PLENARNE SEKCIJE

PLENARY SESSIONS

Sekcija 1 Session 1

BIOHEMIJSKI MARKERI OBOLJENJA | BIOCHEMICAL MARKERS OF THE DISEASES

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Plenarne sekcije
Plenary sessions

POTENCIJAL SRČANIH MARKERA ZA UNAPREĐENJE LEČENJA PACIJENATA

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B-tip natriuretskog peptida (BNP) jeste kvantitativni marker za srčane bolesti. Pokazalo se da upotreba BNP-a kod pacijenata sa dispneom unapređuje lečenje i smanjuje troškove tretmana. Dodatne indikacije koje imaju potencijal da unaprede lečenje pacijenata uključuju praćenje toka lečenja u slučajevima akutnih i hroničnih srčanih bolesti, plućne embolije i koronarne arterijske bolesti.

THE POTENTIAL OF CARDIAC MARKERS TO IMPROVE PATIENT MANAGEMENT

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B-type natriuretic peptide (BNP) is a quantitative marker for heart failure. The use of BNP in patients with dyspnea has consistently shown to improve patient management and reduce treatment cost. Additional indications with the potential to improve patient management include treatment monitoring in acute and chronic heart failure, pulmonary embolism, and coronary artery disease.

NAPREDAK U LEČENJU SRČANIH BOLESNIKA ZAHVALJUJUĆI UPOTREBI SRČANIH MARKERA

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Srčani markeri omogućuju dijagnostikovanje i karakterizaciju srčanih oboljenja. Visokoosetljivi test za troponin specifičan je za infarkt miokarda, dok je mieloperoksidaza moćna alatka za rano otkrivanje nastanka plaka. Homocistein je povezan sa brojnim neurodegenerativnim bolestima, kardiovaskularnim događajima a naročito šlogom. Na osnovu povišenih koncentracija u plazmi može se predvideti ishod i mogu se prepoznati grupe sa visokim rizikom kojima bi pomogle prevencija i terapija. Homocistein se pokazao kao dragocen marker za rano dijagnostikovanje deficita folata i vitamina B koji su uključeni u anemije i brojne hronične bolesti. U ovom radu predstavljeni su inovativni marker MPO, dobro poznati marker troponin i višestruko korisni marker homocistein, i objašnjen je njihov značaj u lečenju srčanih bolesnika.

IMPROVED CARDIAC PATIENT MANAGEMENT THROUGH CARDIAC MARKERS

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Cardiac markers enable the diagnosis and characterization of cardiac diseases. While the highly sensitive troponin assay is specific for myocardial infarction, myeloperoxidase is a powerful for early determination of plaque formation. Homocysteine is independently associated with numerous neurodegenerative diseases, cardiovascular events and stroke in particular. Elevated plasma concentrations predict outcome and help identify high-risk groups most likely to benefit from prevention and therapy. Homocysteine turned out as a valuable marker for early diagnoses of folate and B-vitamin deficiencies that are involved in anemias and numerous chronic diseases. This article presents the innovative marker MPO, the well established marker troponin and the versatile and useful marker homocysteine, and explains their importance in cardiac patient management.

KOŠTANI MARKERI – PRIRODA I KLINIČKA UPOTREBA

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Jedinice za koštano remodelovanje su središna tačka metabolizma kostiju. Pokreće ih sinhronizovana i uravnotežena interakcija osteoklasta i osteoblasta, čija aktivnost izaziva otpuštanje u krv specifičnih supstanci poznatih pod imenom koštani markeri. Markeri resorpcije su rezultat aktivnosti osteoklasta, markeri formacije rezultat su aktivnosti osteoblasta, a markeri prometa mogu nastati od obe vrste ćelija. U kliničkoj praksi se koštani markeri danas koriste za praćenje toka antiresorptivne terapije i odgovora pacijenta na terapiju. Postoje i čvrsti dokazi o tome da su korisni za procenu rizika kad je reč o osteoporozi, gde su komplementarni ustanovljenim »imaging« metodama. Ostale moguće i donekle još nedovoljno istražene indikacije uključuju praćenje neželjenih efekata nekih lekova i onkologiju. Naročito kombinacija markera resorpcije i formacije može omogućiti bolje diferencirani uvid u metaboličko stanje kosti pacijenta. Aktivnosti osteoklasta i osteoblasta pokreću i modulišu brojni faktori, od kojih su neki endokrine prirode. U današnjim laboratorijama se lako mogu izmeriti npr. PTH, kalcitonin i vitamin D. Kalcitonin se ne primenjuje mnogo u osteologiji, ali PTH i vitamin D definišu faktore rizika za ubrzani gubitak koštane mase i narušenu mineralizaciju osteoida, uz srodne bolesti osteoporozu, rahitis i osteomalaciju. Novija dostignuća iz oblasti laboratorijske dijagnostike fokusirana su na reumatske bolesti poput reumatoidnog artritisa, gde je anti-CCP mnogo specifičniji marker od običnih reumatskih faktora.

BONE MARKERS – THEIR NATURE AND CLINICAL USE

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Bone remodeling units are the centerpiece of bone metabolism. They are fueled by a synchronized and well balanced interaction of osteoclasts and osteoblasts, the activity of which releases specific substances known as bone markers into the blood. Resorption markers result from osteoclastic activity, formation markers from osteoblastic activity, and turnover markers from both cell types. In clinical practice, bone markers are today widely used for monitoring of anti-resorptive therapy and patient compliance. There is strong evidence that they are also useful for risk assessment with respect to osteoporosis, here complementing established imaging methods. Other possible and partly not yet investigated indications include monitoring of side-effects of certain therapeutic drugs and oncology. In particular the combination of resorption and formation markers may open up a more differentiated insight into the metabolic situation of a patient's bone. The activity of osteoclasts and osteoblasts is triggered and modulated by numerous factors, some of which are of endocrine nature. Easily measurable in today's laboratory are for instance PTH, calcitonin and vitamin D. While calcitonin is not widely used in osteology, PTH and vitamin D define risk factors for an accelerated loss of bone and impaired mineralization of osteoid with the related diseases of osteoporosis, ricketts and osteomalacia. Recent developments in lab diagnosis of bone diseases focus on rheumatic diseases like rheumatoid arthritis, where anti-CCP is a much more specific marker than the common rheuma factors.

NOVINE NA POLJU GENETIKE ISHEMIJSKOG MOŽDANOG UDARA

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Kao jedan od vodećih uzroka smrtnosti kako u razvijenim tako i u zemljama u razvoju, moždani udar je svetski problem. Oko 80% svih moždanih udara čini ishemijski moždani udar. U patogenezi moždanog udara važnu ulogu imaju genetski faktor, faktori sredine i njihova interakcija. Veliki broj gena-kandidata povezuje se sa ishemijskim moždanim udarom. Geni kandidati koji se ispituju u moždanom udaru dele se u neko-

ADVANCES IN THE GENETIC BASIS OF ISCHEMIC STROKE

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As one of the leading causes of death within both the developed and developing world, stroke is a worldwide problem. About 80% of strokes are ischemic. It is caused by multiple genetic factors, environmental factors, and interactions among these factors. There is a long list of candidate genes that have been studied for a possible association with ischemic stroke. Among the most widely investigated genes are those involved in

liko grupa u zavisnosti od toga da li utiču na hemostazu, inflamaciju, sintezu azot monoksida, metabolizam homocisteina i lipida, renin-angiotenzim-aldosteron sistem. Kombinovanjem linkidž/asocijacionih studija utvrđeno je da geni koji kodiraju PDE4D i ALOX5AP doprinose riziku od dobijanja moždanog udara. U daljem tekstu biće prikazani rezultati studija koje su se bavile ispitivanjem ovih gena, a koji bi mogli da imaju potencijalnu primenu u ranoj dijagnostici, prevenciji i lečenju pacijenata sa ishemijskom bolešću mozga.

haemostasis, inflammation, nitric oxide production, homocysteine and lipid metabolism, renin-angiotensin-aldosterone system. Combined linkage/association studies have demonstrated that genes encoding PDE4D and ALOX5AP confer risk for stroke. We review the studies of these genes which may have potential application on the early diagnosis, prevention and treatment ischemic stroke patients.

Sekcija 2 Session 2

PRIMENA INDIKATORA KVALITETA U MEDICINSKIM LABORATORIJAMA

APPLICATION OF QUALITY INDICATORS IN A MEDICAL LABORATORY

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»IZVESNOST U NEIZVESNOM SVETU« – STAV JEDNOG KLINIČARA O SENZITIVNOSTI I PRECIZNOSTI

P. Tumej

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Klinička praksa evoluira kao i istraživanja. Slično tome, analitičke tehnologije bivaju unapređene svake godine. S pravom ili ne, kliničari sve više pažnje pridaju takvim ispitivanjima na uštrb kliničke istorije i pregleda. Kako se ljudski vek produžava, u porastu je i prevalenca dugotrajnih stanja kao što su bolest štitne žlezde, kardiovaskularna oboljenja i maligniteti. Testovi zasnovani na kliničkoj biohemiji imaju važnu ulogu u tretiranju (skriningu, dijagnostikovanju, prognozi i praćenju) takvih stanja. U Ujedinjenom Kraljevstvu je to postalo očigledno 2004. godine, od kada u primarnoj nezi preko 100 od 550 kliničkih zaključaka zavisi od rezultata testova iz oblasti kliničke biohemije. Rezultati se mogu razlikovati između testova usled odstupanja, preciznosti, specifičnosti i senzitivnosti testa. Do danas se malo značaja pridaje potencijalnom kliničkom efektu preciznosti. Ova prezentacija ispitaće efekte koje preciznost testa može imati na tretiranje važnih dugotrajnih stanja kao što su bolest štitne žlezde, kardiovaskularna oboljenja i maligniteti.

»CERTAINTY IN AN UNCERTAIN WORLD« – A CLINICIAN'S VIEWPOINT OF SENSITIVITY AND PRECISION

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Clinical practice is evolving as research evolves from the bench to the bedside. Similarly, analytical technologies are improving on an annual basis. Rightly or wrongly, increased emphasis is now placed by clinicians on such investigations to the detriment of clinical history and examination. As people live longer, the prevalence of long-term conditions such as thyroid disease, cardiovascular disease and malignancies is increasing. Clinical biochemistry assays play an important part in the management (screening, diagnosis, prognosis and monitoring) of such conditions. This is reflected in the UK since 2004 by the primary care contract where over 100 of the 550 clinical points depend on clinical biochemistry assay results. Inter-assay results may differ due to bias, precision, assay specificity and assay sensitivity. To date, little emphasis has been placed on the potential clinical effect of precision. This presentation will explore the effect that assay precision can have on the management of important long-term conditions such as thyroid disease, cardiovascular disease and malignancies.

GREŠKE U LABORATORIJSKOJ MEDICINI I KAKO IH IZBEĆI

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Medicinsko tumačenje kliničkih laboratorijskih podataka je komparativni proces odlučivanja u kom se rezultat laboratorijskog testiranja pojedinca poredi sa referentnim intervalom izvedenim od referentne populacije. Čini se da ova rečenica opisuje jednostavnu situaciju. Biće predstavljen detaljniji prikaz o tome šta se dešava sa uzorkom pacijenta i kliničkim uzorkom sa uvidom u laboratoriju. Biće prikazane neke granične vrednosti koje potiču direktno sa uzorka i od pacijenta. Nedostatak znanja o kvalitetu uzorka i

ERRORS IN LABORATORY MEDICINE AND WAYS TO AVOID THEM

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The medical interpretation of clinical laboratory data is a comparative decision-making process in which a laboratory test result for an individual is compared with a reference interval derived from a reference population. This sentence seems to describe an easy situation. A closer look to what happens to a patient and to a clinical sample will be discussed in view of the laboratory. One will learn about some limits arriving direct from the sample and the patient. A lack of knowledge about the quality of the sample

posebnim problemima u vezi sa biološkim materijalom stvaraju jaz između laboratorije i kliničara. Za analitički proces bitno je izmeriti materijal za kontrolu kvaliteta. Glavni problem u toj oblasti je nedostatak pravog uzorka i mogućnosti razmene. Kontrolu kvaliteta vršimo uz pomoć veštačkih uzoraka u nadi da će se oni ponašati kao pravi materijal dobijen od pacijenta. Biće detaljnije obrazložena pitanja u vezi sa uzorkom i njegovim kvalitetom. Uz pomoć mnoštva primera iz literature i na osnovu sopstvenog istraživanja, biće prikazana saznanja o:
– upotrebi i pogrešnoj upotrebi referentnih intervala,
– statističkoj prezentaciji i varljivom tumačenju,
– stabilnosti analitika u uzorku,
– uticaju smanjenja zapremine,
– lipemiji, zamućenju, hemolizi i ikterusu,
– kalibraciji,
– posebnim slučajevima interferencije lekova.

Najzad, moraju se bolje ispitati uzorak i proces pred analizu da bi se obezbedili pouzdani rezultati. Konsultacija je važna alatka koja omogućava bolju komunikaciju i pomaže nam da kod kliničara stvorimo potpuno novu sliku o laboratoriji. Moderna analitička alatka – poznata pod nazivom POCT – nudi samo kratkoročno rešenje i ne pokriva deo pre i posle analize. Ko se brine o uzorku o kvalitetu uzorka u toj oblasti? Postoji velika potreba za pokazivanjem vrednosti laboratorije koja razume svoje klijente. Brz i pouzdan rezultat testiranja u kombinaciji sa komentarom ili konsultacijom prikazuje vrednost laboratorijskog rada. Znanje i distribucija znanja obezbeđuju bezbedniju negu pacijenata uz pomoć visokokvalitetnog laboratorijskog rada.

and the special issues with biological material creates a gap between the laboratory and the clinician. To measure quality control material is important for the analytical process. The major concerns in this field are the missing real sample and the commutability. We do quality control with artificial samples and hope they will mimic real patient material. We will move much closer to the issues around the sample and its quality. With a lot of examples from literature and own investigation one will learn more about:
– use and misuse of reference intervals
– statistical presentation and misleading interpretation,
– stability of the analyte in the sample,
– volume displacement effect,
– lipemia, turbidity, hemolysis and icterus,
– calibration,
– special cases of drug interferences.

Finally, we have to better investigate the sample and the preanalytical process to provide reliable results. The consultation is an important tool that opens a field of communication and will help us to bring the laboratory in a new view to the clinician. A modern analytical tool – known as POCT – offers only a short term solution and will not cover the pre- and postanalytical part. Who takes care of the quality of the sample in this field? There is a strong need to show the value of a laboratory that understands its customers. A rapid and reliable test result combined with a comment or a consultation will offer the value of the laboratory's work. Knowledge and sharing the knowledge will result in high quality laboratory work to provide safer patient care.

DILEME I KONTROVERZE U TUMAČENJU LABORATORIJSKIH NALAZA

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Koncentracija mnogih supstanci u krvi je dobar odraz fiziološkog stanja pacijenta. Uobičajeno je da dobijeni rezultati predstavljaju stvarnu koncentraciju ispitivane supstance kod pacijenta, odnosno da predstavljaju njegovo fiziološko stanje. Uticaj nekoliko faktora ukazuje da ova pretpostavka nije uvijek tačna. Greške zbog analitičkih faktora, svode se na najmanju moguću mjeru primjenom kontrole kvaliteta. Takođe, mnogi neanalitički faktori mogu mijenjati koncentraciju jedne ili više supstanci u uzorku, tako da dobijeni rezultati nijesu odraz fiziološkog stanja pacijenta. Rezultati kliničko-biohemijskih određivanja interpretiraju se poređenjem sa referentnim vrijednostima, pa se i zaključak donosi metodom poređenja. Da bi ovaj pro-

DILEMMAS AND CONTROVERSIES IN INTERPRETATION OF LABORATORY RESULTS

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Concentration of many substances in blood is a good indicator of the physiologic state of a patient. It is usual that results obtained represent the real concentration of tested substances of a patient, that is, they represent his physiological state. The influence of some factors indicates that this assumption is not always true. Mistakes owing to analytic factors are reduced to the least possible rate by using the quality control. Also, many nonanalytic factors can change the concentration of one or more substances of the sample, so the results obtained are not the indicator of the physiological state of the patient. Results of clinical-biochemical determination are interpreted by comparing with the referential values, and so the conclusion is

ces mogao pravilno da se izvede potrebno je da postoje referentne vrijednosti za svaki određivani parametar. Ciklične varijacije, fizički napor, stres i drugi faktori imaju značajan uticaj na dobijene vrijednosti analiza. Pri tumačenju rezultata ove specifičnosti moraju biti uzete u obzir, jer će u protivnom biti protumačene kao patološke, što navodi na pogrešan zaključak. Neadekvatna priprema pacijenta za određenu analizu i nepoštovanje pravila koja se odnose na pripremu i analiziranje uzorka, mogu dovesti do drastičnog odstupanja rezultata od stvarne vrijednosti. Iz tih razloga pri tumačenju rezultata nastaju određene dileme i kontroverze.

made by comparing method. In order to perform this process properly, referential values for each specific parameter are necessary to exist. Cyclic variations, physical activity, stress, and other factors significantly affect on obtained result analyses. In interpretation of results, these specificities have to be considered, otherwise they will be interpreted as pathologic, which leads to wrong conclusion. Inadequate preparation of a patient for a certain analysis and disrespect of rules referring preparation and sample analyzing, can lead to drastic deviation of results from the real values. From those reasons there are certain dilemmas and controversies in the result interpretation.

STATUS RAZVOJA I IMPLEMENTACIJE AKREDITACIJE MEDICINSKIH LABORATORIJA U SRBIJI

Lj. Gligić

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Objavljivanjem standarda SRPS ISO 15189:2008 »Medicinske laboratorije: posebni zahtevi za kvalitet i kompetentnost« stvoreni su osnovni preduslovi za njegovu primenu u Srbiji. Primena standarda ISO 15189 danas je prihvaćeni mehanizam za poboljšanje kvaliteta usluga medicinskih laboratorija u Evropi. Na taj način su harmonizovani do tada različiti pristupi poboljšanja kvaliteta medicinskih laboratorija. Funkcionalna organizacija procesa akreditacije medicinskih laboratorija u većini evropskih zemalja sprovodi se kroz saradnju nacionalnih akreditacionih tela, medicinskih eksperata delegiranih iz naučnih društava i resornih ministarstava. Takav tip saradnje pokazao se uspešnim u Velikoj Britaniji, Nemačkoj, Mađarskoj, Francuskoj, Hrvatskoj i dr. Naše iskustvo u akreditaciji medicinskih laboratorija prema standardu SRPS ISO/IEC 17025 (akreditovano je pet laboratorija) i pozitivno iskustvo evropskih zemalja u procesu akreditacije su osnova za razvoj programa akreditacije medicinskih laboratorija u Srbiji. Prvi korak u tom nastojanju je formiranje komisije, sastavljene od eksperata iz različitih oblasti medicine, stručnjaka ATS-a i predstavnika nadležnog ministarstva, i definisanje njenih zadataka, kao što su: izrada potrebne dokumentacije, kvalifikacionih kriterijuma i programa obuke ocenjivača, učestvovanje u izradi šeme eksterne procene kvaliteta preko međulaboratorijskih ispitivanja, praćenje rada evropskih organizacija za akreditaciju, organizovanje zajedničkih ocenjivanja sa domaćim i stranim ocenjivačima i učestvovanje u odlučivanju prilikom dodeljivanja akreditacije.

STATUS OF DEVELOPMENT AND IMPLEMENTATION OF MEDICAL LABORATORY ACCREDITATION IN SERBIA

Lj. Gligić

Accreditation Board of Serbia, Belgrade, Serbia

Through the release of the standard SRPS ISO 15189:2008 entitled »Medical Laboratories –Particular Requirements for Quality and Competence«, conditions have been created for the accreditation of medical laboratories in Serbia. The application of the ISO 15189 standard is an accepted mechanism for improvement of the quality of medical laboratory services in the EU today. In that way, different approaches to the quality improvement of medical laboratories have been harmonised. Functional organisation of the accreditation process of medical laboratories in most European countries is mainly carried out in cooperation with national accreditation bodies, medical experts appointed by scientist associations and the Ministry of Health. This type of collaboration has proven successful in the United Kingdom, Germany, Hungary, France, Croatia, etc. Experiences of the Accreditation Board of Serbia (ABS) in the accreditation of medical laboratories according to the SRPS ISO/IEC 17025 standard (5 laboratories have been accredited) and the positive experiences of the European countries in the accreditation process constitute the basis for the development of a program of medical laboratory accreditation in Serbia. The first step in this direction is the formation of a committee consisting of experts from different fields of medicine, ABS experts and representatives of the competent ministry, and the definition of their tasks, such as: preparation of nessesary documentation, qualification criteria and training programs for assessors, participation in the development of the scheme of external quality assessment through interlaboratory testing, monitoring the work of the European organisations for accreditation, organisation of mutual assessments with national and international assessors and participation in decision making during accreditation.

Sekcija 3 Session 3

BIOHEMIJSKI MARKERI OBOLJENJA | BIOCHEMICAL MARKERS OF THE DISEASES

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BIOHEMIJSKI MARKERI ATEROSKLEROZE

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U ovom radu razmatrani su samo neki lipidni parametri i serumski markeri inflamacije u pogledu njihove prediktivne povezanosti s aterosklerotskom bolešću. Nastavlja se debata o značaju merenja različitih lipida i lipoproteina, uključujući koncentraciju LDL čestica i nivoe apolipoproteina. Takođe, nisu uspostavljene preporuke za apolipoprotein (a) fenotipizaciju i druge lipidne markere. Poslednjih godina preporučuje se simultano merenje nekoliko markera i izračunavanje lipidnih indeksa kao što su lipid tetrada index (LTI), lipid pentada index (LPI) i aterogeni indeks plazme (AIP). Nekoliko cirkulišućih markera inflamacije, npr. C-reaktivni protein, serumski fibrinogen i povišenje broja leukocita, dosledno su udruženi s aterosklerozom. Iako nema dokaza za korisnost merenja C-reaktivnog proteina u široj zajednici, formirane su preporuke za njegovu upotrebu u dijagnostici i lečenju koronarne bolesti srca. Neki proinflamatorni citokini, adhezioni molekuli i markeri leukocitne aktivacije su obećavajući markeri, ali zaslužuju dalja prospektivna ispitivanja. Pitanje koje zahteva odgovor je i da li su ti inflamatorni markeri direktno uključeni u patogeni proces.

BIOCHEMICAL MARKERS OF ATHEROSCLEROSIS

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This paper is a brief review of some lipid parameters and serum markers of inflammation in a view of their predictive relevance for the atherosclerotic disease. A discourse on the importance of measuring different lipids and lipoproteins, concentration of LDL particles and apolipoprotein levels is still underway. Also, the recommendations for apolipoprotein (a), phenotypization and other lipid markers have not yet been established. In recent years the recommendations imply simultaneous measuring of multiple markers and calculating the lipid index values such as lipid tetrad index (LTI), lipid pentad index (LPI) and atherogenic index of plasma (AIP). Several circulating markers of inflammation such as C-reactive protein, serum fibrinogen and elevated leukocyte number, are consistently associated with atherosclerosis. In spite of a lack of evidence on measuring the C-reactive protein in a wide population, the guidelines for its application in diagnostics and therapy of coronary heart disease were developed. Some proinflammatory cytokines, adhesion molecules and markers of leukocyte activation are promising markers, requiring, however, more detailed prospective evaluation. The question to be elucidated is if these inflammatory markers are directly involved in the pathogenic process.

MARKERI INFLAMACIJE I APOPTOZE KOD BOLESNIKA SA ISHEMIJSKOM BOLEŠĆU SRCA

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Ishemijska bolest srca je najčešći uzrok kardiovaskularnog morbiditeta i mortaliteta. Razvija se na terenu ateroskleroze koja se danas smatra hroničnom inflamatornom bolešću. Inflamacija je dokazana praćenjem inflamatornih i imunih biomarkera kao što su C-reaktivni protein (CRP), fibrinogen, neopterin, leukociti, limfociti i drugi koji se značajno menjaju kod bolesnika sa nestabilnom anginom pektoris ili akutnim infarktom miokarda. Najčešće ispitivan marker je CRP. Povećane koncentracije CRP su udružene sa brojnim rizik faktorima. Na osnovu vrednosti CRP mogu se predvideti rekurentni događaji i mortalitet nezavisno od vrednosti kardijačnog troponina, a takođe je nezavisan prediktor kardiovaskularnog događaja nakon korekcije tradicionalnih rizik faktora. Iako se CRP trenutno smatra biološkim markerom koji najviše obećava, još uvek postoje kontroverzije u vezi sa njegovim korišćenjem u kliničkoj praksi. Mada je dokazano da se i nekroza i apoptoza dešavaju u toku aterogeneze malo je dostupnih podataka koji se odnose na markere apoptoze kod bolesnika sa ishemijskom bolešću srca. Sve je više informacija koje ukazuju da se u srcu apoptoza kao i njena regulacija odvijaju, i da se apoptoza može inicirati preko membranskih receptora smrti, ali i oslobađanjem citohroma c iz mitohondrija. Ispitivanje serumskih markera je pokazalo da je proces apoptoze poremećen kod bolesnika sa ishemijskom bolešću srca. TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) je prisutan u stabilnim aterosklerotskim lezijama, povećan u vulnerabilnim plakovima, dok su njegove serumske vrednosti značajno snižene kod bolesnika sa nestabilnom anginom. Koncentracija serumskog Fas je povećana, a FasL snižena kod osoba sa visokim rizikom. Rezultati naše studije su pokazali značajne promene koncentracija Fas, FasL i Bcl-2 u serumu, kao i aktivnost kaspaze-3 u limfocitima u različitim stadijumima ishemijske bolesti srca. Za sada, postoje podaci o efektivnosti statina u regulaciji nekih markera apoptoze. Bolje razumevanje puteva apoptoze i njihove regulacije može omogućiti nov terapijski pristup kardiovaskularnim bolestima.

INFLAMMATORY AND APOPTOTIC MARKERS IN ISCHEMIC HEART DISEASE PATIENTS

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Ischemic heart disease is the most frequent cause of cardiovascular morbidity and mortality. It is develeped on the bases of atherosclerosis which is currently considered a chronic inflammatory disease. It is documented by an increase in inflammatory and immune biomarkers, such as C-reactive protein, fibrinogen, neopterin, leukocytes, lymphocytes and others, that are significantly changed in patients with unstable angina or acute myocardial infarction. CRP is mostly studied. Increased concentrations of CRP are associated with a series of risk factors. CRP may predict recurrent events and mortality independently of cardiac troponin levels, and it is also independent predictor of cardiovascular event after adjustment for traditional risk factors. Although CRP currently appears to be the most promising biological marker, there is still controversy regarding its use in clinical practice. Although both necrotic and apoptotic cell death are documented during atherogenesis the limited data are available about apoptotic markers in ischemic heart disease patients. Increasing evidence supports the existence of apoptotic death initiated by ligation of membrane-bound death receptors or by release of cytochrome c from mitochondria, as well as their regulators in the heart. The studies of serum markers show that apoptotic process is disregulated in ischemic heart disease patients. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is present in stable atherosclerotic lesions, is increased in vulnerable plaques, but its serum levels are reduced significantly in patients with unstable angina. Serum Fas concentrations are increased and FasL are decreased in subjects at high cardiovascular risk. The results of our study show significant changes of serum Fas, FasL, and Bcl-2 concentrations, and lymphocyte caspase-3 activity in different stages of ischemic heart disease. For now, there is evidence that statins are effective in the regulation of some apoptotic markers. The better understanding of the pathways of apoptosis and their regulation is promissing in yielding novel therapeutic targets for cardiovascular disease.

IZOFORME APOPROTEINA(A) I KONCENTRACIJA LP(A) U PLAZMI KOD ČLANOVA ISTE PORODICE

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Apoprotein(a) je multikringel protein u kojem je uočljiv genetski nasledan polimorfizam veličine. Humani gen APO(a) nalazi se u telomeričkom regionu hromozoma 6. Polimorfizam veličine apo(a) glavna je determinanta nivoa Lp(a). Cilj studije je da opiše uticaj veličine apo(a) na nivoe Lp(a) u plazmi tri porodice. K-EDTA je dobijen od svakog člana porodice posle celonoćnog posta. Izoforme apo(a) određene su uz pomoć 3–15% SDS-PAGE-a, a zatim tehnikom »Western imunoblot«. Nivoi Lp(a) kvantifikovani su imunonefelometrijski. Svako dete nasledilo je jednu izoformu od majke a drugu od oca. Deca iz prve porodice nasledila su obe izoforme apo(a) od oba roditelja i kod njih su nivoi Lp(a) bili slični onima koji su izmereni kod njihovih roditelja. Ćerke iz druge i treće porodice nasledile su dominantnu majčinu izoformu S3 apo(a), kao i majčin visok nivo Lp(s). Odredili smo nivoe Lp(a) u plazmi kod članove druge porodice: kod majke 32,3 mg/dl, oca <9,6 mg/dl i ćerke 36 mg/dl, što je viši nivo Lp(a) nego kod njene majke. Kod članova treće porodice, otac je bio nosilac »nula« fenotipa, majka je imala dominantan S3 i izmerili smo veoma visok nivo Lp(a) (74,1 mg/dl) u njenoj krvi; obe ćerke imale su niže nivoe Lp(a) – 46,5 mg/dl i 44,6 mg/dl. Na osnovu tih nalaza zaključili smo da se nasleđivanje izoformi apo(a) odvija prema Mendeljejevom sistemu. Takođe smo otkrili generacijsko snižavanje nivoa Lp(a) kod dece u trećoj porodici.

APOPROTEIN(A) ISOFORMS AND PLASMA LP(A) CONCENTRATION AMONG MEMBERS OF THE SAME FAMILY

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Apoprotein(a) is a multikringle protein which shows a genetically inherited size polymorphism. The human APO(a) gene is located at the telomeric region of chromosome 6. Apo(a) size polymorphism is a major determinant of Lp(a) levels. The aim of this study is to report the influence of apo(a) size on the plasma Lp(a) levels in 3 families. K-EDTA was obtained from every member of the family after an over night fast. Apo(a) isoforms were determined by 3–15% SDS-PAGE followed by Western immunoblot technique. Plasma Lp(a) levels were quantified immuneonephelometrically. Every child inherited one isoform from its mother and the other from its father. The children from the first family inherited the apo(a) isoform each from both parents and had Lp(a) levels similar to those measured in their parents. The daughters from the second and third family inherited the dominant mother s S3 apo(a) isoform, and also mother's high Lp(a) levels, respectively. We have determined plasma Lp(a) levels among the members of the second family: mother 32.3 mg/dl, father <9.6 mg/dl and daughter 36 mg/dl, which was higher than Lp(a) level determined in her mother. Among the members of the third family, the father was null phenotype carrier, mother had a dominant S3 and we measured a very high Lp(a) level (74.1 mg/dl) in her blood; both her daughters had lower Lp(a) levels – 46.5 mg/dl and 44.6 mg/dl respectively. On the basis od these findings we concluded that the inheritance of apo(a) isoforms occurrs according to the Mendeleev system; also, we found a generation decrease of the Lp(a) level in the children of the third family.

ISPITIVANJE IZOFORMI APOLIPOPROTEINA (A) I KONCENTRACIJE LIPOPROTEINA (A) U KORONARNOJ BOLESTI

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Povišena koncentracija lipoproteina (a) [Lp(a)] i prisustvo malih izoformi apolipoproteina (a) [apo(a)] u

EXAMINATION OF APOLIPOPROTEIN (A) ISOFORMS AND LIPOPROTEIN (A) CONCENTRATION IN CORONARY ARTERY DISEASE

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Elevated lipoprotein (a) [Lp(a)] levels and smaller apolipoprotein (a) [apo(a)] isoforms play an important

cirkulaciji imaju značajnu ulogu u procesu ateroskleroze. Pošto ne postoje podaci o veličini apo(a) za zdravu populaciju Srbije, niti za pacijente sa koronarnom arterijskom bolešću (KAB), cilj ovog istraživanja je da se ispita distribucija veličina apo(a), kao i uticaj veličine apo(a) na koncentraciju Lp(a) i na pojavu težeg oblika stenoze na koronarnim krvnim sudovima (KKS). U studiju je uključeno 279 pacijenata sa KAB i 280 zdravih osoba. Pacijenti su na osnovu rezultata koronarne angiografije svrstani u 4 grupe. Grupa 1 je formirana od pacijenata sa stenozom < 50% na KKS. Grupe 2, 3 i 4 su formirane od pacijenata sa stenozom 50% na 1, 2 ili 3 KKS. Izoforme apo(a) su određivane natrijum dodecil sulfat elektroforezom na agarozi i imunoblotingom. Koncentracija Lp(a) određivana je imunoturbidimetrijskom metodom. Pokazano je da je koncentracija Lp(a) u kontrolnoj i u grupi pacijenata pomerena ka nižim vrednostima. Za razliku od koncentracije Lp(a) koja pokazuje log-normalnu distribuciju, distribucija izoformi apo(a) je bimodalna u ispitivanim grupama. Dokazana je obrnuta korelacija između koncentracije Lp(a) i veličine apo(a), kako u kontrolnoj grupi (R=–0,592; P<0,001), tako i u grupi pacijenata (R=–0,553; P<0,001). Koncentracija Lp(a) je viša (167,5 mg/L) u grupi pacijenata u odnosu na kontrolnu grupu (106,1 mg/L, p<0,001), dok su izoforme apo(a) značajno manje u grupi pacijenata u odnosu na kontrolnu grupu (p<0,001). Koncentracija Lp(a) raste sa povećanjem broja KKS zahvaćenih stenozom u slučaju kada nije izvršena korekcija za veličinu izoformi apo(a) (p=0,009), i nakon te korekcije (p=0,001). Međutim, takva veza nije uočena za izoforme apo(a). U studiji je dokazano da izoforme apo(a) imaju najveći uticaj na varijaciju u koncentraciji Lp(a). Male izoforme apo(a) su u vezi sa KAB, ali ne i sa težim oblikom stenoze.

role in atherosclerosis. There were no data regarding apo(a) isoforms within the Serbian population and coronary artery disease (CAD) patients. Therefore, the aim of the study was to examine apo(a) isoform distribution and the impact of apo(a) isoforms on Lp(a) concentration and on the severity of CAD. Two hundred and seventy nine CAD patients and 280 healthy subjects were enrolled in this study. Patients were categorized into 4 groups based on the extent of CAD, assessed by coronary angiography. Group 1 included patients with < 50% luminal narrowing on major vessels. Groups 2, 3 and 4 included patients with luminal stenosis 50% in 1, 2, or 3 major arteries. Apo(a) isoform analysis was performed using sodium dodecyl sulphate agarose gel electrophoresis followed by immunoblotting. The plasma concentration of Lp(a) was measured using immunoturbidimetry. In both patients and controls the distribution of Lp(a) concentrations was skewed towards a lower level. In contrast, the distribution of apo(a) isoforms in both populations was bimodal. Lp(a) concentrations correlated negatively with apo(a) isoforms in both controls (R=–0.592, P<0.001) and patients (R=–0.553, P<0.001). CAD patients compared to healthy subjects, showed significantly higher levels of Lp(a) (106.1 mg/L vs. 167.5 mg/L, p<0.001). Apo(a) isoforms were significantly smaller in CAD patients, compared to healthy subjects (p<0.001). The different number of stenosed vessels was related to increased Lp(a) concentrations, without (p=0.009) and with adjustment (p=0.001) for apo(a) isoforms. However, apo(a) isoforms were not significantly associated with the extent of CAD. This study shows that apo(a) isoforms are the primary factor associated with the variation in Lp(a) concentration. Apo(a) isoforms were related to CAD, but not to the severity of CAD.

POLIMORFIZAM PARAOKSONAZE 1 KOD PACIJENATA SA ANGIOGRAFSKI DOKAZANOM KORONARNOM BOLEŠĆU

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Tradicionalni faktori rizika za razvoj kardiovaskularnih (KVB) bolesti: hiperholesterolemija, starost, pol, hipertenzija, dijabetes, fizička neaktivnost i pušenje, mogu se naći kod 50% osoba obolelih od kardiovaskularnih bolesti. Patogeneza tih bolesti kod ostalih 50% obolelih osoba kod kojih se ne identifikuje nijedan od pomenutih faktora rizika objašnjava se prisustvom malih, gustih LDL čestica, povećanom koncentracijom oksidovanog LDL-a, povišenim nivoom homocisteina, uticajem inflamacije i oksidativnog stresa. Poznato je da zaštitna uloga HDL lipoproteinskih čestica potiče od

PON1 POLYMORPHISM IN PATIENTS WITH ANGIOGRAPHICALLY ASSESSED CORONARY ARTERY DISEASE

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Traditional cardiovascular disease (CVD) risk-factors such as hypercholesterolemia, age, sex, hypertension, diabetes, sedentary way of life, smoking, could be diagnosed in 50% of CVD patients. Coronary heart disease pathogenesis in the other 50% of patients, without traditional risk-factor profiles, could be explained with a predominance of small dense LDL (low dense lipoprotein) particles, increased oxidative LDL concentrations, increased levels of homocysteine, oxidative stress and inflammation influence. It is very important to explain the protective role of HDL particles; it is in

njihovog učešća u reverznom transportu holesterola, ali i prisustva mnogobrojnih antioksidativnih enzima koji su smešteni na njima, a posebno enzima paraoksonaza-1 (PON1). Mi smo ispitivali vezu između statusa enzima PON1 (aktivnost enzima prema dva supstrata, paraoksonu i diazoksonu i fenotip za položaj 192 Q/R) i statusa pacijenata sa angiografski dokazanom koronarnom arterijskom bolešću. Ispitivali smo i status enzima PON1 kod pacijenata i kontrolne grupe u odnosu na oksidativno-stresni status koji smo procenjivali merenjem koncentracija malondialdehida (MDA), nivoa superoksidnog anjona ($O_2^{\cdot -}$), kao i na osnovu aktivnosti enzima superoksid-dizmutaze (SOD) i koncentracije sulfhidrilnih grupa. Distribucija PON1192 fenotipova (QQ, QR, RR) između zdravih osoba i pacijenata sa kardiovaskularnim oboljenjem nije se statistički značajno razlikovala. Rezultati ove studije ukazuju na značajno nižu paraoksonaznu aktivnost enzima PON1 kod pacijenata u odnosu na kontrolnu grupu. Povišene koncentracije MDA i $O_2^{\cdot -}$ kao i snižena aktivnost SOD ukazale su na to da bi jedan od uzroka snižene PON1 aktivnosti mogao biti uznapredovali oksidativni stres kod koronarnih bolesnika. Parametri oksidativnog stresa (MDA i $O_2^{\cdot -}$) bili su u značajnoj korelaciji sa markerima inflamacije (fibrinogen i hsCRP) kod pacijenata sa kardiovaskularnom bolešću, što je ukazalo na to da se oksidativni stres razvija kao posledica hronične niskostepene inflamacije i da se udruženo delovanje oksidativnog stresa i inflamacije odražava na snižavanje aktivnosti PON1 enzima, a time i na smanjivanje zaštitnih sposobnosti HDL čestica.

the well-known antiatherogenic function of this lipoprotein conducted through its involvement in reverse cholesterol transport and also in many antiatherogenic enzymes located on HDL, especially paraoxonase-1 (PON1). We have investigated the relationship between PON1 status ŠPON1 enzymatic activity towards two substrates, paraoxon and diazoxon and also phenotype PON1192 Q/R, coming from the substitution of amino acid glutamine (Q) with arginine (R)Ć and the disease status of angiographycally assessed coronary disease patients. We have also investigated the relation between PON1 status and oxidative stress status which was estimated by measuring malondialdehyde (MDA) concentration, superoxide anion ($O_2^{\cdot -}$) level, and also by superoxide dismutase (SOD) activity and total sulphydril (SH) groups content. PON1192 phenotype distribution (QQ, QR, RR) in the healthy control group was not significantly different from that in coronary heart disease patients. Results of this study indicate significantly lower paraoxonase PON1 activity in CHD patients compared to controls. Increased MDA and $O_2^{\cdot -}$ levels as well as decreased SOD activity showed that one of the reasons for lowered PON1 activity could be exaggerated oxidative stress. Oxidative stress parameters (MDA and $O_2^{\cdot -}$) were in statistically significant, strong correlation with inflammation markers (fibrinogen and hsCRP) in CVD patients, which indicated that oxidative stress is a consequence of chronic low-grade inflammation and their common action influence on PON1 activity lowering and diminished HDL protective capability.

PREPOZNAVANJE PACIJENATA SA VISOKIM RIZIKOM U CILJU PRIMARNE PREVENCIJE ATEROSKLEROZE

D. Pap

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Više od polovine iznenadnih smrti usled koronarne srčane bolesti nastaje kod osoba bez ranije prepoznatih simptoma. Među brojnim novim kardiovaskularnim faktorima rizika koji su ispitani su subklase lipoproteina, faktori vezani za koagulaciju (faktor VIIc, plazminogen aktivator inhibitor 1-PAI 1, Von Willebrandov faktor), faktori vezani za metabolizam (lipoprotein(a), homocistein, insulin) i faktori vezani za inflamaciju – fibrinogen, hsC-reaktivni protein, serumski amiloidni A protein, interleukini, MPO, protein S 100B, holin CD 40L, asimetrični dimetilarginin-ADMA. Cilj rada je procena lipidnog statusa u studentskoj populaciji iz rizičnih porodica i komparativna analiza lipidnog statusa studenata iz porodica bez rizika. Pedeset studenata, starosti 23 godine, iz rizičnih porodica (KB, AIM, hipertenzija, gojaznost, pušenje, dijabetes melitus, HLP) i 50 studenata iste starosne strukture, oba pola, iz porodica bez rizika, izabrano je za ovu studiju. U svim uzorcima su urađene

IDENTIFYING HIGH-RISK PATIENTS FOR THE PRIMARY PREVENTION OF ATHEROSCLEROSIS

D. Pap

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More than half of sudden deaths from coronary heart disease occur in individuals with no prior symptoms recognised. Among a number of new cardiovascular risk factors being evaluated are lipoprotein subspecies, excesssive plasma levels of homocysteine, abnormal blood coagulation characteristics and inflammation – MPO, hsCRP, protein S 100B, choline CD 40L, asymmetric dimethylarginine-ADMA, etc. The aim of the study is to estimate the lipid status in a student population from famillies at risk and to run a comparative analysis of students from families without such a risk. Fifty students from at-risk families (CHD, AMI, hypertension, obesity, smoking, diabetes mellitus, HLP) and 50 students from families without such a risk of both sexes were selected for the study. The following determinations were made: total cholesterol (TCH), triglycerides (TG), HDL-c, low density lipoprotein cho-

sledeće analize: ukupni holesterol (UH), trigliceridi (TG), HDL-hol, lipoproteini male gustine (LDL-hol.), lipoproteini vrlo male gustine (VLDL-hol.), indeks ateroskleroze (IA), i utvrđeni su faktori rizika (FR). Određen je i indeks telesne mase (BMI). Vrednosti UH bile su značajno više u rizičnoj grupi. Vrednosti LDL-hol, triglicerida, indeksa ateroskleroze i faktora rizika značajno su viši u rizičnoj grupi ($p<0,01$). Vrednosti HDL-hol. značajno su niže u rizičnoj grupi ($p<0,01$). Rezultati su pokazali da između BMI i UH, LDL-hol. i TG postoji statistički značajna razlika ($p<0,05$), dok između BMI i HDL-hol. nije utvrđena takva razlika ($p>0,05$). Na ispitivane parametre u obe grupe nije uticao pol ispitanika. Dobijeni rezultati ukazuju na potrebu skrininga lipidnog statusa u studentskoj populaciji iz rizičnih porodica, primenu mera primarne prevencije kroz promenu načina života, promociju zdravog načina života, kao i modifikovanje faktora rizika u cilju sprečavanja progresije ateroskleroze a samim tim i koronarne arterijske bolesti, u pojedinim slučajevima i lečenje utvrđenih kliničkih slučajeva.

lesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c), index of atherosclerosis IA, established risk factors (RF) and body mass index (BMI). The value of TCH was significantly higher in the at-risk group of patients, while LDL-chol. TG, IA and RF were found to be significantly higher in students from families at-risk (p 0.01), HDL-chol. was significantly lower ($p<0.01$) in students from the at-risk group. The results also showed that a statistically significant difference was found ($p<0.5$) between BMI and TCH, LDL-c. and TG, however no statistically significant difference wasn found between BMI and HDL-c ($p>0.05$). These data suggest that screening of the lipid status is necessary in student populations from families at-risk. Primary prevention is very important and can be achieved through lifestyle changes, the promotion of a healthy way of life, as well as modifications of risk factors with the aim of preventing atherosclerosis, coronary heart disease, and in some students through a therapeutic intervention in established clinical cases.

EFIKASNOST RAZLIČITIH TERAPIJSKIH PROTOKOLA ZA SRČANU INSUFICIJENCIJU KOD PACIJENATA SA SRČANOM INSUFICIJENCIJOM I POVIŠENIM NIVOOM NATRIURETSKIH PEPTIDA

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Natriuretski peptidi mogu imati klinički značaj za praćenje terapijskih efekata kod pacijenata sa srčanom insuficijencijom. Cilj ove randomizovane studije je bio da se ispita efikasnost različitih terapijskih protokola za srčanu insuficijenciju kod pacijenata sa srčanom insuficijencijom na osnovu nivoa BNP-a u plazmi pre i posle terapije. Šezdeset dva pacijenta sa srčanom insuficijencijom i povišenim nivoom BNP-a, dobi $55,82\pm9,09$ god, sa II-III NYHA funkcionalnom klasom, ejekcionom frakcijom <45%, primali su 12 nedelja tradicionalnu farmakološku terapiju za srčanu insuficijenciju, i to: ACE inhibitori i ß-blokatori (1. grupa); ACE inhibitori i angiotenzin II receptor blokatori (ARBs) (2. grupa); ß-blokatori i ARBs (3. grupa); ACE inhibitori, ß-blokatori i ARBs (4. grupa). Ispitivali smo nivo BNP-a u plazmi, hemodinamske parametre (pritisak u plućnim kapilarima (PCWP), *cardiac index* (CI), EF i dužinu trajanja opterećenja. Nivo BNP-a u plazmi se značajno snizio u 4. grupi pacijenata, koji su bili na ACE inhibitorima, ß-blokatorima i ARBs, u odnosu na ostale grupe. Takođe je primećen značajan pozitivan uticaj na hemodinamske parametre i trajanje opterećenja u toj grupi pacijenata, u poređenju sa ostalim ispitanicima (rezultati su u tabeli).

THE EFFICACY OF DIFFERENT THERAPY PROTOCOLS FOR HEART FAILURE IN PATIENTS WITH HEART FAILURE AND INCREASED NATRIURETIC PEPTIDE LEVEL

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Natriuretic peptide BNP might be clinically useful for monitoring treatment effects in patients with heart failure (HF). In order to investigate the pharmacological effects of different therapy protocols for patients with HF based on the BNP level before and after therapy, we performed an open randomized comparative trial. Sixty two HF patients with increased natriuretic peptide level, aged 55.82 ± 9.09, II-III NYHA functional classes, ejection fraction (EF) <45%, received a 12-week treatment with either traditional pharmacotherapy for HF with ACE inhibitors and ß-blockers (1st group), or ACE inhibitors and angiotensin II receptor blockers (ARBs) (2^{nd} group), or ß-blockers and (ARBs) (3^{th} group), and ACE inhibitors, ß-blockers and ARBs (4^{th} group). We evaluated the BNP plasma level, hemodynamic state (pulmonary capillary wedge pressure (PCWP), cardiac index (CI), EF and exercise capacity. The BNP plasma level decreased significantly in the 4^{th} group of patients who received ACE inhibitors, ß-blockers and ARBs, in comparison to other groups. A beneficial influence on hemodynamic and exercise capacity was significantly pronounced in this group, compared to the other therapy regimes (the results are in the table).

Promene nivoa BNP-a, hemodinamskih parametara i trajanja opterećenja

	BNP, %	PCWP, %	CI, %	EF, %	trajanje opt.
1. grupa	–13,5*	–9,4*	+6,1*	+8,8**	+11,2**
2. grupa	–14,9*	–6,3*	+4,1*	+7,8**	+7,2**
3. grupa	–15,8*	–13,0**	+5,3*	+7,0*	+6,5*
4. grupa	–39,8**	–20,0**	+11,1**	+14,6**	+14,8**

*–p<0,01; **–p<0,001 u poređenju sa vrednostima pre terapije

Može se zaključiti da terapijski protokol koji uključuje ACE inhibitore, ß-blokatore i ARBs kod pacijenata sa srčanom insuficijencijom i povišenim BNP-om značajno poboljšava kvalitet života, funkciju leve komore, hemodinamske parametre i dužinu trajanja opterećenja. Sve te promene su udružene sa sniženjem nivoa BNP-a u plazmi.

Changes in BNP, hemodynamic parameters and exercise capacity

	BNP, %	PCWP, %	CI, %	EF, %	Exercise cap, %
Group 1	–13.5*	–9.4*	+6.1*	+8.8**	+11.2**
Group 2	–14.9*	–6.3*	+4.1*	+7.8*	+7.2**
Group 3	–15.8*	–13.0*	+5.3*	+7.0*	+6.5*
Group 4	–39.8**	–20.0**	+11.1**	+14.6**	+14.8**

*–p<0.01; ** –p<0.001 compared with baseline

In conclusion, the therapeutic protocol: ACE inhibitors, ß-blockers and ARBs in HF patients with increased natriuretic peptide level significantly improves the quality of life, left ventricular function, hemodynamic parameters and exercise capacity. All these changes were accompanied with a decreasing of the BNP plasma level.

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The Impact of the Pre-analytical Phase on the Quality of the Laboratory Results

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POGREŠNA IDENTIFIKACIJA I OSTALE PREANALITIČKE GREŠKE

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Najveći broj laboratorijskih grešaka događa se u preanalitičkoj fazi uglavnom iz razloga koji se tiču obrazovanja i organizacije. Biće predstavljeno iskustvo naše ustanove, kao i rezultati zajedničkih napora italijanskih laboratorija da se otkriju i umanje greške ili rizik od grešaka u laboratorijskoj medicini.

MISIDENTIFICATION AND OTHER PREANALYTICAL ERRORS

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The largest number of Laboratory Errors occur in the pre-analytical phase and are mainly due to educational and organizational reasons. The experience of our Institution, as well as the results of an Italian interlaboratory effort to detect and reduce errors/risk of errors in Laboratory Medicine will be illustrated.

POBOLJŠANJE PREANALITIČKOG PROCESA: FOKUSIRANJE NA KVALITET UZORKA

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Trendovi u kliničkoj laboratorijskoj praksi postavljaju više zahteva za kvalitet uzoraka pacijenata. Unapređenje analitičkih karakteristika (npr. povećanje automatizacije, smanjenje zapremine uzorka, povećanje osetljivosti određivanja), kao i efikasnosti i smanjenje troškova (npr. prohodnost, vreme obrta testova) je unapredilo medicinsku praksu. Međutim, ove promene su uzrokovale povećanje incidencije preanalitičkih grešaka što zahteva potrebu za uzoraka većeg kvaliteta. Sledeći članak govori o ovim greškama kao dodatak za poboljšanje kvaliteta preanalitičke faze.

IMPROVING THE PREANALYTICAL PROCESS: THE FOCUS ON SPECIMEN QUALITY

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Trends in clinical laboratory practice place more demands on the quality of patient specimens. Advances in analytical performance (i.e., increased automation, reduced sample volume, increased assay sensitivity), as well as efficiency and cost reduction gains (i.e., throughput, test turnaround time) have improved medical practice. These changes, however, have caused an increased incidence of preanalytical errors, dictating the need for higher-quality specimens. The following paper addresses these errors in addition to methodologies for improving the quality of the preanalytical phase.

UPRAVLJANJE PREANALITIČKOM VARIJABILNOŠĆU I OTKRIVANJE GREŠAKA

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U vremenu medicine zasnovane na dokazima, rezultati laboratorijskog testiranja su integralan deo kliničkog odlučivanja, i pomažu u postavljanju dijagnoze, vođenju ili praćenju terapije i predviđanju kliničkog ishoda. S obzirom na sve širu upotrebu laboratorijske dijagnostike i sve veći pritisak da se smanje troškovi, osnovni cilj je postizanje visokog stepena efikasnosti uz što manji uticaj na kvalitet. Iako postoji uvreženo mišljenje da većina grešaka u medicini potiče iz pogrešnih terapija, medicinskih i hirurških, one mogu nastati i u laboratorijskoj dijagnostici, naročito u preanalitičkoj fazi koja podrazumeva najviše manuelnog rada. Kako preanalitička varijabilnost ima veliki uticaj na organizaciju laboratorije, troškove u zdravstvu i konačni ishod pacijenata, upravljanje tom ključnom fazom ukupnog postupka testiranja putem smanjenja neizvesnosti pruža najviše mogućnosti za poboljšanje ukupnog kvaliteta i povećanje zadovoljstva deoničara. Najveći broj preanalitičkih grešaka rezultat su manjkavosti sistema i nedovoljno čestih inspekcija u radu operatera čija je odgovornost prikupljanje/rukovanje uzorcima. Stoga bi se standardizacija i praćenje najvećeg broja, ako ne i svih, preanalitičkih varijabli mogli dovesti u vezu sa najboljim organizacionim i kliničkim prihodima. Najpouzdanija strategija bi dakle trebalo da bude pravljena tako da predvidi nastanak slučajnih događaja (incidenata) i smanji osetljivost preanalitičkih koraka. Glavna stavka i neophodan preliminarni korak u upravljanju preanalitičkom varijabilnošću je identifikacija grešaka. Veliki broj laboratorijskih grešaka, međutim, zahteva uvođenje sveobuhvatnih sistema izveštavanja, koji obuhvataju greške u ukupnom dijagnostičkom postupku, pošto se prethodno definiše lista pokazatelja performansi na lokalnoj osnovi. Najbolji pristup jeste razvijanje sistema koji bi uključio i izvestan broj reprezentativnih mera preanalitičke performanse, na osnovu kriterijuma za prihvatljivost uzoraka. Implementacija sistema za sistematsko praćenje grešaka u svakodnevnoj praksi omogućiće pristupanje specifičnim problemima i odgovornostima, pružiti važne informacije o lokalnim preanalitičkim postupcima koji su najpodložniji greškama, i na taj način dati idealnu osnovu za eliminisanje uskih grla i nedostataka, i preoblikovanje strukture ukupnog postupka testiranja.

GOVERNANCE OF PREANALYTICAL VARIABILITY. ERRORS DETECTION.

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In the age of evidence-based medicine, results of laboratory testing are integral to the clinical decision making, to assist diagnosis, guide or monitor therapy and predict health outcomes. Owing to the increasing demand placed on laboratory diagnostics and the wedging pressure from cost containment policies, the primary goal is to achieve a high degree of efficiency with as little as possible influence on the quality. Although there is widespread perception that most errors in medicine occur from mishandled therapies, both medical and surgical, they can also develop within the laboratory diagnostics, especially in the most manually-intensive preanalytical steps. Since preanalytical variability exerts a strong influence on laboratory organization, healthcare expenditures and patients outcome, governance of this crucial phase of the total testing process by reduction of uncertainty offer the greatest potential for improving total quality and enhance stakeholders satisfaction. Most preanalytical errors result from system flaws and insufficient audit with operators involved in specimen collection/handling responsibilities. Therefore, standardization and monitoring of most, if not all, preanalytical variables would be associated with the best organizational and clinical revenues. The most reliable strategy should hence be tailored to both predict the onset of accidental events (incidents) and decrease the vulnerability of preanalytical steps. The mainstay and necessary preliminary step in governance of preanalytical variability is errors identification. The magnitude of laboratory errors requires, however, the introduction of comprehensive reporting systems, encompassing mistakes falling within the whole diagnostic process, once a list of performance indicators has been defined on a local basis. The most suitable approach is to develop a system including also a variety of representative preanalytic performance measures, based on criteria for specimen acceptability. The implementation of a systematic error tracking system in the daily practice would enable considerations on specific problems and responsibilities, grant meaningful information on the local preanalytic processes more susceptible to errors, thus providing the ideal basis for eliminating bottlenecks and flaws, and redesigning the structure of the total testing process.

BEZBEDNOSNI STANDARDI U UZIMANJU KRVI

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Laboratorijska dijagnostika je zahvaljujući značajnom doprinosu koji daje u postavljanju dijagnoze, praćenju i terapeutskom monitoringu pacijenata presudna za proces kliničkog odlučivanja. Praksa flebotomije se generalno odnosi na uzimanje venske krvi. Iako je uvođenje pravih igala za jednokratnu upotrebu i vakuumskih sistema cevi omogućilo uzimanje uzoraka odgovarajućeg kvaliteta, uz dodatne opšte prednosti kako sa stanovišta bezbednosti tako i u praktičnoj upotrebi, nekoliko različitih aspekata utiče na celokupan postupak u vezi sa uzimanjem uzoraka krvi. Glavni razlog tako velike prevalence problema je što je trenutno teško pratiti većinu preanalitičkih varijabli, uključujući flebotomiju, koje nisu uvek pod direktnom kontrolom ili nadzorom osoblja u laboratoriji. Praksa flebotomije se razlikuje širom sveta; i evropskim oblastima nemačkog govornog područja za sada je samo lekarima dozvoljeno da uzimaju uzorke venske i arterijske krvi i njih stoga obučavaju i nadgledaju starije kolege na odeljenjima. U Britaniji se flebolozi obučavaju kao tehničari i delimično su pod nadzorom profesionalnih laboranata. Zahvaljujući trendu konsolidacije laboratorijskog testiranja, koje će neizbežno obuhvatiti planove za nabavku u vezi sa prikupljanjem i transportom uzoraka, očekuje se da će u budućnosti pojačani nadzor decentralizovanih postupaka u flebotomiji dobiti još veći značaj. Flebotomija je i dalje jedna od najčešće zapostavljanih procedura u zdravstvu, ali je neophodna za dobijanje uzoraka krvi u dijagnostici. Pitanje bezbednosti u prikupljanju uzoraka krvi često je potencirano tokom prethodnih decenija. Međutim, bezbednost pacijenta bila je na prvom mestu u viziji zdravstva u čijem se centru nalazi pacijent, dok su uslovi neophodni za pružanje, održavanje i unapređivanje bezbednosti osoblja u zdravstvu odgovornog za uzimanje krvi bili zanemareni. Flebolozi su integralan deo zdravstvenog sistema, a njihova aktivnost podrazumeva ozbiljne zdravstvene rizike, uglavnom usled slučajnih povreda operatera ubodom igle i uobičajenih ozleda nanesenih pacijentu (ozlede nerva ili tetive), krvarenje, vrtoglavica/sinkopa, limfedem), kao posledica nepravilno izvršenog sečenja vene. Stoga bi gotovo svi zdravstveni sistemi trebalo ozbiljno da se pozabave pitanjem povreda pacijenta i operatera prilikom uzimanja krvi. Pristup bezbednosti u uzimanju krvi neizbežno je višestran, i obuhvata širenje znanja i preporuka, obuku i izdavanje sertifikata flebolozima. Bez obzira na lokalne uslove, radnici u zdravstvu odgovorni za specifične zadatke, poput flebotomije, moraju biti propisno obrazovani i motivisani za obavljanje tih zadataka sa što je manje moguće grešaka i povreda. Glavni ciljevi obuke bi trebalo da im približe aspekte koji su najčešće uključeni u zasecanje vene, uključujući prenošenje znanja o

STANDARDS OF SAFETY IN BLOOD COLLECTION

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Laboratory diagnostics is pivotal to the clinical decision making, since it substantially contributes to diagnosis, follow-up and therapeutic monitoring of patients. The practice of phlebotomy generally refers to the collection of venous blood. Although the introduction of disposable straight needles and evacuated tube systems has allowed collection of specimens of suitable quality, with additional general advantages from both a safety and a practical point of view, the overall procedures linked to blood sample collection, and the phlebotomy success rate itself, are as yet influenced by several aspects. The main reason for such a high prevalence of problems is that it is currently difficult to monitor most of the pre-analytic variables, including phlebotomy, which are not be always under direct control or supervision of the laboratory staff. The phlebotomy activity is rather heterogeneous worldwide; in the German speaking area of Europe only physicians are presently allowed to draw venous and arterial blood and are therefore trained and supervised by the elder colleagues in the ward. In Britain, phlebotomists are educated like technicians and are partially supervised by laboratory professionals. Owing to the expanding trend towards consolidation of laboratory testing, which will inevitably entail outsourcing plans for specimen collection and transportation, improved vigilance of decentralized phlebotomy procedures is expected to gain further relevance in the future. Phlebotomy is still one of the most neglected procedure in healthcare, but it is essential to achieve blood specimens for diagnostics. Much emphasis has been placed on safety issue in blood collection over the past decades. However, patient safety has been foremost in the patient-centred vision of healthcare and the necessary requirements for ensuring, maintaining or enhancing the safety of healthcare personnel with blood collection responsibilities have been overlooked. Phlebotomists are integral parts of the healthcare system, and their activity still involves serious health risks, mainly represented by accidental needlestick injuries to the operator and casual lesions inflicted to the patient (nerve or tendon injury, haemorrhage, vertigo/syncope, lymphedema) as a result of improperly performed venipuncture. Therefore, injuries to both patient and operator while collecting blood should be a matter of concern for most health systems. The approach to safety in blood collection is necessary multifaceted, which includes recommendations and knowledge dissemination, training, and certification of phlebotomists. Regardless of local requirements, healthcare workers responsible for specific tasks, such as phlebotomy, must be properly educated and

anatomiji i fiziologiji (lokacija vena, nerava, tetiva i arterija), osobinama alatki (igle, špricevi, podvezi, dezinfekciona sredstva, vakuumski sistemi cevi), tehnika flebotomije, veština njihovog primenjivanja i hitnim postupcima. Štaviše, zahteva se i osnovno poznavanje psihologije, u cilju uspostavljanja poverljivijeg odnosa sa odraslima i decom. Pre zapošljavanja, flebolozi bi morali steći minimum kliničkog iskustva, na osnovu ponavljanog praktičnog iskustva, koje bi trebalo da im omogući uspešno sprovođenje postupka flebotomije. Dijagnostička industrija u tome ima najvažniju ulogu, a to je da zaposlenima u zdravstvu pruži bezbedna sredstva za rad sa najmanjim mogućim rizikom od povreda ili nezgoda. Takođe se preporučuje da profesionalni laboranti nadziru flebologe, pošto profesionalni laboranti mogu flebolozima da pruže sveobuhvatan uvid u složenost ukupnog postupka testiranja. Na kraju, ciljeve za postizanje najbolje prakse (»BP«), obuhvaćene Standardima za medicinsko osoblje u flebotomiji i pravilnicima OSHA, JCAHO i CDC, treba smatrati osnovnim sredstvima za poboljšanje kvaliteta u celokupnom postupku uzimanja krvi. U Nemačkoj je, na primer, zaživeo program obuke po imenu »DIAPRO« koji je dostupan na CD-romu. Program sadrži tekstovi i primere za obuku budućih medicinskih stručnjaka u sprečavanju grešaka tokom flebotomije. Smanjivanje broja nepotrebnih testova i ograničavanje broja koraka, koji podrazumevaju isporuku uzoraka laboratorijama, obavljanje testova i prenošenje rezultata lekaru koji ih je tražio, možda su dodatni ali ne i protivrečni ciljevi, uz ostala preanalitička pitanja ne nužno povezana sa radom flebologa. Konačno, implementacija sertifikacije flebologa širom sveta podstakla bi visok stepen profesionalizma u tom ključnom delu ukupnog postupka testiranja.

motivated to perform those tasks with as few errors and injuries as possible. The main objectives of training should encompass those aspects most frequently encountered while performing venipunctures, including notions of anatomy and physiology (location of veins, nerves, tendons and arteries), characteristics of devices (needles, syringes, tourniquets, disinfectants, evacuated tube systems), phlebotomy techniques, employability skills and emergency procedures. Moreover, basic notions of psychology, mostly aimed at providing relational, reassuring skills directed either to adults or to children should be given as part of the training. Before entering the work force, phlebotomists must achieve a minimum clinical curriculum, based on repeated practical experience, which should allow them to accomplish the entire phlebotomy process at the best. The diagnostic industry is crucial to this issue, in that it should provide healthcare operators with safety devices with the lowest possible chance of injuries and accidents. Direct supervision of phlebotomists by the laboratory staff would also be advisable, in that the laboratory professionals can provide phlebotomists with a comprehensive view of the complexity of the total testing process. Compliance with Best Practice (»BP«) goals, as included in Phlebotomy Personnel Standards and OSHA, JCAHO and CDC recommendations, should finally be regarded as essential means to improve the quality within the whole blood collection procedures. In Germany, for example, a training program available on CD-Rom and called DIAPRO was started. The program contains texts and examples to train future medical professionals in preventing errors during phlebotomy. Abating the number of unnecessary tests and limiting the number of steps in which specimens are delivered to laboratories, tests are performed, and results provided to the requesting physician might be additional but not alternative objectives, along with other pre-analytic issues not immediately associated with the phlebotomist's activity. Finally, the broad implementation of phlebotomists' certification worldwide would enable a high degree of professionalism in this crucial part of the total testing process.

PREANALITIČKA FAZA U HEMATOLOGIJI

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Preanalitička faza je naročito važna u hematologiji, gde se merenja čestica i ćelija vrše u celoj antikoagulisanoj krvi. Ispravna upotreba kao i koncentracija antikoagulansa je obavezna kako bi se izbegli nejasni rezultati, koji mogu uticati na kliničko odlučivanje.

PREANALYTICAL PHASE IN HAEMATOLOGY

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The preanalytical phase is particularly important in haematology, where counts of particles and cells are performed in whole anticoagulated blood. The correct use and concentration of anticoagulant is mandatory to avoid spurious results, which can influence clinical

EDTA je preporučljivi antikoagulans, mada ima izvesna ograničenja, posebno u vezi sa očuvanjem stabilnosti i oblika pločica. Stabilnost hematoloških parametara je visoka, sa izuzetkom leukocita i retikulocita. Međutim, stabilnost (i instrumentalnu preciznost) treba ocenjivati zajedno sa biološkom varijabilnošću i indeksom individualnosti različitih hematoloških parametara. Na hematološke testove takođe utiču visoke koncentracije lipida i kilomikrona. Postupak mešanja epruveta posle uzimanja krvi a pre analize takođe je ključan za dobijanje tačnih i validnih podataka. Postoje primeri interferencije na automatizovanim hematološkim analizatorima koji se koriste za dijagnostikovanje i praćenje patoloških stanja. Krioglobulini i paraziti eritrocita mogu izazvati nejasne rezultate WBC, RBC i PLT, ali ponavljanje takvih interferencija može se iskoristiti za alarmiranje lekara i može ukazati na prisustvo patoloških proteina ili parazita u krvi. Moderni hematološki analizatori donose nove parametre, direktno definisane ili izračunate na osnovu tradicionalnih mera, ali klinički uticaj tih novih parametara često zavisi od preanalitičkih varijabli.

decision. EDTA is the anticoagulant of choice, but it has some limits, especially for preserving stability and shape of platelets. Stability of haematological parameters is high, with the exception of leukocytes and reticulocytes. However, stability (and instrumental precision) should be evaluated together with biological variability and individuality index of various haematological parameters. Hematological tests are also influenced and interfered by high amounts of lipids and chylomicrons. The mixing procedure of the tubes after blood drawing and before analysis is also crucial for obtaining correct and valid data. There are some examples of interferences on automated haematological analysers which are used for diagnosing and screening pathological conditions. Cryoglobulins and erythrocytes parasites can induce spurious results of WBC, RBC and PLT, but the repeatability of these interferences could be used for alerting the pathologist and could reveal the presence of pathological proteins or blood parasites. New parameters have been proposed by modern haematological analysers, directly defined or calculated from traditional measures, but the clinical impact of these new parameters is often dependent on preanalytical variables.

KVALITET DIJAGNOSTIČKIH UZORAKA, PREPORUKE I NASTAVNA SREDSTVA

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Minhen, Nemačka

Analitički standardi opisani su utvrđenim kriterijumima kontrole kvaliteta, međutim, za prenalitičku fazu takvi standardi ne postoje. S druge strane, mnogi preanalitički aspekti obuhvaćeni su poslednjom Normom EN ISO 15189 za rukovođenje kvalitetom u medicinskim laboratorijama. Preporuke za kvalitet dijagnostičkih uzoraka (1,2) uključuju definiciju optimalne količine uzorka, upotrebu antikoagulanasa i stabilizatora, kao i kriterijume stabilnosti tokom transporta i čuvanja. Osim toga, preanalitičko rukovanje hemoliziranim, lipemičnim i ikteričnim uzorcima se ponovo procenjuju. Tehničke preporuke u pogledu uzorkovanja, transporta i identifikacije su izdate od strane NCCLS, SZO i IFCC. Nedavno su ti standardi objavljeni kao nastavna sredstva na internetu (3). Ta verzija dopušta da se unesu pitanja u vezi sa 350 analita, na koja će se odgovoriti preko podataka koji se odnose na izbor antikoagulansa, stabilnost i potrebnu količinu uzorka. Pored toga, nemački program obuke koji je nedavno objavljen na CD-romu sadrži podatke o preanalitičkim varijablama kao i preporuke za sve procedure uzorkovanja (4). Ti programi su testirani u pet nacionalnih radionica kao materijal za obuku i mogu isto tako služiti za akreditacione procedure medicinskih laboratorija, pošto je preanalitička faza identifikovana kao glavni izvor grešaka u laboratorijama širom sveta.

QUALITY OF DIAGNOSTIC SAMPLES, RECOMMENDATIONS AND EDUCATIONAL TOOLS.

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Whereas analytical standards are described by established quality control criteria, no such standards exist regarding the preanalytical phase. On the other hand, many preanalytical aspects are part of the recent Norm EN ISO 15189 on quality management in the medical laboratory. Recommendations on the quality of diagnostic samples (1,2) include the definition of optimal sample size, the use of anticoagulants and stabilizers, as well as stability criteria regarding transport and storage. Moreover, the preanalytical handling of haemolytic, lipemic and icteric samples has been reevaluated. Technical recommendations regarding sampling and transport identification have been published by NCCLS, WHO and IFCC. Recently, these standards have been edited as educational tools in the internet (3). This version allows to enter questions regarding 350 analytes, which will be answered by data regarding the choice of anticoagulant, stability and sample volume needed. In addition, a German teaching program on CD-Rom has recently been published containing knowledge on the preanalytical variables as well as recommendations for all sampling procedures (4). These programs were tested in five national workshops as teaching material and can likewise serve for accreditation procedures of medical laboratories, after the preanalytical phase was identified as the major source of errors in laboratories worldwide.

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STABILNOST UZORKA

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Kliničke laboratorije su se poslednjih godina našle usred vrtloga promena koje su zadesile medicinu. Predlažu se i implementiraju različite strategije čiji je cilj kontrolisanje i smanjivanje ukupnih troškova laboratorijskih usluga. One uključuju centralizaciju, konsolidaciju i integraciju usluga, reinženjering laboratorije i viši nivo automatizacije, optimalnu upotrebu testova, decentralizaciju postupka testiranja u smeru point-of-care testiranja. Rastući trend spajanja bolnica doveo je do konsolidacije laboratorijskih usluga iz logističkih i ekonomskih razloga. Štaviše, mnoge bolnice, klinike i privatne prakse iz ekonomskih razloga praktikuju slanje uzoraka na testiranje komercijalnim laboratorijama, pošto metode low-volume ili ezoterični testovi nisu ekonomski isplativi. U delovima zemlje gde su mreže zdravstvenih ustanova jake sa agresivnim programima delovanja zatvaraju se laboratorije u manjim bolnicama a testovi se šalju u centralnu laboratoriju. Zahvaljujući izvanrednom porastu broja decentralizovanih službi flebotomije širom sveta, uzorci krvi čak ponekad stižu u centralnu laboratoriju sa različitih udaljenosti, pod različitim uslovima skladištenja i transporta. Kao rezultat, centralne laboratorije koje opslužuju mreže zdravstvenih ustanova iskusile su porast radnog opterećenja i suočene su sa novim problemima u vezi sa stabilnošću uzorka. Nepostojanje standardizovanih postupaka za uzimanje uzorka razlog je najvećeg broja grešaka koje se događaju u celokupnom postupku testiranja. One obuhvataju neodgovarajuće postupke za uzimanje i rukovanje uzorcima, kao što je upotreba pogrešnih alatki, prolongiran zastoj u proticanju krvi tokom sečenja vene, vreme pre centrifugiranja ili analize i konačno neodgovarajući način skladištenja. Osoblje u laboratoriji ne može u potpunosti da kontroliše probleme u pripremi i transportu uzoraka iz periferne ustanove do centralizovane laboratorije. Neke indikaci-

SAMPLE STABILITY

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Over the past years, clinical laboratories have been at the vortex of the maelstrom affecting medicine. Various strategies to contain and reduce overall costs of laboratory services are being implemented or have been advocated. These include centralization, consolidation and integration of services, reengineering the laboratory and increasing the level of automation, optimizing test usage, decentralizing testing to the point-of-care. The growing trend to closures and mergers of hospital have led to consolidation of laboratory services for logistic and economic reasons. Moreover, many hospitals, clinics and physician practices find it economic to send out specimens for testing to commercial laboratories, because performing low volume or esoteric testing is not economically viable. In areas of the country in which healthcare networks with aggressive outreach programs are strong, laboratories in smaller hospitals are being closed, and the tests sent to a central laboratory. Moreover, due to the tremendous growth of decentralized phlebotomy services worldwide, blood samples may arrive in the central laboratory from varying distances, under variable storage and transportation conditions. As a result, central laboratories serving healthcare networks are experiencing an increase in workload and facing new problems related to sample stability. The lack of standardized procedures for sample collection, account for most of the errors encountered within the total testing process. These include inappropriate procedures for collection and handling of the specimens, such as the use of improper collection tools, prolonged stasis during venipuncture, and time before centrifugation or analysis and finally unsuitable storage. The problems in preparation and transporting of samples from a peripheral facility to a centralised laboratory cannot be

je date su od strane Nacionalnog komiteta za standarde u kliničkoj laboratoriji (NCCLS)/Instituta za standarde u kliničkoj laboratoriji (CLSI) u pravilniku o uzimanju uzoraka krvi za laboratorijsko testiranje, ali one ne govore jasno da li uzorke treba skladištiti kao celu krvi ili kao plazmu, a koliko smo mi upućeni samo se mali broj istraživanja bavio pitanjem stabilnosti uzorka u pogledu temperature i vremena pre centrifugiranja za koagulaciju ili kliničko-hemijsku analizu. Opšti problem s kojim se suočavaju kliničke laboratorije jeste integritet necentrifugiranih uzoraka za hemijske analize. Kako je produženi kontakt plazme ili seruma sa ćelijama čest razlog nejasnih rezultata testova, idealno bi bilo odvojiti plazmu i serum što je pre moguće kako bi se sprečio metabolizam ćelijskih konstituenata kao i aktivno i pasivno kretanje analita između plazme ili seruma i ćelijskih pregrada. Međutim, testovi zgrušavanja su naročito podložni lošoj standardizaciji nekoliko postupaka u okviru celokupnog postupka testiranja i na njih verovatno više utiču uslovi skladištenja pre centrifugiranja. U naučnoj literaturi ima izveštaja o izvesnom broju potencijalnih zamki u vezi sa uslovima skladištenja i centrifugiranjem (temperatura i vreme) uzoraka cele krvi. Stabilnost uzorka je posebno kritična, naročito kada uzorci krvi ne mogu biti analizirani odmah ili kada je potrebno ponovno testiranje. Upravljanje ovom preanalitičkom varijablom (tj. stabilnošću uzorka) smanjilo bi laboratorijske troškove i podstaklo poverenje između lekara i osoblja u laboratoriji.

fully controlled by the laboratory staff. Some indications were provided by the National Committee for Clinical Laboratory Standards (NCCLS) / Clinical Laboratory Standard Institute (CLSI) guidelines on the collection of blood specimens for laboratory testing, but they do not clearly stipulate whether samples should be kept as whole blood or plasma and to the best of our knowledge, only few investigations have focused on the sample stability in regard to the temperature and the time before centrifugation for coagulation or clinical chemistry testing. A general problem facing clinical laboratories is the integrity of uncentrifuged specimens for chemical analyses. Because prolonged contact of plasma or serum with cells is a common cause of spurious test results, plasma and serum should ideally be separated from cells as quickly as possible to prevent ongoing metabolism of cellular constituents as well as active and passive movement of analytes between the plasma or serum and cellular compartments. However, clotting assays are particularly susceptible to poor standardization of several process of the total testing process and they are probably more influenced by the conditions of storage before centrifugation. Several pitfalls related to the storage condition and centrifugation (temperature and time) of whole blood samples have been previously reported in scientific literature (1–2). Sample stability may be particularly critical, especially when blood specimens cannot be analyzed immediately or when retesting is required (3–4). The governance of this preanalytical variable (i.e. sample stability), would reduce laboratory costs and enhance the physician-laboratory confidence.

RAZVIJANJE LEAN FILOZOFIJE U KLINIČKIM LABORATORIJAMA

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Ovo je pregled principa *Lean* i *Six Sigma* kao sredstava protoka operacija i poboljšanja produktivnosti. Proizvođački sektori su iskoristili ove koncepte sa mnogo uspeha. Primarni cilj lean inicijative je da se daju kvalitetni proizvodi i usluge odmah i uvek. Da bi se ovo postiglo, sve aktivnosti koje ne doprinose vrednosti (npr. trošak) treba da se eliminišu ili, ako to nije izvodljivo, da se smanje. Zahtevi današnjeg sistema zdravstvene zaštite opravdavaju integraciju sistema upravljanja kvalitetom kao što su oni za prihvatanje povećanja radnog opterećenja, deficit osoblja, i zahtev za brz obrt rezultata uzorka. Ovaj članak diskutuje *Lean* i *Six Sigma* strategije kao i njihove aplikacije za kliničke laboratorije, specifično upotrebljavajući sredstva kao što su 5S, *Kaizen Blitz*, planiranje procesa i planiranje protoka vredno-

DEVELOPING A LEAN CONSCIOUSNESS FOR THE CLINICAL LABORATORY

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This is an overview of the principles of Lean and Six Sigma as a means of streamlining operations and improving productivity. Manufacturing sectors have employed these concepts with much success. The primary goal of a lean initiative is to deliver quality products and services the first time and every time. To accomplish this, all activities that do not add value (i.e., waste) must be eliminated or, if not feasible, reduced. The demands of today's healthcare environment warrant the integration of quality management systems such as these to meet increased workloads, staff shortages, and the demand for rapid turnaround for specimen results. This paper discusses Lean and Six Sigma strategies as well as their application for the clinical laboratory, specifically utilizing tools such as 5S, the

sti. Implementacija ovih sredstava maksimalno povećava protok proces, eliminiše troškove, i prepoznaje varijacije koje mogu sprečiti pružanje visoko-kvalitetnih usluga, zdravstvenim radnicima omogućava dostizanje ciljeva efikasnosti, smanjuje cenu, i obezbeđuje zadovoljstvo korisnika.

Kaizen Blitz, process mapping, and value stream mapping. By implementing these tools to maximize process flow, eliminate waste, and recognize the variations that can hinder the delivery of high-quality services, healthcare professionals can reach their efficiency goals, reduce costs, and provide customer satisfaction.

Sekcija 4 Session 4

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Plenary sessions

KLINIČKI UTICAJ WHO STANDARDIZACIJE NA PSA TESTOVE

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Određivanje nivoa antigena specifičnog za prostatu (PSA) u serumu ima široku primenu u otkrivanju i praćenju toka bolesti kod raka prostate. Pokazalo se da između različitih testova za određivanje PSA koji postoje na tržištu postoji analitička varijabilnost. Utvrđeno je da je neslaganje u rezultatima PSA u vezi sa neekvimolarnim određivanjem ukupnog PSA (tPSA), ali i sa nedostatkom standardizacije testova za određivanje, i može imati ozbiljne kliničke posledice na dijagnostičke karakteristike testiranja PSA. Veruje se da rekalibracija ekvimolarnih testova do uobičajenih referentnih standarda (tPSA SZO 96/670) i tPSA 96/688) pomaže standardizaciju testova za određivanje PSA i ograničava kliničke implikacije varijabilnosti testova. Uporedne studije pokazale su da kalibracija testova za PSA prema standardima SZO značajno unapređuje harmonizaciju analiziranja PSA, ali razlike između testova i dalje postoje. Nedavna ispitivanja kliničkog uticaja analitičkih varijacija zbog kalibracije prema standardu SZO pokazala su da bi se moglo prevideti 15 do 30% slučajeva raka prostate ako bi se koristile zastarele »cut-off« vrednosti. Kako bi se izbegla neprihvatljiva erozija kliničke dijagnostičke performase određivanja PSA u cilju otkrivanja raka prostate pomoću testova kalibrisanih prema SZO, neophodno je odrediti nove specifične tačke za kliničko odlučivanje.

THE CLINICAL IMPACT OF WHO STANDARDIZATION OF PSA ASSAYS

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The determination of serum level of the prostate specific antigen (PSA) is widely used for detection and management of prostate cancer. Analytical variability between the various PSA assays on the market has been reported. This discrepancy in the PSA results was shown to be related to non-equimolar detection of total PSA (tPSA) but also to a lack of assay standardization and could have serious clinical repercussions on the diagnostic performance of PSA testing. The recalibration of equimolar assays to common reference preparations (tPSA WHO 96/670 and fPSA 96/668) was thought to promote standardization of PSA assays and limit the clinical implication of assay variability. Comparison studies have demonstrated that PSA assay calibration to the WHO standard certainly improves the harmonisation of PSA testing, but differences between assays remain. Recent evaluations of the clinical impact of analytical variations induced by a calibration to the WHO standard showed that 15% to 30% of prostate cancer could be missed if the historical tPSA cut-off was used. In order to avoid unacceptable erosion of the clinical diagnostic performance of PSA determination for the detection of prostate cancer with WHO calibrated assays, it is critical to define new specific clinical decision points.

KONCENTRACIJA CISTATINA C U SERUMU PACIJENATA POSLE TRANSPLANTACIJE I TRETMANA IMUNOSUPRESIJE GLUKOKORTIKOIDIMA

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Cistatin C opisan je kao marker koji poseduje mnoge osobine idealnog markera za stopu glomeru-

SERUM CYSTATIN C CONCENTRATION IN TRANSPLANTED PATIENTS TREATED WITH GLUCOCORTICOID IMMUNOSUPPRESSION

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Cystatin C has been described as meeting many of the characteristics of an ideal GFR marker and has

larne filtracije (GFR) i pokazalo se da je u najmanju ruku precizan koliko i kreatinin u serumu koji se obično koristi za otkrivanje oslabljene bubrežne funkcije u različitim grupama pacijenata, uključujući pacijente posle transplantacije bubrega. Cilj studije je da rasvetli uticaj imunosupresije glukokortikoidima na koncentraciju cistatina C u serumu pacijenata posle transplantacije bubrega. Kako bi se ispitao uticaj imunosupresivnih režima, naročito glukokortikoida, na cistatin C u serumu, oformljene su grupe od 38 klinički stabilnih pacijenata na imunosupresivnoj terapiji niskim dozama glukokortikoida, 30 klinički stabilnih pacijenata koji primaju samo ciklosporin A i 18 klinički stabilnih pacijenata koji primaju ciklosporin A zajedno sa azatioprinom. Klinička stabilnost definisana je kao odsustvo akutnog odbijanja, febrilne infekcije i toksičnosti ciklosporina, kao i stabilnost klirensa kreatinina izračunatog pomoću formule Kokrofta i Golta. Članovi tri grupe u kojima je određivan klirens kreatinina (CrCl) birani su na osnovu pola, godina i vremena koje je prošlo od transplantacije. Da bi se smanjio uticaj poznatih bioloških varijacija cistatina C, kod svih pacijenata obavljeno je šest merenja tokom uzastopnih poseta koja su pokazala stabilno kliničko stanje. Srednje vrednosti cistatina C, kao i procenjenog CrCl i CysCGFR izračunate su i korišćene u analizi podataka. Analiziranjem deset pacijenata koji primaju kratkoročnu terapiju visokim dozama metilprednizolona (500 mg dnevno intravenski tokom tri dana) za smanjenu bubrežnu funkciju uočen je potencijalni doznozavisni efekat administracije glukokortikoida. U grupi koja prima kratkoročnu terapiju visokim dozama metilprednizolona rezultati su određivani na osnovu četiri dostupne vremenske tačke: a) pre početka terapije metilprednizolonom (medijana, 15 dana); b) na dan početka terapije metilprednizolonom (pre davanja leka); c) posle tri dana terapije metilprednizolonom; i d) na kontroli 9–10 dana posle poslednje doze. Cistatin C u serumu određivan je metodom particle-enhanced turbidimetric immunoassay (»PETIA«, »Dako«) na analizatoru »Cobas Mira« (»Roche«). Kreatinin u serumu izmeren je modifikovanom kinetičkom Jaffeovom metodom. Klirens kreatinina izračunat je pomoću formule Kokrofta i Golta, a cistatin C GFR pomoću Grubbove formule ($CysCGFR = 84{,}69, CysC^{-1.680}$). Kod pacijenata koji primaju dugoročnu terapiju niskim dozama glukokortikoida otkrivene su više koncentracije cistatina C nego u kontrolnoj grupi (2,25; 1,9–2,9, $P<0{,}05$). Visoke doze metilprednizolona date intravenski dovele su do značajnih razlika u vrednostima cistatina C u različitim vremenskim tačkama (pre administracije, posle tri doze, i posle 8 dana od prestanka terapije; $P<0{,}001$). Posle tri dnevne doze od 500 mg, koncentracije cistatina C skočile su sa 2,13 mg/L (1,72–2,80) na 2,69 mg/L (2,34–3,5; $P<0{,}05$). Osam dana posle prestanka terapije, koncentracije cistatina C su se značajno snizile na 1,96 mg/L (1,63–2,4; $P<0{,}05$). U tim vremenskim tačkama, ni procenjena vrednost ClCr (54 ± 13 mL·min^{-1} · 1,73 m^{-2}, 51 ± 15 mL · min^{-1} · 1,73 m^{-2}, i 56 ± 14 mL · min^{-1} · 1,73 m^{-2}; $P=0{,}05$), niti koncentracije kreatinina u serumu (165 μmol/L, been reported to be at least as accurate as the commonly used serum creatinine to detect impaired renal function in various patient groups, including renal transplant patients. The present study aimed to elucidate the influence of glucocorticoid immunosuppression on cystatin C concentration in the serum of renal transplant patients. To evaluate the influence of immunosuppressive regimens, especially glucocorticoids, on serum cystatin C, 38 clinically stable patients on immunosuppression therapy with low-dose glucocorticoids were matched with 30 clinically stable patients receiving cyclosporin A alone and 18 clinically stable patients receiving cyclosporin A together with azathioprine. Clinical stability was defined as the absence of acute rejection, febrile infection, and cyclosporin A toxicity, as well as stability of creatinine clearance as estimated by the formula of Cockroft and Gault. The three groups were matched for estimated creatinine clearance (CrCl) and had comparable gender, age, and time since transplantation. To reduce the influence of the known biologic variation of cystatin C, all patients had six measurements during subsequent visits that demonstrated a stable clinical condition. Means from cystatin C reciprocals, as well as from CrCl estimates and CysCGFR were calculated and used for data analysis. Furthermore, 10 patients receiving a short course of high-dose methylprednisolone (500 mg intravenously per day for 3 days) for deteriorating renal function were analyzed to observe a possible dose-dependent effect of glucocorticoid administration. The group receiving short-course, high-dose methylprednisolone had results from four time points available: a) before methylprednisolone commencement (median, 15 days); b) the day methylprednisolone was started (before medication); c) after 3 days of methylprednisolone therapy; and d) on a follow-up 9-10 days after last dose. Serum cystatin C was measured by a particle-enhanced turbidimetric immunoassay (PETIA; Dako) on a Cobas Mira (Roche). Serum creatinine was measured with a modified kinetic Jaffe method. Creatinine clearance was estimated by the formula of Cockroft and Gault, and the cystatin C GFR was measured by the formula of Grubb ($CysCGFR = 84.69.CysC^{-1.680}$). Patients receiving long-term, low-dose glucocorticoid therapy showed higher cystatin C concentrations than controls (2.25; 1.9–2.9, $P<0.05$). High-dose methylprednisolone given intravenously led to significant differences in cystatin C values at different time points (before administration, after three doses, and 8 days after discontinuation; $P<0.001$). After three daily doses of 500 mg, cystatin C concentrations increased from 2.13 mg/L (1.72–2.80) to 2.69 mg/L (2.34–3.5; $P<0.05$). Eight days after discontinuation, cystatin C concentrations significantly decreased to 1.96 mg/L (1.63–2.4; $P<0.05$). At these time points, neither the CrCl estimate (54 ± 13 mL · min^{-1} · 1.73 m^{-2}, 51 ± 15 mL · min^{-1} · 1.73 m-2, and 56 ± 14 mL · min^{-1} · 1.73 m^{-2}; $P = 0.05$). The serum creatinine concentrations (165 μmol/L,158 μmol/L, and 162 μmol/L, $P=0.19$) underwent signif-

158 μmol/L, i 162 μmol/L, P=0,19) nisu se značajno promenile. Studija je pokazala da pacijenti posle transplantacije bubrega na terapiji glukokortikoidima imaju viši cistatin C nego preostale dve grupe na imunosupresivnoj terapiji bez glukokortikoida. Kako su pacijenti koji primaju 500 mg metilprednizolona imali značajno više vrednosti cistatina C nego pacijenti koji primaju 10 mg prednizona, može se govoriti o dozno-zavisnom uticaju datih doza glukokortikoida. Dakle, glukokortikoidi dovode to sistematski pogrešno procenjenih nižih vrednosti stope glomerularne filtracije kod pacijenata posle transplantacije bubrega. Primena glukokortikoida kod odraslih pacijenata posle transplantacije bubrega povezana je na dozno-zavisan način sa povišenim cistatinom C, što dovodi do sistematski pogrešnog utvrđivanja niže stope glomerularne filtracije. To ne isključuje mogućnost korišćenja cistatina C u otkrivanju oslabljene bubrežne funkcije kod pacijenata posle transplantacije bubrega na glukokortikoidima, pošto su ova i druge studije pokazale da je cistatin C značajno precizniji u otkrivanju oslabljene bubrežne funkcije kod te grupe pacijenata. Štaviše, naši podaci ukazuju na potrebnu za specifičnim referentnim intervalima kod pacijenata na terapiji glukokortikoidima. U rutinskoj kliničkoj praksi, kao i u budućim kliničkim studijama, važno je uzeti u obzir glukokortikoide prilikom tumačenja koncentracija cistatina C u serumu pacijenata posle transplantacije bubrega a potencijalno i u ostalim grupama pacijenata.

icant changes. This study demonstrates that renal transplant patients receiving glucocorticoid medication have higher cystatin C than two comparable groups with glucocorticoid-free immunosuppression. Because patients receiving 500 mg of methylprednisolone had significantly higher cystatin C values than patients receiving 10 mg of prednisone, a dose-dependent influence of the administered glucocorticoid dose is suggested. Thus, glucocorticoid medication leads to systematic underestimation of GFR in renal transplant patients. Glucocorticoid medication in adult renal transplant patients is associated in a dose-dependent manner with increased cystatin C, leading to systematic underestimation of GFR. This does not preclude the use of cystatin C in detecting impaired renal function in renal transplant patients with glucocorticoids, because this study and others showed cystatin C to be significantly more accurate in detecting impaired renal function in this patient group. Moreover, our data illustrate the need for specific reference intervals in patients on glucocorticoid therapy. In clinical routine settings, as well as in future clinical studies, it is important to take glucocorticoid medication into account when interpreting serum cystatin C concentrations in renal transplant patients and presumably in other patient groups.

ULOGA GLUTATION S-TRANSFERAZA U TUMORIMA URINARNOG TRAKTA

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Jedan od etioloških faktora za nastanak karcinoma bubrega svetlih ćelija i karcinoma prelaznog epitela mokraćne bešike je izloženost potencijalnim kancerogenima. Karcinom bubrega svetlih ćelija pripada tumorima sa visokim stepenom rezistencije na hemioterapiju, a karcinom prelaznog epitela mokraćne bešike grupi rekurentnih i multifokalnih tumora. Citosolne glutation S-transferaze (GST) su superfamilija enzima koji u zdravim ćelijama imaju zaštitnu ulogu od potencijalnih kancerogena, katališući reakcije konjugacije elektrofilnih jedinjenja sa glutationom. Neki izoenzimi GST poseduju i hidroperoksidaznu aktivnost. Najbolje okarakterisane klase GST su Alfa (GSTA), Mi (GSTM), Pi (GSTP) i Teta (GSTT), a svaka od ovih klasa sadrži više različitih izoenzima. Uočeno je nekoliko alelskih varijacija unutar različitih klasa, usled kojih GSTM1 nulti i GSTT1 nulti genotip imaju za posledicu odsus-

THE ROLE OF GLUTATHIONE S-TRANSFERASES IN URINARY TRACT TUMORS

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Exposure to potential carcinogens is among etiological factors for renal cell carcinoma (RCC) and transitional cell carcinoma (TCC) of urinary bladder. RCC is very resistant, while TCC exhibits high recurrence rate and multifocality. Cytosolic glutathione S-transferases (GST) are a superfamily of enzymes which protect normal cells by catalyzing conjugation reactions between electrophylic compounds, including carcinogens, and glutathione. Some GST enzymes posses hydroperoxidase activity. The most well characterized classes have been named Alpha (GSTA), Mu (GSTM), Pi (GSTP) and Theta (GSTT) and each of these classes contain several different isoenzymes. Several types of allelic variation have been identified within classes, among which GSTM1-null and GSTT1-null confer impaired catalytic activity. Individuals with the GSTM1 null genotype carry a substantially higher risk for blad-

tvo katalitičke aktivnosti. Kod osoba koje su homozigoti za GSTM1 nulti alel uočen je značajno veći rizik za nastanak karcinoma mokraćne bešike. Uticaj polimorfizma GSTT1 na povećanu osetljivost za nastanak karcinoma bubrega svetlih ćelija i karcinoma prelaznog epitela mokraćne bešike u velikoj meri zavisi od izloženosti određenim hemijskim agensima koji se metabolišu pomoću GSTT1-1. U procesu nastanka tumora bubrega dolazi do smanjenja ekspresije izoenzima, pripadnika GST klase alfa, sa posledičnom promenom redoks statusa, što pogoduje razvoju karcinoma bubrega svetlih ćelija. Enzimska aktivnost GST pi je neizmenjena u karcinoma bubrega svetlih ćelija i može doprinositi hemorezistenciji ovih tumora. U nastanku malignog fenotipa karcinoma prelaznog epitela mokraćne bešike značajnu ulogu ima povećana ekspresija različitih klasa GST. Povećana ekspresija GSTT1-1 i GSTP1-1 može značajno uticati na rast tumora, pomeranjem ravnoteže između pro- i antioksidanasa ka redukovanom stanju, što pogoduje inhibiciji apoptotskih puteva.

der carcinogenesis. The effects of glutathione S-transferase T1 polymorphism on the increased susceptibility to RCC and TCC of urinary bladder depend on the presence of specific chemical exposures to compounds metabolized via GSTT1-1 pathway. In the process of kidney cancerisation expression of GST alpha isoenzymes tends to decrease, consequently favoring prooxidant environment necessary for growth of RCC. GST pi enzyme activities are generally retained in RCC and might contribute to their chemotherapy resistance of RCC. In the malignant phenotype of TCC of urinary bladder up regulation of various GST classes occurs. Up regulation of GSTT1-1 and GSTP1-1 might have important consequences on the tumor growth, by providing reduced environment and inhibition of apoptotic pathways.

Sekcija 5 Session 5

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FUNKCIONALNA HRANA-ULOGA U UNAPREĐENJU ZDRAVLJA

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Funkcionalna hrana je hrana koja ima povoljan uticaj na ljudsko zdravlje mimo uobičajenih nutritivnih funkcija. Biološki aktivna jedinjenja su nosioci povoljnog dejstva funkcionalne hrane. Brojni naučni dokazi govore u prilog tome da je ishrana bogata pojedinim namirnicama (kao što su to na primer voće i povrće) direktno u vezi sa smanjenim rizikom od hroničnih, nezaraznih bolesti, tako da se na tim saznanjima razvio koncept funkcionalne hrane. Otkrivaju se funkcionalne osobine tradicionalnih namirnica, ali se dizajniraju i nove funkcionalne namirnice. Uobičajene izjave koje prate tu vrstu namirnica mogu se svrstati u dve kategorije: (1) izjave o odnosu strukture i funkcije (engl. Structure and function claims) moraju da budu istinite i da ne dovode u zabludu potrošača. Te izjave ne moraju da budu odobrene od strane FDA. (2) Zdravstvene izjave (engl. Health claims ili disease-specific claims) moraju da budu autorizovane od strane FDA i da poseduju značajnu naučnu potvrdu (Hillovi kriterijumi). Neophodno je rangiranje dokaza različitih tipova studija koje podržavaju zdravstvenu izjavu. Veliki broj biološki aktivnih jedinjenja su nestabilna tokom tretmana i čuvanja. Ona podležu mnogobrojnim hemijskim reakcijama, kao što su to oksidacija, hidroliza, termička degradacija i Maillardova reakcija, što rezultira smanjenjem bioiskoristljivosti. Povoljan efekat biološki aktivnih jedinjenja direktno zavisi od primenjenog tretmana.

THE FUNCTIONAL FOODS – THE ROLE IN HEALTH IMPROVEMENT

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Functional foods are foods that may provide a health benefit beyond basic nutrition. Numerous scientifically proven pieces of evidence in many epidemiological studies indicate that nutrition abundant in certain foods (e.g. fruits and vegetables) is directly correlated with a decreased risk of degenerative diseases. Biologically active compounds in functional foods may impart health benefits or desirable physiological effects. Functional attributes of many traditional foods are being discovered, while new food products are being developed with beneficial components. These results are closely related to nutrition's potentials in preventing chronic diseases. Based on these facts the concept of functional foods has been developed. Rigorous scientific investigation has to confirm the positive physiological effects of these compounds upon health. Labeling claims that are used on functional foods are of two types: (1) structure and function claims, which describe effects on normal functioning of the body, but not claims that the food can treat, diagnose, prevent, or cure a disease (claims such as »promotes regularity«, »helps maintain cardiovascular health«, and »supports the immune system« fit into this category); and (2) disease-risk reduction claims, which imply a relationship between dietary components and a disease or health condition. Structure and function claims do not require preapproval by the FDA, and they require much less stringent scientific consensus than disease-risk reduction claims. Many biologically active compounds are unstable during treatments and storage. They undergo many common chemical reactions such as oxidation, hydrolysis, thermal degradation and Maillard reaction, contributing to the lowering of bioavailability. Anyhow, beneficial effect of bioactive compounds depends directly on the applied treatment in the production of foods.

TAJNI ŽIVOT MASTI: ŠTA MASNE ĆELIJE ČINE ZA REGULISANJE METABOLIZMA

A. Končeva

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Adipozno tkivo se dugo smatralo organom čija je jedina svrha skladištenje viška energije u obliku triglicerida i otpuštanje energije u obliku slobodnih masnih kiselina, što samo po sebi predstavlja neophodni sistem samoodbrane u cilju preživljavanja tokom gladovanja. Takav stav je danas izmenjen, a masno tkivo se pokazalo kao endokrini i sekretorni organ koji utiče na nekoliko metaboličkih puteva. Njegova glavna endokrina funkcija je lučenje izvesnog broja hormona, pre svega leptina i adiponektina. Adipozno tkivo takođe otpušta adipokine uključene u inflamaciju i hemostazu: faktore rasta (TNFα, transformišući faktor rasta-beta, nervni faktor rasta, VEGF), citokine (IL-1β, IL-6, IL-10), hemokine (IL-8), proteine akutne faze (haptoglobin, serumski amiloid A) i protrombotski faktor (plazminogen aktivatora inhibitor-1). Cilj ove studije je da predstavi odabrane predmete skorašnjih istraživanja adipokina koji bi mogli imati poseban značaj.

THE SECRET LIFE OF FAT: WHAT FAT CELLS DOING FOR REGULATION OF METABOLISM

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Adipose tissue has long been regarded as an organ the sole purpose of which was to store excess energy as triglycerides, and release energy as free fatty acids, which itself is an essential self-defense system for survival during starvation. This point of view has now changed, fat tissue has emerged as an endocrine and secretory organ affecting more than one metabolic pathway. Its major endocrine function is secreting several hormones, notably leptin and adiponectin. Also adipose tissue releases adipokines involved in inflammation and hemostasis: growth factors (TNFα, transforming growth factor-beta, nerve growth factor, VEGF), cytokines (IL-1β, IL-6, IL-10), chemokines (IL-8), acute-phase proteins (haptoglobin, serum amyloid A) and prothrombotic factor (plasminogen activator inhibitor-1). This review aims to present some of the recent topics of selected adipokine research that may be of particular importance.

KLINIČKI ZNAČAJ INHIBICIJE CITOHROMA P450 3A4

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Citohrom P450 3A4 (CYP3A4) je enzim uključen u metabolizam velikog broja lekova. Indukcija ili inhibicija enzima mogu uzrokovati sniženje ili povećanje koncentracija lekova u plazmi. Ukoliko su plazma koncentracije lekova značajno izmenjene može biti ugrožena efikasnost terapije ili pacijent može imati povećani rizik od toksičnosti. Klinički značajne interakcije lekova koje u osnovi imaju inhibiciju CYP3A4 najverovatnije će se pojaviti kod lekova sa uskim terapijskim indeksom ili niskim stpenom bioraspoloživosti. Ozbiljna neželjena dejstva uzrokovana inhibicijom CYP3A4 su poznata. Među najznačajnijima se nalaze ventrikularne aritmije (torsades de pointes), rabdomijoliza, pojačana sedacija, ataksija, ergotizam i fatalna hipotenzija. Zabeležena su takođe neželjena dejstva nakon inhibicije CYP3A4 koja se u odsustvu inhibitora ne javljaju poput akutne hepatične insuficijencije kod primene budesonida. Izostanak terapijske efikasnosti do koje dolazi pri rezistenciji na klopidogrel se takođe pripisuje inhibiciji CYP3A4. Nasuprot tome, interakcije lekova mogu imati i pozitivan ishod poput sniženja neophodne doze skupe terapije ciklosporinom nakon

CLINICAL IMPACT OF CYTOCHROME P450 3A4 INHIBITION

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Cytochrome P450 3A4 (CYP3A4) is an enzyme involved in the metabolism of the majority of drugs. Induction or inhibition of the enzyme may induce reduction or elevation of drug plasma concentrations. If plasma levels are significantly altered treatment efficacy may be jeopardized or the patient may be at risk of increased toxicity. Clinically significant drug interactions which have the inhibition of CYP3A4 as the underlying mechanism are likely to occur in drugs with narrow therapeutic index or low bioavailability. Several severe adverse effects due to CYP3A4 inhibition have been reported. Among the most important are ventricular arrhythmias (torsades de pointes), rhabdomyolysis, excessive sedation, ataxia, ergotism and fatal hypotension. Drugs may also develop side effects after CYP3A4 inhibition which normally do not occur such as acute hepatic insufficiency after administration of budesonide. Also lack of treatment efficacy such as resistance to clopidogrel has been attributed to CYP3A4 inhibition. Nevertheless, drug interactions may also have a positive outcome, such as decrease of necessary dose of the high cost treatment with cy-

inhibicije CYP3A4. Farmaceuti moraju imati aktivnu ulogu u prevenciji ili potenciranju interakcija lekova, a za to moraju uzeti u obzir osobine lekova, karakteristike pacijenta i vreme primene lekova.

closporine after CYP3A4 inhibition. Pharmacists should have an active role in preventing or potentiating drug interactions and should therefore take into consideration drug properties, patient characteristics and time of drug administration.

MALE NEKODIRAJUĆE RNK – NOVINE U DIJAGNOSTICI I TERAPIJI

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Nekodirajuće RNK predstavljaju specifičnu klasu RNK koje ne služe u procesu translacije proteina. One učestvuju u nizu različitih regulatornih procesa vezanih za proces transkripcije i posttranskripcioni nivo. Pomenuta familija RNK sadrži različite tipove, ali su gotovo sve oko 20–30 nukleotida dugačke, nastale iz prekursora veće dužine. Neke od njih, kao što je siRNK (mala interferentna RNK) formiraju se nakon razgradnje dvostrukospiralnih RNK (dsRNK) i udružene su sa virusnom infekcijom. Druge familije, poznate kao mikro RNK (miRNK), kodiraju se od strane specifičnih gena. Glavna funkcija je inhibicija translacije, razgradnja mRNK ili indukcija razgradnje mRNK, poznata kao utišavanje mRNK. Prepoznavanje target mRNK moguće je po principu komplementarnosti baza. Stepen komplementarnosti određuje uspešnost utišavanja. Korišćenje iRNK u antivirusnom odgovoru predstavlja novi terapijski izazov. Dok neke siRNK imaju zadatak da suprimiraju razvoj oboljenja (virus), druge su uključene u razvoj humanih kancera. Stoga je njihova identifikacija važna sa dijagnostičkog i terapijskog aspekta kod različitih tipova karcinoma i različitih stadijuma oboljenja. Neki tipovi miRNK su udruženi sa razvojem degenerativnih i metaboličkih oboljenja. S obzirom na to da pravilan razvoj nervnog sistema, insulinska sekrecija, kao i važni metabolički procesi zahtevaju preciznu kontrolu na nivou ekspresije gena, kontrola stabilnosti i translacije mRNK putem malih nekodirajućih RNK predstavlja dominantnu regulaciju različitih metaboličkih, endokrinih i neuroloških procesa.

SMALL NON-CODING RNAs – DIAGNOSTIC AND THERAPEUTIC NEWS

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Non-coding RNAs represent a specific type of RNAs that are non translated into proteins. They participate in a number of different regulatory processes concerning the transcriptional and posttranscriptional level. This family contains different types, each mainly 20-30 nucleotides long, excised from longer precursors. Some of them, such as siRNAs (small interfering RNAs) are formed during longer double-stranded dsRNA cleavage associated with the viral infection. Another family, named micro RNAs (miRNA) are encoded by specific genes. The main function is to repress or degrade mRNA translation or to induce mRNA degradation, know as RNA silencing. They recognize target mRNA according to the sequence-specific base pairing. The degree of complementarity directs the outcome of the reaction. The use of RNAi as a defence against different viruses was documented as a new therapeutic tool. Some siRNAs have a potential of silencing disease (virus) specific RNAs, while miRNAs are involved in development of human cancers. Their profiling can be used as diagnostic and prognostic tool in different cancer types and different cancer stages. Some of the miRNA-associated genes are implicated in the development of degenerative and metabolic diseases. Since the proper development of the nervous system, insulin secretion and important metabolic processes requires precise control of gene expression, control of the translation and stability of many mRNAs by the small non-coding RNAs is emerging as an important regulator of various metabolic, endocrine and neurological processes.

ZNAČAJ ODREĐIVANJA URINARNIH MARKERA U PRAĆENJU DIJABETESNE NEFROPATIJE

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Oksidativni stres može se smatrati jedinstvenim faktorom koji povezuje dijabetes melitus (DM) i njegove komplikacije, uključujući nefropatiju (DN). Cilj ovog istraživanja bio je da se u urinu pacijenata sa DN odrede parametri oksidativnog oštećenja lipida i proteina kao i aktivnost ektoenzima. Istraživanjem je obuhvaćeno 40 pacijenata: 10 pacijenata sa dijabetes melitusom tipa 2 i mikroalbuminurijom (DMT2-MIA), 10 dijabetičnih pacijenata tipa 2 sa makroalbuminurijom (DMT2-MAA), 10 pacijenata sa dijabetom tipa 1 i mikroalbuminurijom (DMT1-MIA) i 10 zdravih osoba (kontrola). U urinu je određivana koncentracija TBA reaktivnih supstanci (TBARS), reaktivne karbonilne grupe (RCG) i aktivnost ektoenzima *N*-acetil-β-d-glukozaminidaze (NAG), plazma ćelijski diferencirajući antigen (PC-1), aminopeptidaza N (APN) i dipeptidil peptidaza IV (DPP IV). Veća koncentracija TBARS u urinu nađena je u DMT2-MIA i DMT1-MIA grupi, u odnosu na kontrolu ($p<0,001$ i $p<0,05$). Koncentracija RCD u urinu pokazuje slične vrednosti sa statistički značajnim povećanjem u DMT2-MAA i DMT1-MIA grupi u odnosu na DMT2-MIA ($p<0,001$) i kontrolnu grupu ($p<0,001$). Aktivnost NAG, APN i DPPIV značajno je veća u urinu pacijenata sa DMT2-MAA u odnosu na kontrolu ($p<0,01$). Aktivnost PC-1 pokazuje lagani, ali ne i signifikantan porast u toj grupi pacijenata. Zaključujemo da praćenje markera oksidativnog stresa i aktivnost *brush border* ektoenzima u urinu mogu biti korisni, neinvazivni i lako primenljivi testovi u praćenju DN.

THE SIGNIFICANCE OF URINARY MARKERS IN THE EVALUATION OF DIABETIC NEPHROPATHY

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Oxidative stress is considered to be a unifying link between diabetes mellitus (DM) and its complications, including nephropathy (DN). The aim of this study was to determine the parameters of oxidative injury of lipids and proteins as well as the activity of ectoenzymes in the urine of DN patients. The study included 40 individuals: 10 patients with type 2 diabetes mellitus and microalbuminuria (DMT2-MIA), 10 type 2 diabetic patients with macroalbuminuria (DMT2-MAA), 10 patients with type 1 diabetes and microalbuminuria (DMT1-MIA) and 10 age- and sex-matched healthy subjects (control). In the urine we determined TBA reactive substances (TBARS), reactive carbonyl groups (RCG), and the activity of ectoenzymes *N*-acetyl-β-d-glucosaminidase (NAG), plasma cell differentiation antigen (PC-1), aminopeptidase N (APN) and dipeptidyl peptidase IV (DPP IV). A higher concentration of TBARS in the urine was found in DMT2-MIA and DMT1-MIA, compared to the control group ($p<0.001$ and $P<0.05$). The urine concentration of RCD shows similar results with a significant elevation in the groups with DMT2-MAA and DMT1-MIA, compared to the DMT2-MIA ($p<0.001$) and control group ($p<0.001$). Activities of NAG, APN and DPPIV were significantly higher in the urine of DMT2-MAA, compared to the control ($p<0.01$). The activity of PC-1 was slightly increased in that group, but not significantly. In conclusion, the level of oxidative stress markers and activities of brush border ectoenzymes in the urine may be a useful non-invasive and easily repeatable test in DN.

DIJAGNOSTIČKI ZNAČAJ ANTI-CIKLIČNIH CITRULINIRANIH PEPTIDNIH ANTITELA, AKTIVNOSTI ADENOZIN DEAMINAZE I DRUGIH POTENCIJALNIH BIOMARKERA U OTKRIVANJU I PRAĆENJU REUMATOIDNOG ARTRITISA

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Cilj rada je bio utvrđivanje prisustva anti-cikličnih citruliniranih peptidnih antitela (anti-CCP2) kod pacijenata sa i bez reumatoidnog artritisa (RA) i poređenje tih auto-antitela s drugim markerima kao što su reumatoidni faktor (RF) i C-reaktivni protein (CRP). Određivana je katalitička aktivnost adenozin deaminaze (ADA) u serumima pacijenata sa reumatoidnim artritisom, bez terapije i sa terapijom metotreksatom. Cilj rada bio je i utvrđivanje mogućnosti uvođenja navedenih biohemijskih parametara u dijagnostikovanje i praćenje reumatoidnog artritisa u našu regiju. Anti-CCP2 su analizirana ELISA testom, katalitička aktivnost ADA određivana je spektrofotometrijskom metodom uz adenozin kao supstrat. RF je određivan Latex testom. CRP je meren imunoturbidimetrijskom metodom. Određivanje anti-CCP2 je korisno u dijagnostikovanju RA zbog visoke osetljivosti i specifičnosti, međutim, kombinovano određivanje anti-CCP2 sa RF je mnogo korisnije. Katalitička aktivnost ADA u serumu može da se koristi kao marker upalnog procesa u RA. Određivanje CRP u serumu pacijenata sa RA nema dijagnostički značaj.

THE DIAGNOSTIC VALUE OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES, ADENOSINE DEAMINASE ACTIVITY AND OTHER POTENTIAL BIOMARKERS FOR PREDICTING AND MONITORING RHEUMATOID ARTHRITIS

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The aim of this study was to investigate the presence of anti-cyclic citrullinated peptide antibodies (anti-CCP2) in RA and non-RA patients and to evaluate the combination of these auto-antibodies with some other markers such as RF and CRP. We examined the enzymatic activity of ADA in RA patients without therapy and RA patients treated with methotrexate. Therefore, the aim of this study was also to assess the possibility of introducing these biochemical parameters in the diagnosis and monitoring of the RA patients in this region. Serum antibodies directed to cyclic citrullinated peptide were analyzed using an anti-CCP2 antibody ELISA. Serum ADA activity was measured by a spectrophotometer using adenosine as substrate (Giusti method). Rheumatoid factor (IgG-RF) was analyzed using a latex agglutination test. Serum CRP was measured by an immunoturbidimetric assay. The measurement of anti-CCP2 by itself, is useful for the diagnosis of RA for its high sensitivity and specificity, however, a combined use of anti-CCP2 with RF is much more useful. Serum ADA activity can be used as a biochemical marker of inflammation in RA. Measuring CRP in the RA patients has no diagnostic value.

OKSIDATIVNI STRES – KLINIČKO-DIJAGNOSTIČKI ZNAČAJ

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Povećano stvaranje slobodnih radikala (SR) i/ili nedovljna antioksidativna zaštita stimuliše ćelijski odgovor na nastali oksidativni stres (OS). Produženi oksidativni insult ili u intezivnoj formi ostvaren prevazilazi ćelijski odbrambreni kapacitet, dolazi do oštećenja makromolekula, gibi se ćelijska funkicja, oštećuju se membrane što dovodi do smrti ćelije. Oksidativni stres kao patofiziološki mehanizam je potvrđen u brojnim patolo-

OXIDATIVE STRESS – CLINICAL-DIAGNOSTIC SIGNIFICANCE

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Increased free radical (FR) production and/or insufficient antioxidative defense stimulates cellular oxidant stress responses. Sustained oxidative insult or sufficient intensity overcome cell defenses, damage to macromolecules can accumulate, leading to loss of cell function, membrane damage, and ultimately to cell death. Oxidative stress (OS) as a pathophysiological mechanism is confirmed in numerous pathologies, poi-

gijama, trovanjima, starenju i stanjima organizma kao što su povećana fizička aktivnost, izloženost zagađenju čovekove okoline, ksenobioticima, pušenje itd. Najdominantnije reaktivne vrste u organizmu, reaktivne kiseonične vrste (RKV) i reaktivne azotove vrste (RNS), endogeno ili egzogeno stvorene, mogu lako da napadnu sve klase biomolekula (proteni, DNK, nezasićene masne kiseline). Narušen oksido-reduktivni milje, kroz povećanje lipidne peroksidacije, promenu aktivnosti direktnih ili indirektnih antioksidativnih enzima, kao i smanjenog sadržaja neenzimskih antioksidanasa može biti prepoznat u presimptomatskoj fazi brojnih bolesti, i u tom smislu može biti pokazatelj izmenjenih metaboličkih i funkcionalnih zbivanja. U svakodnevnoj kliničko-dijagnostičkoj praksi analize parametara OS u biološkom materijalu bi trebalo da imaju svoje mesto, radi prevencije bolesti i unapređivanja terapije bolesti.

sonings, ageing and body conditions such as enhanced physical exercise, exposure to environmental pollution and xenobiotics, smoking, etc. Principal reactive species, reactive oxygen species (ROS) and reactive nitrogen species (RNS), endogenously or exogenously produced, can readily attack all classes of macromolecules (proteins, DNA, unsaturated fatty acid). Perturbated oxidative-reductive milieu, proceeded through lipid peroxidation development, changed activities of antioxidative enzymes (AOE) and depletion of non enzymatic endogenous antioxidants can be recognized in presymptomatic phase of many diseases, and thus might be marker of changed metabolic and functional disorder. Therefore, in daily clinical- diagnostic practice, it would be useful to routinely analyze OS parameters in order to prevent and/or advance adequate disease treatment.