biomarkera kao i njihovu upotrebljivost u kliničkoj praksi u većim studijama.

DETERMINATION OF BIOMARKERS IN SERUM AND URINE AND THEIR SIGNIFICANCE IN DIAGNOSTICS KIDNEY DISORDERS

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Chronic kidney disease (CKD) is becoming a major public health problem worldwide due to the epidemic increase of patients on renal replacement therapy and their high cardiovascular morbidity and mortality. The only effective approach to this problem is prevention and early detection of CKD. In addition, despite significant improvements in therapeutics, the mortality and morbidity associated with acute kidney injury (AKI) remain high. A major reason for this is the lack of early markers for AKI, and hence an unacceptable delay in initiating therapy. Therefore, there is a pressing need to develop biomarkers (proteins and other molecules in the blood or urine) for renal disease, which might assist in diagnosis and prognosis and might provide endpoints for clinical trials of drugs designed to slow the progression of renal insufficiency. Besides serum creatinine, promising novel biomarkers for AKI include a plasma panel (neutrophil gelatinase-associated lipocalin-NGAL and cystatin C) and a urine panel (NGAL, kidney injury molecule-1, interleukin-18, cystatin C, alpha1-microglobulin, Fetuin-A, Gro-alpha, and meprin). For CKD, these include a similar plasma panel and a urine panel (NGAL, asymmetric dimethylarginine, and liver-type fatty acid-binding protein). Increased plasma and urinary TGF-β1 levels might contribute to the development of chronic tubulointerstitial disease, indicating the possible therapeutic implications. Furthermore, to differentiate lower urinary tract infection and pyelonephritis interleukin-6 and serum procalcitonin levels were introduced. It will be important in future studies to validate the sensitivity and specificity of these biomarker panels in clinical samples from large cohorts and in multiple clinical situations.

BIOHEMIJSKI MARKERI KARDIOVASKULARNIH BOLESTI U HRONIČNOJ BOLESTI BUBREGA

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Kod pacijenata sa hroničnim oboljenjem bubrega, kardiovaskularni morbiditet i mortalitet su značajno povišeni. Iako se ne može smatrati ekvivalentom rizika za kardiovaskularne bolesti, veruje se da je bubrežna insuficijencija nezavisni prediktor povećanog kardiovaskularnog rizika i da se taj rizik povećava sa slabljenjem bubrežne funkcije. Ova udruženost je veoma kompleksna i danas se široko koristi termin kardiorenalni sindrom. Kardiovaskularna bolest u hroničnoj bolesti bubrega obično se ispoljava kao ishemijska bolest srca (u obliku angine, akutnog koronarnog sindroma ili nagle srčane smrti), cerebrovaskularna bolest, periferna vaskularna bolest i kongestivna bolest srca. Vaskularna bolest obuhvata aterosklerozu i vaskularne kalcifikacije, dok kardiomiopatija obuhvata hipertrofiju leve komore, kardijalnu fibrozu i sistolnu i dijastolnu disfunkciju leve komore. Pored dobro poznatih tradicionalnih faktora rizika kao što su hipertenzija, dislipidemija, insulinska rezistencija i diabetes mellitus, u osnovi ove udruženosti je i sinergističko delovanje netradicionalnih faktora rizika kao što su povećanje odnosa kalcijum-fosfor, hiperparatireoidizam, anemija, hemodinamsko opterećenje, pothranjenost, zapaljenje, hiperhomocisteinemija, izmenjena sinteza azot-monoksida i povećan oksidativni stres. U radu se razmatraju dosadašnja saznanja o značaju pojedinih uremijskih toksina, natriuretičkih peptida, biohemijskih markera poremećaja u homeostazi kalcijuma i fosfora, sistemske inflamacije, oksidativnog stresa i dislipidemije.

CARDIOVASCULAR BIOMARKERS IN CHRONIC KIDNEY DISEASE

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Cardiovascular morbidity and mortality are markedly increased in chronic renal failure patients. Although it cannot be regarded as a cardiovascular disease risk equivalent, kidney dysfunction is considered an independent predictor of increased cardiovascular risk that increases with deteriorating kidney function. The association is a very complex one, and the term cardiorenal syndrome is now widely used. Cardiovascular disease in chronic kidney disease patients usually manifests as ischemic heart disease (in the form of angina, acute coronary syndrome or sudden cardiac death), cerebrovascular disease, peripheral vascular disease, and congestive heart failure. Vascular disease includes atherosclerosis and vascular calcifications, and cardiomyopathy comprises left ventricular hypertrophy, cardiac fibrosis and left ventricular systolic and diastolic dysfunction. In addition to the well-established traditional risk factors such as hypertension, hyperlipidemia, insulin resistance and diabetes mellitus, the association is supported by synergistic action of non-traditional risk factors such as excessive calcium-phosphorus load, hyperparathyroidism, anemia, hemodynamic overload, malnutrition, inflammation, hyperhomocysteinemia, altered nitric oxide synthase and increased oxidative stress. This paper summarizes the current understanding of the significance of specific uremic retention solutes, natriuretic peptides, biochemical markers of disorders in calcium-phosphorus homeostasis, systemic inflammation, oxidative stress, and dyslipidemia.

URINSKI NGAL – NOVI BIOMARKER ZA RANO OTKRIVANJE AKUTNOG BUBREŽNOG OŠTEĆENJA

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Akutno oštećenje bubrega (AKI) je glavni faktor rizika za intra-hospitalni mortalitet. U svakodnevnoj kliničkoj praksi dijagnoza AKI je obično zasnovana na određivanju serumskog kreatinina i urinskog uzorka.

URINE NGAL – A NOVEL BIOMARKER FOR THE EARLY DETECTION OF ACUTE KIDNEY INJURY

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Acute kidney injury (AKI) is a major risk factor for in-hospital mortality. In every day clinical practice the diagnosis of AKI is usually based on measu rements of serum creatinine and urinary output. Since these

Pošto ovi markeri reflektuju funkciju pre nego oštećenje, dijagnoza akutnog oštećenja bubrega je često u zakašnjenju do 48 sati. Nekoliko markera akutnog tubularnog oštećenja je predloženo, koji se određuju u urinu i serumu. Od njih, marker koji najviše obećava, koji trenutno ulazi u kliničku praksu, je NGAL (lipokalin udružen sa neutrofilnom želatinozom). Istraživanja genoma su pokazala da je NGAl jedan od najčešće ushodno regulisanih gena u tubularnom oštećenju. NGAL je ekspresovan od strane oštećenih renalnih tubula distalnog nefrona i sekretuje se bazolateralno u krv i apikalno u urin. Prema tome, urinarni NGAL ukazuje na oštećenje i proksimalnog i distalnog nefrona. Urinarni NGAL značajno se povećava za manje od dva sata posle renalnog oštećenja i dostiže svoj koncentracioni pik posle 24 sata. Nivo indukcije je u korelaciji sa stepenom oštećenja koji često dovodi do povećanja za više od 30 puta. Laboratorijski testovi za urinarni NGAL se razvijaju i procenjuju za dijagnozu akutnog oštećenja bubrega u različitim kliničkim situacijama, uključujući perioperativni AKI, kontrastom indukovani AKI, post-transplantacioni AKI i ostale uzroke AKI u urgentnoj službi. Većina ovih kliničkih ispitivanja je pokazala dobre do odlične karakteristike testa za NGAL da detektuje AKI, i superiornije performanse u poređenju sa drugim bubrežnim biomarkerima i ustanovljenim testovima. Važno je da NGAL nije samo prediktivni marker akutnog bubrežnog oštećenja (utvrđenog na osnovu kriterijuma zasnovanih na kreatininu), već takođe i dodatnih kliničkih ishoda, uključujući uvođenje terapije renalne transplantacije i intrahospitalni mortalitet. Nivoi NGAL u urinu, pojedinačno ili kada se kombinuju sa drugim kliničkim informacijama, uticaće na praćenje pacijenata i olakšaće razvoj novih terapeutskih strategija zasnovanih na ranom otkrivanju AKI. Prema tome, za ovaj marker se očekuje da će uskoro imati glavnu ulogu u dijagnozi, prognozi i tretmanu AKI.

markers reflect function rather than injury, the diagnosis of acute kidney injury is frequently delayed by up to 48 hours. Several biomarkers of acute tubular damage have been proposed, which are measured in urine and serum. The most promising of these markers, which is currenty entering clinical practice, is *neutrophil gelatinase-associated lipocalin* (NGAL). Genome-wide screens have demonstrated that NGAL is one of the most up-regulated genes in tubular injury. NGAL is expressed by damaged renal tubules of the distal nephron and secreted basolaterally to enter blood and apically to enter urine. Circulating NGAL undergoes glomerular filtration and, in the setting of injury-induced impediment to its proximal tubular uptake, is rapidly excreted into urine. Thus, urinary NGAL indicates damage of both the proximal and the distal nephron. Urine NGAL is markedly increased as early as two hours following renal injury and reaches its peak concentration at 24 hours. The level of induction is correlated with the degree of damage frequently resulting in more than 30-fold increases. Laboratory tests for urinary NGAL have been developed and evaluated for the diagnosis of acute kidney injury in various clinical settings, including perioperative AKI, contrast-induced AKI, post-transplant AKI and all-cause AKI in the emergency room setting. Most of these clinical studies have revealed good to excellent test characteristics of NGAL to detect AKI, and superior performance compared with other biomarkers and established tests. Importantly, NGAL is not only predictive of acute kidney injury (defined by creatinine criteria), but also of additional clinical endpoints, including renal replacement therapy initiation and in-hospital mortality. Urine NGAL levels, alone or when combined with other clinical information, will affect patient management and facilitate the development of novel therapeutic strategies based on an early detection of AKI. Thus, this marker is expected to soon play a major role in diagnosis, prognosis and treatment of AKI.

ZNAČAJ ODREĐIVANJA KIM-1 U TKIVU I URINU BOLESNIKA SA RAZLIČITIM BOLESTIMA BUBREGA

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U poslednje vreme je postala očigledna potreba za novim bubrežnim biomarkerima za monitoring oštećenja proksimalnih tubula jer je pokazano da promene u tubulointersticijumu značajno doprinose progresiji hronične bubrežne slabosti i vode ka terminalnoj fazi bolesti. Jedan od njih je i kidney injury molecule-1 (KIM-1), novi, specifičan biomarker

THE IMPORTANCE OF KIM-1 DETERMINATION IN TISSUE AND URINE OF PATIENTS WITH DIFFERENT KIDNEY DISEASES

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There is an urgent need for early renal biomarkers for the monitoring of proximal tubular injury because tubulointerstitial disease accompanies many processes leading to chronic and end stage kidney disease. One of these is kidney injury molecule-1 (KIM-1) a new specific histological biomarker for diagnosing early tubular injury from renal biopsies but

za dijagnozu ranih tubulskih oštećenja koji se određuje u tkivu bubrega ali i u urinu bolesnika. Ovaj transmembranski tubulski protein sa nepoznatom funkcijom se ne detektuje u zdravim bubrezima, ali je obeležje skoro svih proteinuričnih, toksičnih i ishemičnih bolesti bubrega. Nedavna istraživanja su ukazala na njegovu eventualnu patofiziološku ulogu u moduliranju tubulskog oštećenja i reparaciji. U ovom preglednom članku biće predstavljen strukturalni i biohemijski aspekt KIM-1, njegova ekspresija i patofiziološka uloga u bubrežnim bolestima. Takođe će biti razmatrana i njegova prognostička uloga u odnosu na proteinuriju kao i uloga biomarkera bubrežnog oštećenja i prediktora smanjenja bubrežne funkcije ali i perspektive za monitoring odgovora na terapiju.

also in urine. This transmembrane tubular protein with unknown function is undetectable in normal kidneys, but is the hallmark of virtually all proteinuric, toxic and ischaemic kidney diseases. Recent data revealed its possible pathophysiological role in modulating tubular damage and repair. This review is focused on the structural and biochemical aspects of KIM-1, its expression pattern and its pathophysiological role in renal disease. Also, the prognostic value of KIM-1 in relation to urinary protein excretion will be discussed, as well the potential of KIM-1, as the biomarker of renal damage, as a predictor of renal function decline and its perspectives for monitoring therapy response.

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Plenarne sekcije
Plenary sessions

PRIMENA KVALITETA I EFIKASNOSTI LABORATORIJSKE AUTOMATIZACIJE

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Krajnji cilj za kliničke laboratorije je unapređenje nege pacijenata putem tačnosti i doslednosti laboratorijskog određivanja. U kliničkom laboratorijama, kvalitet je neophodan uslov za zdravlje pacijenta. Paralelno sa očekivanjima o kvalitetu, postoji sve veći pritisci na laboratorije da obezbede povećanje produktivnosti i isplativosti. Optimalna laboratorijska efikasnost se postiže korišćenjem automatizacije u kombinaciji sa informatikom i *six sigma* kvalitet/*lean* principima. Laboratorijska automatizacija koristi mehaničke i kompjuterske tehnologije u izvršavanju planiranog niza zadataka koji povećavaju tačnost, pouzdanost i propusnost laboratorijskih određivanja. Komponente automatizovane laboratorije uključuju mehanizme za pripremu uzoraka, transport, određivanje, skladištenje, kao i kontrolu i informacioni sistem. Postoje tri najvažnija oblika automatizacije kliničkih laboratorija: totalna laboratorijska automatizacija, integrisana modularna laboratorijska automatizacija i laboratorijske automatizacija pored postelje pacijenta (point of care, POC). Potpuno integrisana totalna laboratorijska automatizacija uključuje sortiranje, usmeravanje, centrifugiranje, pripremu alikvota, određivanje, a ponekad i post-analitičko skladištenje i pretraživanje. Modularni sistemi laboratorijske automatizacije omogućavaju fleksibilno korišćenje prostora ili pozicioniranja funkcija u postojećim objektima, kao i mogućnost modifikacije u budućem proširenju kliničke laboratorije. Trend u automatizaciji se promenio na modularni pristup iz više razloga: velika ulaganja, nefleksibilnost i dug proces implementacije totalne laboratorijske automatizacije. Drugi modeli automatizacije kliničkih laboratorija uključuju decentralizaciju pomoću automatizovanog POC testiranja. Potencijalni problemi u POC testiranju su korišćenje manje tačnih instrumenata, neadekvatna upotreba kontrole kvaliteta i nedovoljna stručnost laboratorijske ekspertize. Očekuje se da POC analizatori prerastu u još manje, lakše za rukovanje i tačnije instrumente. Ciljne platforme laboratorijske automatizacije se poklapaju sa četiri

IMPLEMENTING LABORATORY AUTOMATION QUALITY AND EFFICIENCY

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The overall goal for clinical laboratories is to improve patient care through accuracy and consistency in laboratory analyses. In clinical laboratories, quality is a necessary condition for patient health. In parallel with expectations about quality, there are increasing pressures for laboratories to ensure increasing laboratory productivity and cost effectiveness. Optimal laboratory efficiency can be achieved through using automation in combination with informatics and six sigma quality/lean principles. Laboratory automation uses mechanical and computer technologies to perform a scheduled series of tasks that increase the accuracy, reliability and throughput of laboratory tests. Components of an automated laboratory include the mechanisms for sample preparation, transport, analysis, storage and the control and information system. There are three most important forms automation of clinical laboratories: total laboratory automation, integrated modular laboratory automation and point-of care (POC) testing laboratory automation. Fully integrated total laboratory automation systems include sample sorting, routing, centrifugation, aliquot preparation, analysis, and sometimes, post-analytical storage and retrieval. Modular systems of laboratory automation allow more flexible use of space or positioning of functions in existing facilities and can be modified for future expansion of clinical laboratory. The trend in automation has moved to a modular approach for a number of reasons: large investment, inflexibility and long implementation time of total laboratory automation. Other models of automation of clinical laboratories include the decentralization through the use of automated POC testing. Potential problems in POC testing are use a fewer accurate instruments, inadequate use of quality control and insufficient laboratory expertise. POC analyzers are expected to evolve into even smaller, easier to operate and more accurate instruments. The target test platforms of laboratory automation are in four areas coinciding

tradicionalna laboratorijska odseka: klinička hemija, imunohemija, rutinska hematologija i koagulacija. Analizatori koji integrišu kliničku hemiju i platforme za imunoodređivanja imaju široki test »meni«, uključuju opštu kliničku hemiju, tumorske markere, hormone, praćenje koncentracije i zlouptrebe lekova, specifične proteine i serologiju. Automatizacija preanalitičkih i post-analitičkih poslova koji predstavljaju mesto javljanja većine grešaka u laboratoriji, može da ima značajnu ulogu u smanjenju broja medicinskih grešaka i u poboljšanju sigurnosti pacijenta. Automatizovani transport uzoraka eliminiše ručno rukovanje uzorcima, smanjuje rizik od infekcije zaposlenih, obezbeđuje pozitivnu identifikaciju uzoraka, kao i procesuiranje rezultata na najefikasniji mogući način. Automatsko izdavanje rezultata (autoverifikacija) sa kliničkih instrumenata primenom algoritama se obavlja preko laboratorijskog informacionog sistema/»middlevare«-a može poboljšati efikasnost, smanjuje ukupno vreme obrta (*turnaround time*) i može pozitivno da utiče na radni protok u laboratoriji. U zaključku, laboratorijska automatizacija eliminiše većinu ručnih procesa, smanjuje izloženost ljudi opasnim materijama, smanjuje potrebu za treniranim obučenim tehničarima. Zbog toga, pacijentima su dostupni precizniji i pouzdaniji rezultati zbog veće dostupnosti i efikasnosti kliničke laboratorije.

with traditional laboratory divisions: clinical chemistry, immunochemistry, routine hematology and coagulation. Analyzers that integrate clinical chemistry and immunoassay testing platforms have broad test menu, including general clinical chemistry, tumor markers, hormones, TDM, drug of abuse, specific proteins and serology. Automating pre- and post-analytical tasks, which is where most errors in the lab occur, can play a significant role in the reduction of number of medical errors and in the improvement of patient safety. Automated sample transport eliminates manual sample handling, minimizes the risk of infection to staff members, ensures positive sample identification, and processes results in most efficient way. The automatic release of results (autoverification) from clinical instruments via algorithms running in a laboratory information system/»middleware« may improve efficiency, reduce overall turnaround time, and can positively affect workflow in the laboratory. In conclusion, the laboratory automation eliminates most of the manual processes, decreases human exposure to hazardous material, decreases need to find and train skilled technicians. Consequently, patients get more precious and reliable results because of greater availability and efficiency of clinical laboratory.

MEDICINSKE GREŠKE: PRE-ANALITIČKA FAZA I BEZBEDNOST PACIJENTA

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U poslednjih nekoliko decenija značajno je smanjena stopa analitičkih grešaka u kliničkim laboratorijama, dok sve veći broj dokaza pokazuje da su pre- i post-analitički koraci u ukupnom postupku testiranja (TTP) podložniji greškama od analitičke faze. Preciznije, većina grešaka je otkrivena u pre-preanalitičkim koracima, izvan laboratorije i van njene kontrole. Međutim, u okviru pristupa pružanju zdravstvenih usluga orijentisanog na pacijenta postoji potreba da se istraži, u ukupnom postupku testiranja, svaki potencijalni nedostatak koji može negativno uticati na pacijenta, nezavisno od toga o kom se koraku radi i da li greška zavisi od laboratorije (npr. kalibracija ili greška u testiranju) ili nelaboratorijskog osoblja (npr. neodgovarajući zahtev za test, greška u identifikaciji pacijenta i/ili uzimanju krvi). U pre-analitičkoj fazi učestalost pogrešne identifikacije pacijenta/uzorka i prisustvo potencijalnih razloga za odbijanje uzorka (hemoliza, zgrušavanje, nedovoljna zapremina itd.) predstavljaju važan rizik za bezbednost pacijenta. Sprečavanje grešaka u pre-analitičkim koracima zahteva kako

MEDICAL ERRORS: PRE-ANALYTICAL ISSUE IN PATIENT SAFETY

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The last few decades have seen a significant decrease in the rates of analytical errors in clinical laboratories, while a growing body of evidence demonstrates that the pre- and post-analytical steps of the total testing process (TTP) are more error-prone than the analytical phase. In particular, most errors are identified in pre-pre-analytic steps outside the walls of the laboratory, and beyond its control. However, in a patient-centred approach to the delivery of health care services, there is the need to investigate, in the total testing process, any possible defect that may have a negative impact on the patient, irrespective of which step is involved and whether the error depends on a laboratory professional (e.g. calibration or testing error) or a non-laboratory operator (e.g. inappropriate test request, error in patient identification and/or blood collection). In the pre-analytic phase, the frequency of patient/specimens misidentification and the presence of possible causes of specimen rejection (haemolysis, clotting, insufficient volume, etc.) represent a valu-

tehnološki razvoj (narukvice, barkodovi, pre-analitičke radne stanice) tako i blisku saradnju u kliničkom svetu, radi postizanja efikasnog timskog rada. Najvažnija lekcija koju smo naučili je, dakle, da se laboratorijske greške i radnje štetne za pacijenta mogu sprečiti preoblikovanjem sistema koji zdravstvenim radnicima u svim koracima ukupnog postupka testiranja otežavaju pravljenje grešaka.

PRE-ANALITIČKE RADNE STANICE KAO SREDSTVO ZA REDUKCIJU LABORATORIJSKIH GREŠAKA

G. Da Rin

Laboratory Medicine – ASL n°3 Bassano del Grappa

Redukcija grešaka i poboljšanje kvaliteta su integralni deo laboratorijske medicine. Laboratorijsko testiranje, vrlo složen postupak koji se često naziva procesom totalnog testiranja (TTP), obično se deli na tri tradicionalne faze: pre-analitičku, intra-analitičku i post-analitičku. Niz radova objavljenih počev od 1989. skrenuo je pažnju laboratorijskih stručnjaka na pre-analitičku fazu, koja se trenutno čini najpodložnijom greškama. Stoga bi pre-analitička faza trebalo da bude glavna meta za dalje poboljšanje kvaliteta. Prepoznavanje kritičnih koraka u pre-analitičkoj fazi preduslov je za stalno unapređenje kvaliteta, dalju redukciju grešaka, kao i za unapređenje bezbednosti pacijenta. Korišćenje automatizovanih sistema kad god je to moguće i uvid u redukciju grešaka/poboljšanje kvaliteta kao faktor pri izboru instrumenata jesu glavna sredstva kojima raspolažemo u nastojanju da osiguramo visok kvalitet i smanjimo broj grešaka u pre-analitičkoj fazi. Razlozi za automatizaciju pre-analitičke faze postali su toliko jaki da je ona sada neophodna, a ne više samo prednost u odnosu na konkurenciju. Takvi sistemi mogu uticati na klinički/laboratorijski interfejs i odraziti se na delotvornost, efikasnost i kvalitet nege.

PRE-ANALYTICAL WORKSTATIONS AS A TOOL FOR REDUCING LABORATORY ERRORS

G. Da Rin

Laboratory Medicine – ASL n°3 Bassano del Grappa

Reducing errors and improving quality are an integral part of Laboratory Medicine. Laboratory testing, a highly complex process commonly called the total testing process (TTP), is usually subdivided into three traditional (pre-, intra-, and post-) analytical phases. A series of papers published from 1989 drew the attention of laboratory professionals to the pre-analytical phase, which currently appears to be more vulnerable to errors than the other phases. Consequently, the preanalytical phase should be the main target for further quality improvement. Therefore, identifying the critical steps in the pre-analytical phase is a prerequisite for continuous quality improvement, further error reduction and thus for improving patient safety. Use of automated systems where feasible, and use of error reduction/improved quality as a factor when selecting instrumentation are the main tools we have to insure high quality and minimize errors in the pre-analytical phase. The reasons for automation of the pre-analytical phase have become so compelling that it is no longer simply a competitive advantage for laboratories, but rather a competitive necessity. These systems can impact on the clinical/laboratory interface and affect the efficiency, effectiveness and quality of care.

PROGRESIVNA AUTOMATIZACIJA – IZBOR REŠENJA ZA POBOLJŠANJE LABORATORIJSKE EFIKASNOSTI

J.M. Valid

Beckman Coulter International SA, Nyon, Switzerland

Primarni je cilj svake laboratorije da umanji one procese koji su izvor grešaka. Ovo se postiže primenom različitih metoda, počev od tradicionalnih načina

PROGRESSIVE AUTOMATION – THE SOLUTION OF CHOICE FOR IMPROVING LAB EFFICIENCY

J.M. Valid

Beckman Coulter International SA, Nyon, Switzerland

The primary goal of every laboratory should be to reduce those processes that present an opportunity for error. This can be achieved through a variety of meth-

konsolidacije i funkcionalne integracije do potpune automatizacije. Zavisno od izbora načina automatizacije laboratorija će povećati kvalitet, umanjiti varijabilnosti i povećati konzistentnost rezultata. Potpuno automatizovani proces laboratorijskog ispitivanja dovešće do unapređenja proeca rada te će ovo podsticati bolnice da investiraju u laboratorije. Da bi se ovaj proces podsticao izrađena su uputstva koja će omogućiti laboratorijama da automatizuju svoj proces rada. Primenom LEAN metodologije postignut je jedan od najboljih načina automatizovanja laboratorijskog procesa. Laboratorijski rezultati se dobijaju mnogo brže, održava se konzistentno turnaroud vreme (TAT) i poboljšava se celokupni proces rada. Pacijenti se dijagnostikuju i zbrinjavaju brže, što dovodi i do bržeg oporavka. Vreme provedeno u bolnici se smanjuje, a protok pacijenata je veći.

KONCEPTI IN VITRO DIJAGNOSTIKE: KONSALTING USLUGE PRI RAZVOJU EKONOMIČNIH I VISOKOKVALITETNIH IN VITRO DIJAGNOSTIČKIH REŠENJA

G. Wirl

Roche Diagnostics Deutschland GmbH, Mannheim, Germany

U gotovo svim evropskim zemljama sve veći pritisak na javno zdravstvo da snizi troškove primorao je bolnice da uvedu ekonomičnija rešenja koja će to omogućiti. Kako bi se snizili troškovi opreme i osoblja u bolničkoj laboratoriji, testiranje uzoraka se sve češće seli u velike referentne laboratorije, ponekad bez temeljnog razmatranja posledica. Donošenje odluka, umesto upravnika laboratorije, postaje zadatak upravnih odbora (CEO, CFO, MD). Novi igrači, npr. preduzeća sa privatnim kapitalom, stupaju na scenu, čak i ako bolnica nastavi da pruža neke osnovne usluge laboratorijskog testiranja za hitnu medicinsku pomoć, ograničena je fleksibilnost za dodatne zahteve pojedinačnih bolnica. Umrežavanje bolničkih laboratorija moglo bi biti alternativa opisanom scenariju. Implementacija integrisanih sistemskih rešenja u glavnoj laboratoriji i umrežena *point-of-care* rešenja u perifernim laboratorijskim ustanovama otvoreno podržavaju gorepomenuti pristup konsolidacije. Roche Diagnostics nudi dokazane koncepte za konsalting u cilju izrade individualnih ekonomičnih i visokokvalitetnih *in vitro* dijagnostičkih rešenja.

ods, from traditional workstation menu consolidation and function integration to full automation. Regardless of the choice, automating processes will help the laboratory increase quality, decrease variability and increase consistency. Streamlining testing processes in this way provides a compelling argument for hospitals to invest in their laboratories so they can benefit from automation. To support this, there are independent guidelines for laboratories to use when taking the first steps towards deciding which processes to automate. Greater use of LEAN analysis has confirmed that one of the best ways of achieving this is by automating laboratory processes. Test results are delivered more quickly, maintaining a more consistent turnaround time (TAT) and improving the overall patient care process. Patients are then diagnosed and admitted more quickly for timely treatment resulting in improved recovery. The time spent in hospital is reduced, with the flow of patients to the wards managed more efficiently.

CONCEPTS FOR AN IN VITRO DIAGNOSTIC ORGANIZATION: CONSULTING SERVICES TO DEVELOP CUSTOMIZED ECONOMICAL AND HIGH QUALITY IN VITRO DIAGNOSTIC SOLUTIONS

G. Wirl

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The increasing cost pressure in public health care in nearly all European countries forces hospitals to implement more cost-efficient solutions. In order to reduce cost for hospital laboratory equipment and personnel, sample testing is more and more outsourced to large reference labs, sometimes without thoroughly considering the consequences. Decisions have moved from lab director towards management board (CEO, CFO, MD). New players, e.g. private equity companies are entering the arena. Even if the hospital keeps some basic laboratory testing service for emergency medical aid, there is limited flexibility for additional individual hospital requirements. The networking of hospital labs could be an alternative to the above-described scenario. The implementation of integrated system solutions in the core laboratory and cross-linked Point of Care solutions in peripheral lab facilities would clearly support the above-described consolidation approach. Roche Diagnostics delivers proven Consulting Concepts for tailored economical and high quality In Vitro Diagnostic Solutions.

KONCEPT ORGANIZACIJE LEAN LABORATORIJE

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U poslednjih nekoliko decenija, bolničke laboratorije suočene su sa zahtevima za smanjenje troškova laboratorijskih postupaka i istovremeno i) pružanje bržih i dostupnijih usluga i ii) obrađivanje šireg spektra parametara i iii) veće frekvencije uzoraka. Ovi zahtevi potiču od pacijenata, lekara, bolničkih uprava i vladinih agencija. Tako se od uprave laboratorije očekuje da snizi troškove, poboljša efikasnost i omogući zadovoljstvo klijenata, pri čemu kvalitet ima presudnu ulogu. Pored glavnih poslova laboratorije (npr. analiziranje uzoraka pacijenata, tumačenje rezultata, stručno savetovanje kliničara), važni zadaci i odgovornosti tiču se upravljanja kvalitetom, edukacije tehničara i medicinskog osoblja, istraživanja i razvoja, kao i razvijanja ekonomskih strategija. Organizacija »Lean« laboratorije važan je uslov za uspešno obavljanje tih zadataka. Koncept »Lean« laboratorije mora obuhvatiti pre-analitički, analitičku i post-analitičku fazu. Strateške odluke o planiranju moraju biti dugoročne i pre svega zasnovane na informacijama iz spoljašnje sredine. Koncept »Lean« laboratorije uvek podrazumeva holistički pristup, koji uključuje medicinske zahteve i ekonomske aspekte. Biće dat primer na koji način koncept »Lean« laboratorije utiče na organizaciju, efikasnost i delatnost bolničke laboratorije.

CONCEPTS FOR LEAN LABORATORY ORGANIZATION

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In the last decades, hospital laboratories are beset on all sides by demands to lower the costs of laboratory procedures and at the same time to provide (i) more rapid and usable services, (ii) a broader spectrum of parameters, and (iii) process a higher frequency of specimens. These demands are voiced by patients, physicians, hospital administrators, and governmental agencies. Thus, laboratory management is required to decrease costs, increase efficiency, and promote customer satisfaction under the consideration of quality to be of primary importance. Beside the main task of a laboratory (i.e. the analysing of patient specimens, interpretation of results, expert advice for clinicians), quality management, education of technicians and medical staff, research and development, and development of economic strategies are important duties and responsibilities. A lean laboratory organisation is an important condition to cope these duties. Lean laboratory concepts have to include the pre-analytical, analytical and post-analytical period. Strategic planning decisions have to be based primarily on information derived from the external environment and have to be long-term. Lean laboratory concepts always have a holistic view, including medical demands and economic aspects. An example will be shown of how lean laboratory concepts influence the organisation, efficacy and performance of a hospital laboratory.

AUTOMATIZACIJA, LEAN, SIX SIGMA: SINERGIJA U POBOLJŠANJU LABORATORIJSKE EFIKASNOSTI

D. Villa

Monza, Italy

Patološke službe širom sveta, okružene proizvodima, tragaju za rešenjima. Pristup ovom cilju je u bliskoj saradnji između medicinskih radnika i laboratorijskih stručnjaka. Uprkos budžetima ograničenim na 2–3% ukupnih troškova zdravstva, laboratorije pružaju informacije za >70% medicinskih postupaka. »Peri-analitika« postaje fokus, razumevanje protoka informacija i uzoraka kroz čitavo putovanje i procese. Analiza procesa je glavna stavka za razumevanje i oblikovanje najbolje kombinacije komponenata u dizajniranju finansijski zaista povoljnog rešenja za laboratoriju. Metodologije poput Lean (ili Toyota Production System) i Six Sigma nedavno su počele da se usvajaju u zdravstvu kao i u laboratorijskom okruže-

AUTOMATION, LEAN, SIX SIGMA: SYNERGIES FOR IMPROVING LABORATORY EFFICIENCY

D. Villa

Monza, Italy

The Pathology Services worldwide, surrounded by products are today requesting solutions. The approach aims towards the brain-to-brain cycle between caregivers and laboratory professionals. Despite budgets limited to 2–3% of total healthcare expenses, Laboratories are providing information for >70% of medical actions. »Peri-analytics« is becoming the focus; understanding information and sample flow in the whole journey and processes. Process analysis is the main component to understand and shape the best combination of components in designing a truly cost-effective Laboratory solution. Methodologies like Lean (or Toyota Production System) and Six Sigma have started recently to be

nju. Posle razvoja u drugim sektorima, te tehnike su u zdravstvu pokazale uspešnu primenu. Njihove alatke obraćaju se definicijama »vrednosti«, »otpada«, »protoka« kao ključnim pokretačima za poboljšanje performansi. Sinergija između metoda dozvoljava donosiocima odluka da prepoznaju koji je stepen automatizacije stvarno potreban u njihovoj laboratoriji, uz modernizovane procese. Različite platforme napravljene u industriji, za *in vitro* dijagnostičko testiranje, mogle bi postati finansijski nepristupačne i neefikasne bez pažljive procene potreba, putanja i varijabli vezanih za vrednost. Ukupna laboratorijska automatizacija ili samostalna »ostrva« za sisteme mogu se identifikovati i izabrati posle mapiranja procesa i preporuka primenjenih u tehnikama Lean i Six Sigma. Ovaj članak ističe neke ključne koncepte Leana i njegovog mesta u laboratorijskoj organizaciji, kao metodologije koju treba implementirati pre odabira i usvajanja automatizovanog sistema.

adopted also in healthcare and in the Laboratory environment. Those techniques showed already successful implementations in healthcare, after their development in other sectors. Their tools are addressing the definition of »value«, »waste«, »flow« as key drivers to improve performances. The synergy among the methods allows decision makers to identify the degree of automation really necessary in their laboratory, with streamlined processes. The different platforms made available by industries, for *in vitro* diagnostic testing, could become not cost-effective or efficient without a careful assessment of needs, pathways and value-related variables. Total laboratory automation or stand-alone islands for systems can be identified and chosen after process mapping and recommendations deployed with Lean and Six Sigma techniques. This article highlights some key concepts of Lean and their fit in laboratory organization, as methodologies to be implemented before selecting and adopting automated systems.

ANALIZA PRIMERA LEAN SIX SIGMA PROCESA U MIKROBIOLOŠKOJ LABORATORIJI

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Suočene sa smanjenjem budžeta, rastom obima posla i nedostatkom osoblja, mikrobiološke laboratorije se sve više okreću automatizaciji, sa ciljem maksimizacije učinka i efikasnosti. Najbolji koncept poboljšanja procesa danas je Lean Six Sigma. Ovaj koncept izvlači brojne koristi iz automatizacije. Lean proces u laboratorijama se usredsređuje na ispitivanje proizvoda i materijala, da bi se na efikasan način dobili najbolji rezultati što se tiče vremena ciklusa i troškova, ili obe komponente zajedno. Planirani rezultat Lean laboratorije podrazumeva manje napora, manje resursa i manje vremena za ispitivanje uzoraka, sa ciljem oslobađanja ljudskog potencijala. S druge strane, Six Sigma koncept obezbeđuje tok procesa i proizvoda/usluga bez grešaka. Lean Six Sigma pristup analizira tok aktivnosti u laboratorijama radi utvrđivanja neefikasnosti i otkrivanja prilika za oslobađanje kapaciteta, kao i radi smanjenja vremena i troškova. Dokazane Lean Six Sigma tehnike povećavaju produktivnost u okruženju laboratorije i osiguravaju najbolje rezultate. U radu se analizira identifikovani značajni proces, definiše pristup i daje pregled rezultata dobijenih korišćenjem Lean Six Sigma koncepta. Analiziran je proces u mikrobiološkoj laboratoriji. Tradicionalni proces koji primenjuje standardne metode analize obuhvata jedan broj aktivnosti koje ne dodaju vrednost, zahteva mnogo vremena i pruža prilike za pojavu grešaka. Snimanjem

LEAN SIX SIGMA SAMPLE ANALYSIS PROCESS IN A MICROBIOLOGY LABORATORY

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Faced with shrinking budgets, growing volumes, and personnel shortages, clinical laboratories are increasingly moving to automation to maximize output and efficiency. The best tool for improvement is the Lean Six Sigma concept. The concept reaps the full benefits of automation. A Lean process in a laboratory is focused on testing products and materials to deliver results in the most efficient way in terms of cost, speed, or both. The goal of a Lean laboratory is to use less effort, less resources and less time to test incoming samples. On the other hand, the Six Sigma concept provides process workflow and products/ services without defects. The Lean Six Sigma approach analyzes laboratory workflow to help identify inefficiencies and uncover opportunities to free capacity, reduce turn-around time and lower costs. The assessment examines the end-to-end process looking closely at workflow as well as overall laboratory efficiency. The proven techniques of Lean and Six Sigma enhance productivity in the laboratory environment and ensure the best outcomes. This article analyzes a particular process, defines the approach, and gives a review of results obtained by deployment of the Lean Six Sigma concept. The article discusses a sample analysis process in a microbiology laboratory. A traditional process that applies standard analysis methods has a number of non-value-added activities, takes too much time, and

postojećeg procesa korišćenjem SIPOC modela identifikovano je 12 aktivnosti. Primenom Lean alata identifikovane su četiri aktivnosti, koje nisu potrebne ako se koristi novi sistem. Šest aktivnosti pruža prilike za poboljšanje u pogledu značajnog smanjenja vremena trajanja procesa i uštede resursa. Samo dve aktivnosti u postojećem tradicionalnom procesu su bile optimalno rešene korišćenjem standardnih metoda i nisu zahtevale redizajn ni uklanjanje. Primena Lean Six Sigma koncepata i automatizovani sistemi analize u novom procesu utvrđuju samo do 9 aktivnosti u procesu, pa je tako potrebno mnogo manje vremena i resursa. Ovde se opisuju osnovni principi, praksa i metode korišćenih Lean i Six Sigma koncepta. Posebno su analizirani Lean alati 5S i spaghetti dijagram. Za Six Sigma koristi se DMAIC metodologija i daje se pregled primenjenih alatki za poboljšanje kvaliteta za pojedine faze poboljšanja procesa.

has opportunities for defects. By mapping an existing process using a SIPOC model, 12 activities were identified. With the use of Lean tools four non-value-adding activities, which are not needed if a new system is used, were identified. Six activities had opportunities for improvement in terms of significant reduction in process time, and saving resources. Only two activities in the existing traditional process, with the use of standard analysis methods were optimally solved, and this did not require redesign or removal. The application of Lean Six Sigma concepts and automated analysis systems on a new process led to only nine activities in the process that now takes much less time and uses less resources. This article presents a description of the main principles, practices, and methods used in Lean and Six Sigma. The Lean tools particularly discussed here are 5s and spaghetti diagram. For Six Sigma, DMAIC methodology is used, and a review of applied quality tools for certain process improvement phases is given.

Sekcija 5

ANALIZA PROTEINA NA MOLEKULARNOM NIVOU: OD FUNDAMENTALNIH ISTRAŽIVANJA DO PRIMENE U MEDICINI

Session 5

PROTEIN ANALYSIS AT THE MOLECULAR LEVEL: FROM FUNDAMENTAL RESEARCH TO APPLICATION IN MEDICINE

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Plenarne sekcije
Plenary sessions

INDIVIDUALIZOVANA TERAPIJA: ULOGA PROTEINSKIH I GENETSKIH VARIJANTI TIOPURIN S-METILTRANSFERAZE

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Tiopurin S-metiltransferaza (TPMT: EC 2.1.1.67) jeste enzim koji metaboliše imunosupresivne tiopurinske lekove, koji se koriste za lečenje autoimunih bolesti, malignih oboljenja i u transplantacionoj medicini. Aktivnost enzima TPMT kod pojedinih ljudi je izrazito smanjena ili povećana u odnosu na normalni nivo aktivnosti. Istraživanja strukture i biohemijskih karakteristika proteina TPMT su ukazala na postojanje određenih proteinskih varijanti koje imaju izmenjenu aktivnost. Otkriveni su polimorfizmi u genu za TPMT koji daju različite TPMT alozime. Smanjenoj aktivnosti enzima može doprineti i manja količina sintetisanog proteina, što zavisi i od transkripcione aktivnosti promotora gena za TPMT. Polimorfizmi u samom promotoru, kao što je promenjiv broj tandemskih ponovaka (VNTR), mogu da modulišu transkripciju. Primena tiopurinskih lekova kod pacijenata sa određenim genetskim varijantama TPMT izaziva tešku hematološku toksičnost. Da bi se toksičnost izbegla, terapija se modifikuje u skladu sa genotipom TPMT (farmakogenetika). Mi smo izučavali polimorfizme u egzonima i regulatornim elementima (promotor) gena za TPMT koji dovode do promene aktivnosti enzima TPMT u srpskoj populaciji. Koristili smo metodologiju baziranu na PCR i DNK sekvenciranje za detekciju genetskih varijanti TPMT. Pokazali smo da su u našoj populaciji prisutne genetske varijante u egzonima koje ukupno daju 7,5% varijantnih alozima TPMT koji imaju smanjenu enzimsku aktivnost. Terapija za pacijente koji imaju ove farmakogenetičke markere je modifikovana, što je doprinelo uspešnijem lečenju. Funkcionalnim esejima *in vitro* smo pokazali da aktivnost promotora gena za TPMT, a samim tim i količina sintetisanog enzima TPMT, zavisi od arhitekture (broja i tipa) VNTR u promotoru. Promotor gena za TPMT specifično odgovara na tretman ćelija K562 tiopurinom zavisno od tipa VNTR. Izučavanje interakcija DNK i proteina je otkrilo da transkripcioni faktori Sp1 i Sp3 interaguju sa VNTR. Naša istraživanja ukazuju na to da bi region VNTR u promotoru gena za TPMT mogao postati novi farmakogenetički marker od kliničkog značaja za individualizaciju tiopurinske terapije.

INDIVIDUALIZED THERAPY: ROLE OF THIOPURINE S-METHYLTRANSFERASE PROTEIN AND GENETIC VARIANTS

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Thiopurine S-methyltransferase (TPMT: EC 2.1.1.67) is an enzyme that metabolizes immunosuppressive thiopurine medications, used in the treatment of autoimmune diseases, cancer and in transplantation medicine. In some individuals, TPMT enzyme activity is significantly increased or decreased compared to the normal TPMT activity level. Structural and biochemical analyses of the TPMT protein revealed the existence of certain protein variants with altered activity. It has been shown that certain TPMT gene polymorphisms exist, that define different TPMT allozymes. Decreased TPMT enzyme activity can also be a consequence of lower protein synthesis, which depends on the promoter transcription activity. Promoter polymorphisms, such as variable number of tandem repeats (VNTR), can modulate the transcription. Administering thiopurine drugs in patients with certain genetic TPMT variants leads to severe hematologic toxicity. To avoid toxicity, therapy is being modified according to the TPMT genotype (pharmacogenetics). We investigated the polymorphisms in exons and regulatory elements (promoter) of the TPMT gene which affect TPMT enzyme activity in the Serbian population. We used PCR-based methodology and sequencing in the detection of genetic variants on TPMT gene. We showed that genetic variants in exons account for 7.5% of all TPMT variants with decreased enzyme activity. The therapy for patients with these pharmacogenetic markers was modified, which contributed to the efficiency of treatment. Functional assays *in vitro* showed that the TPMT promoter activity and, therefore, the quantity of TPMT protein synthesized, depended on the architecture of VNTRs (i.e. number and type) in the promoter. Promoter of the TPMT gene specifically responds to mercaptopurine treatment of K562 cells in a VNTR-dependent manner. Study of DNA-protein interactions revealed that Sp1 and Sp3 transcription factors interact with VNTRs. Our research pointed out that the VNTR promoter region of the TPMT gene could become a new pharmacogenetic marker, clinically significant for the individualization of thiopurine therapy.

PROTEINI FAMILIJE MARP: MOGUĆA ULOGA U MOLEKULARNIM MEHANIZMIMA TUMOROGENEZE

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Familiju MARP (muscle ankyrin repeat proteins) čine tri strukturno slična proteina: CARP/Ankrd1, Ankrd2/Arpp i DARP/Ankrd23. Sva tri proteina poseduju ankirinske ponovke preko kojih ostvaruju protein–protein interakcije kao i signal za lokalizaciju u jedru. Članovi familije MARP imaju strukturnu i regulatornu funkciju i mogu biti lokalizovani i u jedru i u citoplazmi mišićne ćelije. Učestvuju u signalnoj transdukciji kao molekulski glasnici koji prenose informacije mehaničkog stresa sa sarkomere do jedra, gde učestvuju u regulaciji genske ekspresije. Nivo proteina CARP/Ankrd1 i Ankrd2/Arpp je izmenjen u mišićnim bolestima koje karakteriše atrofija mišića, kao što su Dišenova mišićna distrofija, kongenitalna miopatija i spinalna mišićna atrofija. Mutacije u genu za CARP/Ankrd1 su otkrivene u pacijenata sa dilatiranom i hipertrofičnom kardiomiopatijom. Promene u ekspresiji ovih proteina su takođe uočene u tumorima kao što su rabdomiosarkom, onkocitom bubrega i kancer ovarijuma. U cilju funkcionalne karakterizacije proteina familije MARP, pokazali smo da oba proteina interaguju sa supresorom tumora p53, a geni za CARP/Ankrd1 i Ankrd2/Arpp su pozitivno regulisani ovim transkripcionim faktorom. Rezultati su ukazali na moguću ulogu proteina CARP/Ankrd1 i Ankrd2/Arpp u molekularnim mehanizmima tumorogeneze, čime se otvara novo polje istraživanja ove familije proteina.

MARP PROTEIN FAMILY: A POSSIBLE ROLE IN MOLECULAR MECHANISMS OF TUMORIGENESIS

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The MARP (muscle ankyrin repeat protein) family comprises three structurally similar proteins: CARP/Ankrd1, Ankrd2/Arpp and DARP/Ankrd23. They share four conserved copies of 33-residue ankyrin repeats and contain a nuclear localization signal, allowing the sorting of MARPs to the nucleus. They are found both in the nucleus and in the cytoplasm of skeletal and cardiac muscle cells, suggesting that MARPs shuttle within the cell enabling them to play a role in signal transduction in striated muscle. Expression of MARPs is altered under different pathological conditions. In skeletal muscle, CARP/Ankrd1 and Ankrd2/Arpp are up-regulated in muscle in patients suffering from Duchene muscular dystrophy, congenital myopathy and spinal muscular atrophy. Mutations in *Ankrd1* gene (coding CARP/Ankrd1) were identified in dilated and hypertrophic cardiomyopathies. Altered expression of MARPs is also observed in rhabdomyosarcoma, renal oncocytoma and ovarian cancer. In order to functionally characterize MARP family members CARP/Ankrd1 and Ankrd2/Arpp, we have found that both proteins interact with the tumor suppressor p53 both *in vivo* and *in vitro* and that p53 up-regulates their expression. Our results implicate the potential role of MARPs in molecular mechanisms relevant to tumor response and progression.

NEUROENDOKRINI TUMORI – LABORATORIJSKA DIJAGNOZA

A. Tzontcheva

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Neuroendokrini tumori (NETs) jesu heterogena grupa neoplazmi poreklom iz endokrinih ćelija, koje odlikuju prisustvo sekretornih granula i sposobnost produkcije biogenih amina i polipeptidnih hormona. Ovi tumori potiču od endokrinih žlezda kao što su adrenalna medula, hipofiza i paratiroide, kao i endokrinih insula u okviru tiroide ili pankreasa i raspršenih endokrinih ćelija u respiratornom ili gastrointestinalnom traktu. Kliničko ponašanje NETs varira u znatnoj meri. Oni mogu biti funkcionalni ili nefunkcionalni, kao i spororastući (uspešno diferentovani

NEUROENDOCRINE TUMORS – LABORATORY DIAGNOSIS

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Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms originating from endocrine cells, which are characterized by the presence of secretory granules as well as the ability to produce biogenic amines and polypeptide hormones. These tumors originate from endocrine glands such as the adrenal medulla, the pituitary, and the parathyroids, as well as endocrine islets within the thyroid or the pancreas, and dispersed endocrine cells in the respiratory and gastrointestinal tract. The clinical behavior of NETs is extremely variable; they may be functioning or not

NETs), koji čine većinu, ili veoma agresivni i vrlo zloćudni tumori (nedovoljno diferentovani NETs). NETs gastrointestinalnog trakta obično se dele na dve glavne grupe: 1) karcinoide, i 2) endokrine tumore pankreasa (EPTs). Većina neuroendokrinih tumora proizvodi i sekretuje peptidne hormone i amine. Neke od tih supstanci izazivaju specifične kliničke sindrome: karcinoidni, Zolinger-Elisonov, hiperglikemijski, glukagonom i Verner-Morisonov. Specifični markeri za te sindrome su bazalni i/ili stimulisani nivoi 5-HIAA u urinu, gastrina, insulina, glukagona i vazoaktivnog intestinalnog polipeptida u serumu ili plazmi. Neki karcinoidni tumori kao i otprilike trećina endokrinih tumora pankreasa ne daju nikakve kliničke simptome i nazivaju se »nefunkcionalnim« tumorima. Stoga se uobičajeni tumorski markeri, poput hromogranina A, pankreasnog polipeptida, neuron-specifične enolaze u serumu i podjedinica glikoproteinskih hormona, koriste za skrining kod pacijenata bez jasnih kliničkih simptoma vezanih za hormone. Među uobičajenim tumorskim markerima kao veoma osetljiv i specifičan serumski marker za različite tipove neuroendokrinih tumora pokazao se pankreasni polipeptid hromogranin A, iako njegova funkcija još nije ustanovljena. Razlog tome je što može biti povišen i u mnogim slučajevima nedovoljno diferentovanih tumora neuroendokrinog porekla koji ne luče poznate hormone. Hromogranin A se u ovom trenutku smatra najboljim neuroendokrinim markerom u serumu ili plazmi koji je dostupan za dijagnozu i terapeutsku evaluaciju. Povišen je kod 50–100% pacijenata s različitim neuroendokrinim tumorima i njegovi nivoi u serumu ili plazmi odražavaju stanje tumora. Hromogranin A može se smatrati nezavisnim prognostičkim markerom kod pacijenata s karcinoidima srednjeg probavnog trakta.

functioning, ranging from very slow-growing tumors (well-differentiated NETs), which are the majority, to highly aggressive and very malignant tumors (poorly differentiated NETs). Classically, NETs of the gastrointestinal tract are classified into 2 main groups: (1) carcinoids and (2) endocrine pancreatic tumors (EPTs). Most neuroendocrine tumors produce and secrete a multitude of peptide hormones and amines. Some of these substances cause a specific clinical syndrome: carcinoid, Zollinger-Ellison, hyperglycemic, glucagonoma and WDHA syndrome. Specific markers for these syndromes are basal and/or stimulated levels of urinary 5-HIAA, serum or plasma gastrin, insulin, glucagon and vasoactive intestinal polypeptide, respectively. Some carcinoid tumors and about one third of endocrine pancreatic tumors do not present any clinical symptoms and are called 'nonfunctioning' tumors. Therefore, general tumor markers such as chromogranin A, pancreatic polypeptide, serum neuron-specific enolase and subunits of glycoprotein hormones have been used for screening purposes in patients without distinct clinical hormone-related symptoms. Among these general tumor markers chromogranin A, although its precise function is not yet established, has been shown to be a very sensitive and specific serum marker for various types of neuroendocrine tumors. This is because it may also be elevated in many cases of less well-differentiated tumors of neuroendocrine origin that do not secrete known hormones. At the moment, chromogranin A is considered the best general neuroendocrine serum or plasma marker available both for diagnosis and therapeutic evaluation, and is increased in 50–100% of patients with various neuroendocrine tumors. Chromogranin A serum or plasma levels reflect tumor load, and it may be an independent marker of prognosis in patients with midgut carcinoids.

rRNK METILTRANSFERAZE I NJIHOVA ULOGA U REZISTENCIJI NA ANTIBIOTIKE

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Metiltransferaze (MTaze), koje čine veliku proteinsku superfamiliju, kao donatora metil grupe najčešće koriste S-adenozil-L-metionin (SAM). SAM-zavisne MTaze metiluju nukleinske kiseline (DNK, RNK) i proteine, modulišući tako njihovu aktivnost, funkciju i strukturnu organizaciju. Metilacija G1405 ili A1408 baza u 16S rRNK mikroorganizama koji proizvode

rRNA METHYLTRANSFERASES AND THEIR ROLE IN RESISTANCE TO ANTIBIOTICS

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Methyltransferases (MTases), a large protein superfamily, commonly use S-adenosyl-L-methionine (SAM) as the methyl group donor. SAM-dependant MTases methylate both nucleic acids (DNA, RNA) and proteins, and thus modulate their activity, function and folding. Methylation of G1405 or A1408 nucleotides of 16S rRNA in aminoglycoside-producing microorganisms confers the resistance to

aminoglikozide obezbeđuje rezistenciju na sopstvene toksične proizvode. Ovaj mehanizam rezistencije je donedavno bio opisan samo kod proizvođača antibiotika. Od 2003. godine i kod patogenih bakterija beleži se neprestan porast rezistencije na aminoglikozide putem ovog mehanizma, što predstavlja veliku pretnju efikasnoj upotrebi aminoglikozida u kliničkoj praksi. Jedno od mogućih rešenja problema leži u razvoju novih jedinjenja koja bi efikasno delovala na nova mesta u okviru ribozoma. Drugi pristup rešavanju ovog problema uključuje razvoj inhibitora MTaza odgovornih za rezistenciju, sa idejom da se onemogući modifikacija bakterijske rRNK i na taj način vrati terapeutska efikasnost postojećim aminoglikozidima. Fundamentalna istraživanja vezana za proteinsku ekspresiju, potpuno razumevanje mehanizma rezistencije kao i razrešenje tercijarne strukture proteina su neophodan preduslov za primenu inhibitora 16S rRNK MTaza u medicini.

their own toxic product(s). This mechanism of resistance has been considered as unique to antibiotics producers until recently. Since 2003, methylation of 16S rRNA as a mechanism of resistance is increasingly emerging in pathogenic bacteria. This represents a major threat towards the usefulness of aminoglycosides in the clinical practice. A potential solution to the problem involves the design of novel compounds that would act against new ribosomal targets. The second approach to the issue includes the development of resistance MTases' inhibitors, with the idea to prevent them from modifying the bacterial rRNA, and thus reinstate the therapeutic power of existing aminoglycosides. As the latter approach has considerable potential, it is obvious that fundamental research related to protein expression, in-depth understanding of the mechanism of action and resolving a tertiary structure of 16S rRNAs MTases are prerequisites for application in medicine.

SPECIJALNO PREDAVANJE
SPECIAL LECTURE

Science at the crossroads: Fact or Fiction

Professor David M Goldberg
Toronto, Canada

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NAUKA NA RASKRŠĆU: ČINJENICA ILI FIKCIJA?

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Moderna nauka se uglavnom zasniva na formulisanju pretpostavki koje se potom potvrđuju kroz opažanje i eksperimente. Malo mesta ostaje za *znatiželju* koja je na ranom stupnju razvoja nauke imala važnu ulogu. Rezultate koji nose negativne implikacije nije lako objaviti, dok pretpostavke postepeno poprimaju oblik religijskih mantri. Nauka na akademskom nivou suočava se na mnogim frontovima sa pritiscima kojih u prošlosti gotovo da nije bilo. Pored plate, na raspolaganju su veoma visoke novčane nadoknade, kao što su honorari za konsultante, sudske veštake, za razvoj patenata, čak i osnivanje privatnih preduzeća. Komercijalno finansiranje zamenjuje vladine i nekomercijalne izvore, zbog čega se često gubi kontrola nad protokolima istraživanja kao i sloboda da se rezultati objave. Medijska pažnja donosi slavu i prestiž na čijem sticanju pojedini naučnici marljivo rade, neretko uz podršku univerzitetskih resursa i organizovanje konferencija za štampu pre ili u času izlaska publikacije. Naučnici su odavno stalno zaposleni u vladinim ministarstvima, ali ta ministarstva sve češće nude ugovore za istraživanja akademskom osoblju na bazi honorarnog rada. Takvi pritisci i prilike, uz prioritet koji istraživanju daju univerzitetski komisije za mandate i unapređivanje, praktično umanjuju želju naučnika da preuzmu druge važne odgovornosti poput podučavanja i administracije. Za nekoliko decenija, univerzitetski naučnici su se od elite pretvorili u biznismene, pri čemu mnogi od njih opslužuju više gospodara. Gornji scenario može doneti veću finansijsku dobit i omogućiti istraživanja koja bi bez tih spoljašnjih izvora bila preskupa. Ipak, javljale su se i negativne posledice, koje mogu naučnike, ne njihovom krivicom, navesti da postanu saučesnici pri uvođenju lekova i suplemenata koji: a) ne uspevaju da ostvare obećani boljitak, b) povećavaju rizik od nekih drugih bolesti; c) imaju opasna neželjena dejstva nepoznata ili neprijavljena u trenutku uvođenja. Neki od primera su terapija zamene hormona i antioksidantni vitamini (A i E) radi zaštite od koronarne srčane bolesti; prehrambena vlakna za sprečavanje raka

SCIENCE AT THE CROSSROADS: FACT OR FICTION?

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Modern Academic Science is largely based on the formulation of hypotheses that are then confirmed through observations and experiments. There is little scope for *curiosity* that played an important role in early Science. Results carrying negative implications are not easy to publish, and hypotheses have a tendency to take on the mantra of religious beliefs. Academic Science is facing on many fronts pressures that hardly existed in the past. Financial rewards apart from salary can be very high, in the form of fees for consultants, expert legal witnesses, patent development, and even the establishment of private companies. Commercial funding is replacing Government and non-commercial sources, but this often leads to loss of control over research protocols and freedom to communicate the results. Media attention confers fame and prestige that is assiduously sought out by some individual scientists, often supported by University resources, and Press Conferences prior to or synchronous with actual publication. Scientists have long been employed full-time by Government Departments, but research contracts are being increasingly offered by the latter to academic staff on a part-time basis. These pressures and opportunities, together with the priority given to research by most University Tenure and Promotion Committees, are tending the diminish the appetite of scientists for other important responsibilities such as teaching and administration. In a few decades, University scientists have moved from the »Ivory Tower« to the High Street, and many are serving more than one master. The above scenario may bring increased remuneration and the possibility of research that would be too expensive without these external sources, but adverse consequences have also occurred. They may lead to the complicity of scientists, through no fault of their own, in the introduction of drugs and supplements that: a) fail to deliver the benefits claimed; b) increase the risk of some unrelated illness; c) possess dangerous side effects not know or reported at the time of

kolona; i potencijalno suplementi za kalcijum u cilju lečenja osteoporoze. Događalo se da naučnici posluže kao paravan proizvođačima prilikom objavljivanja falsifikovanih izveštaja u kojima se umanjuje rizik od ozbiljnih neželjenih dejstava leka kako bi se obezbedilo zvanično odobrenje, kao što je bio slučaj sa Vioxxom za lečenje artritisa i Seroquelom za depresiju. Pojedinačne prevare ili zloupotrebe češće su nego što se pretpostavlja, jer većina incidenata nema veliki odjek i biva zataškana od strane univerziteta i agencija za finansiranje. Pravi skandali su retkost, ali mogu imati ozbiljne posledice u javnosti i okaljati ugled nauke. Nedavni primeri uključuju: polemiku oko hladne fuzije (niskoenergetska nuklearna reakcija); nameru AndrewaWakefielda da poveže autizam sa vakcinacijom protiv rubeola; zloglasno stvaranje stem ćelija somatskim ćelijskim nuklearnim transferom koje je lažno prijavio Hwang Woo-Suk. Prevare komercijalnih kompanija podležu sili zakona, ali kako se nauka tretira kao struka koja samu sebe reguliše, kazne koje se dele su relativno banalne. U suštini, nauka je pre 1950, naročito u severnoj Americi, išla putem na kojem je saobraćaj bio podeljen na komercijalni, vladin i akademski, prolazeći kraj inspirativnih pejzaža i zelenih pašnjaka. Kasnije je stigla na raskršće odakle je alternativni put vodio na tržište i na kom podela na navedena tri toka nije bila sprovedena. Sada je to glavni put za nauku širom sveta, ali se osnovano veruje da je to povećalo incidencu opasne vožnje i saobraćajnih nezgoda u vidu sukoba interesa, neetičkog ponašanja, zloupotrebe, pa i prevare. Možda je prekasno da se nauka vrati na raskršće i nastavi prvobitnim putem, ali vraćanje originalnoj podeli na tri toka može vredeti mnogo više, uz uspostavljanje, kao i sprovođenje, strožih pravila ponašanja.

introduction. Examples include hormone replacement therapy and antioxidant vitamins (A and E) to protect against Coronary Heart Disease; dietary fibre to prevent colon cancer; and arguably calcium supplements to treat osteoporosis. On occasions, academic scientists have served as fronts for the publication by the manufacturers of falsified reports minimizing the risk of serious drug side-effects to ensure Regulatory Approval, as occurred with Vioxx in the treatment of arthritis and Seroquel for depression. Individual fraud or misconduct is more frequent than suspected, because most incidents are without major impact and are suppressed by Universities and Funding Agencies. Major scandals are rare, but may have serious repercussions for the general public and bring science into disrepute. Recent examples include: the Cold Fusion controversy (Low Energy Nuclear Reaction); the linkage by Andrew Wakefield of autism with Rubella vaccination; the infamous creation of stem cells by somatic cell nuclear transfer falsely reported by Hwang Woo-Suk. Fraud by commercial companies is subject to the full force of the law, but Science is treated as a self-regulating profession, and as such the punishments handed out are relatively trivial. In essence, Science prior to 1950, except in North America, proceeded along a highway that segregated the traffic into Commercial, Government and Academic, and passed through inspiring landscapes and green pastures. It later came to a crossroads from which the alternative road led to the Marketplace, and on which segregation into the above three streams was not enforced. It has now become the main thoroughfare for Science world-wide, but there are reasons to believe that this has increased the incidence of dangerous driving and traffic accidents in the form of conflicts of interest, unethical behaviour, misconduct and even fraud. It may be too late to return to the crossroads and continue along the original highway, but there could be considerable merit in restoring the original segregation between the three streams of Science and developing, as well as enforcing, a stricter code of behaviour.

Sekcija 6 Session 6

GENETSKI POLIMORFIZMI KAO MARKERI PODLOŽNOSTI ZA NASTANAK OBOLJENJA

GENETIC POLYMORPHISMS AS MARKERS OF SUSCEPTIBILITY TO DISEASE

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Plenarne sekcije
Plenary sessions

INTERAKCIJA GEN–SREDINA: GENETSKO-EPIDEMIOLOŠKI PRISTUP

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Klasična epidemiologija se bavi izučavanjem distribucije i determinanti bolesti i faktora koji doprinose njenoj pojavi s ciljem da se bolest prevenira. Kako genetski tako i faktori sredine mogu doprineti tome da osoba bude podložna datoj bolesti, ali se još uvek nedovoljno zna o njihovim interakcijama i uticajima na rizik od oboljenja. Genetska epidemiologija inkorporira koncepte i metode različitih disciplina, uključujući epidemiologiju, genetiku, biostatistiku, kliničku i molekularnu medicinu, što ima ključnu ulogu u razumevanju zajedničkih uticaja genetskih i sredinskih faktora u procesu nastanka bolesti. Izučavanje interakcije gen–sredina zauzima centralno mesto u genetskoj epidemiologiji. Definiše se kao »različit efekat izloženosti faktorima sredine za pojavu bolesti u osoba sa različitim genotipovima«, ili, ekvivalentno, kao »različit uticaj genotipa na rizik od bolesti u osoba sa različitom izloženošću faktorima sredine«. Opisano je pet biološki prihvatljivih modela koji povezuju genotipove i izloženost faktorima sredine u smislu njihovih zajedničkih efekata na rizik od bolesti. Proučavanje interakcije gen–sredina doprinosi povećanju tačnosti i preciznosti procene uloge genetskih i faktora sredine, posebno kod zdravstvenih poremaćaja čija etiologija nije dovoljno poznata. Genetska epidemiologija igra značajnu ulogu na različitim nivoima prevencije bolesti.

GENE–ENVIRONMENT INTERACTION: A GENETIC-EPIDEMIOLOGICAL APPROACH

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Classical epidemiology addresses the distribution and determinants of diseases in populations, and the factors associated with disease causation, with the aim of preventing disease. Both genetic and environmental factors may contribute to susceptibility, and it is still unclear how these factors interact in their influence on risk. Genetic epidemiology is the field which incorporates concepts and methods from different disciplines including epidemiology, genetics, biostatistics, clinical and molecular medicine, and their interaction is crucial to understanding the role of genetic and environmental factors in disease processes. The study of gene–environment interaction is central in the field of genetic epidemiology. Gene–environment interaction is defined as »a different effect of an environmental exposure on disease risk in persons with different genotypes,« or, alternatively, »a different effect of a genotype on disease risk in persons with different environmental exposures.« Five biologically plausible models are described for the relations between genotypes and environmental exposures, in terms of their effects on disease risk. Therefore, the study of gene–environment interaction is important for improving accuracy and precision in the assessment of both genetic and environmental factors, especially in disorders of less defined etiology. Genetic epidemiology is also applied at the various levels of disease prevention.

GENSKI POLIMORFIZMI KAO MARKERI PREDISPOZICIJE ZA OBOLJENJA

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Najrasprostranjenije bolesti savremenog čoveka imaju poligensku tj. multifaktorsku osnovu, koja uključuje genetičke faktore predispozicije i činioce iz spoljne sredine. Takav je slučaj sa kardiovaskularnim bolestima, malignitetom, dijabetesom itd. Treba imati na umu da faktori rizika obično obuhvataju poremećaje koji su sami po sebi takođe multifaktorski, što dodatno ukazuje na kompleksnost patofizioloških mehanizama. U okviru istraživanja genetičkih činilaca kod poligenskih bolesti pristupa se studijama asocijacije sa određenim genskim polimorfizmima. Pod genskim, odnosno DNK polimorfizmom podrazumevaju se razlike u naslednoj osnovi koje se normalno sreću u humanim populacijama. Genom čoveka se sastoji od $3x10^9$ nukleotidnih (baznih) parova a smatra se da je u proseku svaki 1000. nukleotid polimorfan, tj. da se razlikuje između dva lokusa ili dve osobe. Najčešći tip genskih polimorfizama su polimorfizmi pojedinačnih nukleotida (engl. single nucleotide polymorphism – SNP). Iako genski polimorfizmi predstavljaju izraz normalnih varijacija u naslednoj osnovi, zanimljiv je njihov uticaj na fenotip, a naročito je aktuelno povezivanje sa sklonošću ka određenim bolestima. U studijama asocijacije ispituje se učestalost pojedinih genskih varijanti, tj. genskih polimorfizama u grupi obolelih i upoređuje sa podacima u zdravoj populaciji. Rezultati su često protivrečni, pa je još uvek mali broj polimorfizama sa jasno potvrđenom ulogom genetičkog markera predispozicije. U radu iznosimo iskustva naše laboratorije u ispitivanju genskih polimorfizama kao faktora predispozicije za pojavu trombofilije i ateroskleroze i njenih kliničkih manifestacija.

GENE POLYMORPHISMS AS MARKERS OF DISEASE SUSCEPTIBILITY

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The most widespread diseases of modern man have a polygenic basis, including genetic predisposition and factors in the external environment. Such is the case with cardiovascular disease, malignancy, diabetes and so on. It should be borne in mind that risk factors usually include disorders that are themselves multifactorial, which further indicates the complexity of pathophysiological mechanisms. In the investigation of genetic factors in polygenic diseases studies are underway to determine the association with specific gene polymorphisms. Genetic or DNA polymorphisms are differences in the hereditary basis which are normally found in human populations. The human genome consists of $3x10^9$ nucleotide (base) pairs, and it is considered that, on average, every 1000th nucleotide is polymorphic, i.e. varies between two loci or two individuals. The most common type of gene polymorphisms is the single nucleotide polymorphism (SNP). Although gene polymorphisms are an expression of normal variations in the hereditary basis, their effect on the phenotype is interesting, especially the association with proneness to certain diseases. Association studies examine the incidence of certain genetic variants, i.e. genetic polymorphisms in a group of patients, and compare it with the data of a healthy population. The results are often contradictory, so the number of polymorphisms whose role as markers of genetic predisposition has been clearly confirmed is still small. In this paper we review literature data and present experiences from our laboratory in studying genetic polymorphisms as susceptibility factors for the occurrence of thrombophilia and atherosclerosis and its clinical manifestations.

GENETSKA PREDISPOZICIJA ZA TIP 1 DIABETES MELLITUSA – ULOGA STRESA ENDOPLAZMATSKOG RETIKULUMA U ETIOPATOGENEZI OBOLJENJA ČOVEKA

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Stalni porast incidence diabetes mellitusa u svetu i u našoj zemlji, predstavlja značajan stimulans za

GENETIC PREDISPOSITION FOR TYPE 1 DIABETES MELLITUS – THE ROLE OF ENDOPLASMIC RETICULUM STRESS IN HUMAN DISEASE ETIOPATHOGENESIS

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The increasing incidence of diabetes mellitus worldwide has prompted a rapid growth in the pace

ubrzanje naučnih otkrića koja doprinose uvidu u kompleksne mehanizme uključene u etiopatogenezu ovog multifaktorijalnog oboljenja. Brojna istraživanja ukazuju da je stres endoplazmatskog retikuluma značajan faktor u patogenezi dijabetesa, koji doprinosi apoptozi beta ćelija pankreasa, kao i rezistenciji na insulin. Wolfram sindrom predstavlja autozomno recesivno neurodegenerativno oboljenje, koje karakteriše razvoj insulin-zavisnog diabetes mellitusa i progresivne atrofije optičkog nerva. Wolfram sindrom je retko neurodegenerativno genetsko oboljenje, nepoznate patogeneze. Gen za wolframin (WFS1 lokus) mapiran je na hromozomu 4p16.1, međutim postoje značajni dokazi za genetsku heterogenost, uz prisustvo delecija u mitohondrijalnom genomu kod malog procenta pacijenata. Analize sprovedene primenom strategije pozicionalnog kloniranja dovele su do identifikacije drugog lokusa (WFS2) i uzročne mutacije CISD2 gena za WFS2, na hromozomu 4q24, koji kodira mali intermembranski protein lokalizovan u endoplazmatskom retikulumu. Naši rezultati su dobijeni analizom porodica koje pripadaju specifičnoj populaciji, sa članovima obolelim od Wolframovog sindroma (WFS1). Identifikovali smo novu genetsku alteraciju WFS1 gena, dvostruku »non-synonymous & frameshift« mutaciju, kao dodatnu potvrdu genetske heterogenosti ovog sindroma. Novoidentifikovane mutacije doprinose razumevanju patogeneze Wolfram sindroma, kao i utvrđivanju kompleksnih mehanizama uključenih u nastanak diabetes mellitusa.

of scientific discovery of the mechanisms involved in the etiopathogenesis of this multifactorial disease. Accumulating evidence suggests that endoplasmic reticulum stress plays a role in the pathogenesis of diabetes, contributing to pancreatic beta cell loss and insulin resistance. Wolfram syndrome is an autosomal recessive neurodegenerative disorder accompanied by insulin-dependent diabetes mellitus and progressive optic atrophy. The pathogenesis of this rare neurodegenerative genetic disease is unknown. A Wolfram gene (WFS1 locus) has recently been mapped to chromosome 4p16.1, but there is evidence for locus heterogeneity, including the mitochondrial genome deletion. Recent positional cloning led to identification of the second WFS locus, a mutation in the CISD2 gene, which encodes an endoplasmic reticulum intermembrane small protein. Our results were obtained by the analysis of a families belonging to specific population, affected by Wolfram syndrome. We have identified the newly diagnosed genetic alteration of WFS1 locus, a double non-synonymous and frameshift mutation, providing further evidence for the genetic heterogeneity of this syndrome. Newly identified mutations may contribute to the further elucidation of the pathogenesis of Wolfram syndrome, as well as of the complex mechanisms involved in diabetes mellitus development.

ULOGA POLIMORFIZMA GLUTATION S-TRANSFERAZA M1 I T1 KOD PACIJENATA SA KARCINOMOM BUBREŽNOG PARENHIMA

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Genetski polimorfizam je prisutan kod mnogih članova superfamilije glutation-S transferaza. U toku su istraživanja koja ispituju ulogu GST kao biomarkera za nastanak različitih karcinoma, uključujući karcinom bubrežnog parenhima (KBP). U ovoj studiji je ispitivana uloga *GSTM1* i *GSTT1* polimorfizma u nastanku KBP, nezavisno ili udruženo sa poznatim faktorima rizika za ovaj karcinom. DNK je izolovana iz krvi 182 kontrolna subjekta i 76 bolesnika sa KBP. Polimorfizam *GSTM1* i *GSTT1* je određivan metodom PCR-a. Dobijeni rezultati su analizirani u odnosu na faktore rizika za KBP, uključujući pušenje i profesionalnu izloženost. Učestalost *GSTM1*-nultog genotipa je bila viša kod bolesnika sa KBP (60,5%)

THE ROLE OF GSTM1 AND GSTT1 POLYMORPHISM IN PATIENTS WITH RENAL CELL CARCINOMA

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Members of the glutathione S-transferase (GST) superfamily exhibit polymorphic expression. GSTs are investigated as biomarkers of risk for various cancers, including renal cell carcinoma (RCC). The aim of this study was to test the association between *GSTM1* and *GSTT1* polymorphism and susceptibility to RCC, independently or in conjunction with known risk factors. Genomic DNA was isolated from 182 controls and 76 patients with RCC. *GSTM1* and *GSTT1* genotypes were determined by multiplex PCR. Data obtained were analyzed with respect to RCC risk factors including smoking and occupational exposure. The frequency of *GSTM1*-null genotype was higher in patients with RCC (60.5%) compared to controls

nego kod kontrola (47,2%). Prisustvo *GSTT1*-nultog genotipa je utvrđeno kod 28,6% kontrola i 27,6% bolesnika sa KBP. Nosioci *GSTM1*-nultog genotipa imaju 1.9-puta veći rizik za KBP (95% CI: 1,06–3,33). Prisustvo *GSTT1* aktivnog genotipa je udruženo sa povećanim rizikom za KBP kod profesionalno izloženih subjekata kada su kao referentna grupa uzeti neizloženi nosici *GSTT1*-nultog genotipa (OR: 2,48; 95% CI: 1,05–5,86). Nije otkrivena povezanost između nedostatka aktivne forme *GSTM1* i *GSTT1* i pušenja kod obolelih od KBP. Studija izvedena u Srbiji je pokazala da prisustvo *GSTM1* aktivnog genotipa štiti od nastanka KBP, dok prisustvo *GSTT1* aktivnog genotipa povećava rizik kod profesionalno izloženih osoba.

(47.2%). *GSTT1*-null genotype was found in 28.6% controls and 27.6% of cases. *GSTM1*-null individuals exhibit 1.9-fold increased risk of RCC (95% CI: 1.06–3.33). The presence of *GSTT1* active genotype was associated with increased risk of RCC in occupationally exposed subjects when unexposed *GSTT1*-null subjects were used as a comparison group (OR: 2.48; 95% CI: 1.05–5.86). No association was found between the inactive form of *GSTM1* and *GSTT1* and smoking in RCC patients. In a Serbian cohort of patients, the presence of a *GSTM1* active genotype is protective against RCC, whereas a *GSTT1* active genotype increases RCC risk in occupationally exposed subjects.