

P1 – Prikaz slučaja, P1-1

**Neusklađeni rezultati pretraga štitnjače:
prikaz slučaja**

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

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P1 – Case report, P1-1

**Incompatible thyroid test results:
case report**

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

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P1-2

Tromboembolija u 14-godišnjeg dječaka

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Četranaestgodišnji dječak primljen je u hitnu dječju ambulantu, a potom na odjel Klinike za pedijatriju zbog otoka desne potkoljenice. Unatrag tri tjedna dječak je bio u mirovanju kod kuće zbog infekta liječenog antibioticima. Dokazana je duboka venska tromboza (DVT) potkoljeničnih vena desne noge. Uvedena je terapija niskomolekularnim heparinom. Od petog dana boravka osjeća zaduhu i bol u lijevoj strani prsišta. Dijagnosticirana je plućna embolija. Uvodi se terapija nefrakcioniranim heparinom, a potom i oralnim antikoagulantom (Marivarin(R)). Bolesnik je uspješno reagirao na terapiju, heparinska terapija je isključena i liječenje nastavljeno Marivarinom (R). Prikazuju se rezultati dijagnostičke obrade koji upućuju na mogući uzrok nastanka tromboembolije u ovog bolesnika. U bolesnika su načinjene slijedeće pretrage: SE, KKS, APTV, PV, TV, fibrinogen, test fibrinolize, D-dimeri, antitrombin III (ATIII), protein C (PC), protein S (PS), čimbenici zgrušavanja FII-FXIII, plazminogen (PG), inhibitor aktivatora plazminogena-1 (PAI-1), APC rezistencija (APCR), lupus antikoagulant (LAC), antikardiolipinska antitijela (ACA) i antitijela na dvostruku uzvojnici DNA (anti-dsDNA) i genetski čimbenici rizika koagulacijskih poremećaja: genotipizacija polimorfizama PAI-1, FII zgrušavanja, MTHFR i mutacija FV Leiden. Određivanje APTV i PV rađeno je svakodnevno prema protokolu za praćenje dvojne antikoagulantne terapije DVT. Utvrđene su povišene vrijednosti D-dimera (1,3-1,9 mg/L), PAI-1 (4,9 U/mL), LAC (3,0 uz produljenje APTV heparinom i 2,76 nakon heparinske terapije), ACA-IgG (104,7 GPL-U/mL); snižene vrijednosti broja trombocita ($90-106 \times 10^9/L$), aktivnosti FXIII (50,6%), aktivnosti PS (50,1%) i APCR (0,81). Vrijednosti unutar referentnog raspona: aktivnosti FII-FXII (73,4-117,2%), PC (93,7%), ATIII (92%), PG (89,5%), ACA-IgM (5,1 MPL-U/mL) i anti-dsDNA (16,3 IU/mL). Molekularnom dijagnostikom utvrđen je genotip 4G/5G (heterozigot za PAI-1 polimorfizam), uz uredan nalaz drugih molekularnih biljega. Dijagnostičkom obradom dokazano je više čimbenika rizika za nastanak tromboembolije u ovog bolesnika: heterozigotnost za PAI-1 polimorfizam uz povišenu koncentraciju PAI-1, smanjena APCR uz sniženu aktivnost PS, pozitivna LA i ACA autoantitijela.

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P1-2

Thromboembolism in a 14-year-old boy

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A 14-year-old boy was admitted to the emergency pediatric clinic and then transferred to the University Department of Pediatrics ward for edema of the right lower extremity. Three weeks before, the boy was at rest for infection treated by antibiotics. Deep venous thrombosis (DVT) of the lower right extremity was confirmed. Therapy with low-molecular weight heparin was introduced. From hospitalization day 5 he felt dyspnea and pain in the left chest. Pulmonary embolism was diagnosed. Therapy with unfractionated heparin was initiated, followed by oral anticoagulants (Marivarin(R)). The patient responded well to this therapy, heparin was discontinued, and treatment was continued with Marivarin (R). Results of diagnostic work-up are presented, suggesting the possible cause of thromboembolism in this patient. The following tests were performed: ESR, CBC, APTT, PT, TT, fibrinogen, fibrinolysis test, D-dimer, antithrombin III (ATIII), protein C (PC), protein S (PS), FII-FXIII, plasminogen (PG), plasminogen activator inhibitor-1 (PAI-1), APC resistance (APCR), lupus anticoagulants (LA), anticardiolipin antibodies (ACA), antibodies to double-stranded DNA (anti-dsDNA) and genetic risk factors for coagulation disorders: genotyping polymorphisms for PAI-1, coagulation FII, MTHFR and Factor V Leiden mutation. APTT and PT were determined according to the protocol for monitoring of double anticoagulant therapy of DVT. Increased values were measured for: D-dimer (1.3-1.9 mg/L), PAI-1 (4.9 U/mL), LA (3.0 by heparin APTT prolongation and 2.76 after heparin therapy), ACA-IgG (104.7 GPL-U/mL); decreased values were measured for: platelets ($90-106 \times 10^9/L$), activities of FXIII (50.6%) and PS (50.1%), APCR (0.81); values within the reference range were measured for: activities of FII-FXII (73.4-117%), PC (93.7%), ATIII (92%) and PG (89.5%), ACA-IgM (5.1 MPL-U/mL), anti dsDNA (16.3 IU/mL). Molecular diagnosis confirmed the 4G/5G genotype (heterozygous for PAI-1 polymorphism) with no detected polymorphism for other molecular markers. Thus, diagnostic work-up confirmed several risk factors for thromboembolism in this patient: heterozygosity for PAI-1 polymorphism with elevated PAI-1 concentration, decreased APCR with lower PS activity, and positive LA and ACA autoantibodies.

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P1-3

Kronična mijeloična leukemija - važnost praćenja terapije: prikaz slučaja

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

P1-3

Chronic myelogenous leukemia – therapy monitoring: case report

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

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

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P1-4

Waldenströmova makroglobulinemija i vrijednosti leukocita

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Waldenströmova makroglobulinemija prvi puta je opisana 1944. godine. Danas taj oblik ne-Hodgkinova limfoma čini 2% svih hematoloških karcinoma. Iako se javlja u svim životnim dobima i nije spolno određena, bolest je učestalija u muškaraca starijih od 50 godina. Žena u dobi od 77 godina hospitalizirana je putem hematološke ambulante zbog simptoma anemije s otežanim disanjem, slabošću i malaksalošću, te nepodnošenjem ikakvog napora uz ranije potvrđenu šećernu bolest i hipertenziju. Pri zaprimanju u bolnicu učinjene su slijedeće pretrage: sedimentacija eritrocita, glukoza, kreatinin, diferencijalna krvna slika (DKS). Pri izradi DKS na hematološkom brojaču Cell Dyn 3500 (Abbott, SAD) uz oznake upozorenja uočene su velike razlike u vrijednosti leukocita mjerene optičkim i impedancijskim načinom, pa je DKS učinjena ručno. Dobivene vrijednosti DKS uz prisutnost *rouleaux* formacija i izrazito visoku sedimentaciju, određenu elektroforezu serumskih proteina u kojoj se isticala povećana gama frakcija, izmjerene vrijednosti imunoglobulina u kojima se isticala vrijednost imunoglobulina M ukazivale su na postojanje Waldenströmove makroglobulinemije. Naknadnom obradom učinjena je imunoelektroforeza sa specifičnim protutijelima za lake i teške lance, pri čemu je potvrđena prisutnost monoklonskog imunoglobulina klase M tipa lakog lambda lanca. Terapija osnovne bolesti započeta je Alkeranom i prednisonom. Tijekom tromjesečne terapije vrijednosti leukocita mjerene impedancijom kretale su se unutar referentnih vrijednosti za ženski spol i navedenu dob (medijan $4,53 \times 10^9/L$), dok su vrijednosti određene optičkim načinom rasle od 39,2 do $149 \times 10^9/L$ (medijan $87,2 \times 10^9/L$). Porast vrijednosti leukocita mjerene optičkim načinom korelirao je s porastom gama frakcije, odnosno imunoglobulina M, što je potvrdilo izostanak terapijskog odgovora na primijenjene lijekove te je terapija promijenjena.

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

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P1-4

Waldenström's macroglobulinemia and leukocyte values

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Waldenström's macroglobulinemia was first described in 1944. Today, this form of non-Hodgkin lymphoma accounts for about 2% of all hematologic cancers. Although it occurs in all age groups and is not sex-determined, it is more common in men over fifty years of age. A 77-year-old woman was hospitalized through hematologic clinic with symptoms of anemia, difficult breathing, weakness, and intolerance of even minor effort. Diabetes mellitus and hypertension were confirmed previously. On admission, the following tests were done: erythrocyte sedimentation rate, glucose, creatinine, and differential blood count. Differential blood count was done on a Cell Dyn 3500 (Abbott, USA) blood counter. During the test, there were great differences between leukocyte values measured optically and with impedance. Therefore differential blood count was done manually. Along with the presence of *rouleaux* formations and extremely high sedimentation, serum protein electrophoresis with considerably increased gamma fraction, the measured immunoglobulin values with marked M immunoglobulin value were indicative of Waldenström's macroglobulinemia. Additionally, immunoelectrophoresis with specific antibodies for light and heavy chains confirmed the presence of monoclonal M class immunoglobulin of the light lambda chain type. The underlying disease was treated with Alkeran and prednisone. During three months, leukocyte values measured with impedance oscillated within the reference range for women and age (median $4.53 \times 10^9/L$), while those measured optically increased from 39.2 to $149 \times 10^9/L$ (median $87.2 \times 10^9/L$). The increase in optically measured leukocyte values correlated with the increase of gamma fraction, i.e. M immunoglobulin. It confirmed the lack of therapeutic response to the medications administered, and the patient's therapy was changed accordingly.

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P1-5

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

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Novorođenče u dobi od 15 dana primljeno je u Gastroenterološki odjel Klinike za pedijatriju zbog aholičnih stolica i sumnje na kolestazu. Nije primijećeno žutilo kože, a mokraća je bila žute boje.

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

P1-5

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

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

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

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P1-6

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

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

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

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P1-6

Combined megaloblastic and AIHA (immuno-hemolytic anemia) – case report

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A female patient aged 55 presented with weakness and vertigo. For ten years now she had been treated for schizophrenia and rheumatoid arthritis (RA). The patient was pale, adynamic, eupneic, cardiopulmonarily compensated, without peripheral lymphadenopathy, and adipose. Body weight 117 kg, height 162 cm, moving with difficulty. The abdomen normal on palpation. Blood test results on admission were as follows: RBC $1.68 \times 10^{12}/\text{L}$, Hb 65 g/L, Hct 0.189 L/L, MCV 112.7 fL, MCH 38.9 pg, MCHC 345 g/L, RDW 32.8, Plt $87 \times 10^9/\text{L}$, MPV 7.4 fL, WBC $5.4 \times 10^9/\text{L}$. Red blood cell assessments showed profound alterations: dimorphic erythrocyte population, microerythrocytes, and erythrocyte fragments-schizocytes were found, whereas erythrocytes showed pronounced anisopoikilocytosis, hypochromia and polychromasia. LD showed extremely high values (26 900 U/L), while bilirubin and urobilinogen were in the normal range. IAT was negative. Serum level of folic acid was normal, whereas that of vitamin B 12 was decreased (41 pg/mL). On admission, bone marrow aspiration cytology was not performed because the clinical

je liječena kao megaloblastična anemija s 1000 µg vitamina B12 i 5 mg folne kiseline; nakon 24 sata terapije LD je značajno pao na 4715 U/L. Vrijednosti feritina i željeza su uredne, a gastrin je povišen (534 µU/mL). Gastroskopijom (EGD) je utvrđen kronični gastritis, bez infekcije *H. pylori*. Nakon 8 dana liječenja hematološki nalazi se neočekivano pogoršavaju: E $1,27 \times 10^{12}/L$, Hb 54 g/L, Htc 0,157 L/L, MCV 123,4 fL, MCH 42,1 pg, MCHC 341 g/L, RDW 30,3, Rtc 0,148, Tr $76 \times 10^9/L$, L $6,6 \times 10^9/L$. Citološki nalaz koštane srži (učinjen je nakon 21 dana terapije) nije ukazao na promjene tipične za megaloblastičnu anemiju. Kako nije došlo do očekivanog poboljšanja ponovljeni su transfuziološki testovi koji su sada bili pozitivni: IAT +, anti IgG, anti C3d, DAT + (direktni antiglobulinski test). U terapiju je uključen metilprednizolon u dozi od 16 mg i nakon 48 sati dolazi do poboljšanja. Nalazi nakon izmijenjene terapije: E $2,40 \times 10^{12}/L$, Hb 80 g/L, Htc 0,242 L/L, MCV 101,0 fL, MCH 33,4 pg, MCHC 331 g/L, RDW 22,3, Tr $441 \times 10^9/L$, MPV 7,4 fL, L $6,7 \times 10^9/L$, Rtc 0,049. Prilikom otpusta transfuziološki testovi su i dalje pozitivni. Kod naše bolesnice pojavila se iznimno rijetka udružena megaloblastična anemija s AIHA za koju smatramo da se razvila u okviru osnovne bolesti (RA i shizofrenije). Moguće objašnjenje intenziviranja hemolize kao i konverzije IAT negativnih u pozitivne je pojava nove populacije eritrocita inicirane terapijom vitaminom B12 s izmijenjenim epitopima antigena na eritrocitnoj membrani.

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picture and low vitamin B12 serum concentration were highly suggestive of megaloblastic anemia. The patient was treated with vitamin B12 and folic acid, and after 24 hours of therapy LD decreased significantly to 4715 U/L. The values of ferritin and iron were normal, while gastrin was increased (534 µU/mL). Chronic gastritis was found by gastroscopy (EGD). After eight days, the findings changed surprisingly to the worse: RBC $1.27 \times 10^{12}/L$, Hb 54 g/L, Hct 0.157 L/L, MCV 123.4 fL, MCH 42.1 pg, MCHC 341 g/L, RDW 30.3, Rtc 0.148, Plt $76 \times 10^9/L$, WBC $6.6 \times 10^9/L$. Bone marrow cytology (done after 21 days of therapy) showed no changes typical of megaloblastic anemia. Since the expected improvement failed to occur, transfusion medicine tests were repeated with IAT, anti-IgG, anti-C3d, and DAT (Coombs test) positive. Methylprednisolone in a daily dose of 160 mg was introduced and after 48 hours the patient's condition improved: RBC $2.40 \times 10^{12}/L$, Hb 80 g/L, Hct 0.242 L/L, MCV 101.0 fL, MCH 33.4 pg, MCHC 331 g/L, RDW 22.3, Rtc 0.049, Plt $441 \times 10^9/L$, MPV 7.4 fL, WBC $6.7 \times 10^9/L$. Therapy with methylprednisolone and folic acid was continued, upon which the findings improved. In conclusion, our patient with combined megaloblastic and autoimmune hemolytic anemia with two mechanisms of hemolysis (dyssynchronization of the cytoplasm and DNA maturation, and immune mechanism) is one of the rare cases described so far. Immunotransfusal conversion could be explained by change of the emerging erythrocyte population with fully expressed membrane antigens, or by primary insensitivity of the method. Apart from these parameters, very useful in recognizing hemolysis are the parameters of IRF and MRV.

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P2 – Hematologija, P2-1 (UP2-1)

Praćenje broja trombocita u bolesnika s opeklinama

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Trombociti imaju značajnu ulogu u održavanju hemostaze te sudjeluju u imunog odgovoru. U bolesnika s opeklinama dolazi do značajnog narušavanja hemostaze i imunog odgovora. Cilj ispitivanja bio je pratiti broj trombocita u vremenu s obzirom na različit postotak opečene površine tijela (%TBSA) te odrediti značenje praćenja broja trombocita s obzirom na ishod bolesti.

Ispitivanu skupinu činilo je 68 bolesnika primljenih na Odjel za opeklina; ispitanici su podijeljeni na skupinu A

P2 – Haematology, P2-1 (UP2-1)

Platelet count monitoring in burn patients

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

The study included 68 burn patients divided into group

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

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P2-2

Vrijednosti hematoloških parametara u djece predškolske dobi

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

A (32 patients with mild burns, <10% TBSA) and group B (36 patients with moderate/severe burns, >10% TBSA). Whole blood samples were obtained on days 1, 4, 7, 14, 21 and 28 of the injury (depending on the length of hospital stay). Platelet count was determined on a Sysmex XT-1800i hematology analyzer. Data were analyzed using the Medcalc software. The level of significance was set at $p < 0,05$. On admission, platelet count did not differ significantly between group A and group B ($p = 0,1697$). On day 4, the values decreased in both groups, the decline being more pronounced in group B ($p = 0,0003$). From day 7 to day 21, platelet count continued to rise in both groups ($p = 0,0452$; $p = 0,1995$). Platelet count continued to rise on day 21 in group A, but declined in group B, without significant between group difference ($p > 0,10$). On day 28, platelet count declined in both groups ($p > 0,10$). According to outcome, a statistically significant difference in platelet count between the survived and deceased patients was recorded on days 1, 4, 7, 14, 21 and 28 ($p < 0,05$). Platelet count was lower in patients with lethal outcome. Study results indicated a decline in platelet count on day 4 in both groups of patients, attributable to hemodilution and increased production of microaggregates, with a note that platelet count was significantly lower in group B. Platelet count rose on day 7 due to the acute phase reaction stimulation by inflammation in both groups. The highest platelet count was recorded in group A on day 21 and in group B on day 14. In patients with lethal outcome platelet count was lower throughout the monitoring period.

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P2-2

Values of hematology parameters in preschool children

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

obuhvaćeno 462 djece (242 dječaka i 220 djevojčica) rođene 1999. i 2000. godine s područja grada Pule i okolice, koja su u laboratorij upućena zbog redovnog sistematskog pregleda za upis u prvi razred osnovne škole. Svoj djeci određena je krvna slika na hematološkom brojaču Sysmex XT2000i (Sysmex Inc., Japan). Izmjerene vrijednosti leukocita, eritrocita, hemoglobina, hematokrita, MCV, MCH, MCHC, RDW, trombocita, MPV iskazane su srednjom vrijednosti, medijanom, standardnom devijacijom, koeficijentom varijacije. Odnos prema referentnim vrijednostima iskazan je značajnošću razlike (p).

Statističkom obradom podataka dobivene su slijedeće vrijednosti izražene medijanom: $E=4,74 \times 10^{12}/L$, $Hb=131$ g/L, $Hct=0,376$ l/L, $MCV=794$ fL, $MCH=27,7$ pg, $MCHC=347$ g/L, $L=6,97 \times 10^9/L$, $Tr=307 \times 10^9/L$, $RDW=13,1\%$, $MPV=9,7$ fL. Utvrđena je statistički značajna razlika za sve izmjerene parametre u odnosu na referentne vrijednosti za ovu dobnu skupinu ($p<0,001$). Vrijednosti svih izmjerenih hematoloških parametara kretale su se unutar u literaturi preporučenih referentnih intervala za ovu dobnu skupinu, te iako postoji statistički značajna razlika, klinički značajna razlika nije potvrđena.

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P3 – Koagulacija, P3-1 (UP1-1)

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

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

(242 boys and 220 girls) from Pula and its surroundings, born in 1999 and 2000. The children were referred to the laboratory for the regular medical check-up prior to school enrolment. Blood count was done on a Sysmex XT2000i (Sysmex Inc., Japan) blood counter. The measured values of leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets and MPV were expressed as mean, median, standard deviation and coefficient of variation. The relation to reference values was shown by the significance of difference (p). On statistical data processing, the following values were obtained (as expressed in median): $E=4.74 \times 10^{12}/L$, $Hb=131$ g/L, $Hct=0.376$ l/L, $MCV=794$ fL, $MCH=27.7$ pg, $MCHC=347$ g/L, $L=6.97 \times 10^9/L$, $Plt=307 \times 10^9/L$, $RDW=13.1\%$, and $MPV=9.7$ fL. A statistically significant difference from reference values for this age group was found for all measured parameters ($p<0.001$).

The values of all measured hematology parameters were within the recommended reference intervals for this age group. In spite of the statistically significant differences, no clinically significant difference was confirmed.

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P3 – Coagulation, P3-1 (UP1-1)

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

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

simalna agregacija (V_{max}). U ispitivanje je bilo uključeno 76 zdrave djece u dobi od 2 do 12 godina bez poremećaja hemostaze, s normalnim brojem trombocita (raspon $210-425 \times 10^9/L$) i bez uzimanja lijekova koji bi inhibirali funkciju trombocita. Referentni intervali (percentili 0,025-0,975) za induciranu agregaciju trombocita su bili za ADP 68,6-92,3%, adrenalin 67,6-95,0%, kolagen 72,1-89,0% i ristocetin 79,8-98,7%. Određivanje inducirane agregacije trombocita na BCT s različitim agonistima je potpuno automatizirana i vrlo precizna analiza, izvodi se s malim volumenom plazme i standardiziranim brojem trombocita. Važan je dijagnostički parametar za utvrđivanje uzroka krvarenja, procjenu disfunkcije trombocita i utjecaja lijekova na primarnu hemostazu.

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P3-2

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

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Zbog visoke učestalosti Faktora V Leiden (FVL) ispitivanje rezistencije na aktivirani protein C (RAPC) sastavni je dio analiza probiranja na trombofiliju. Pouzdani test za ispitivanje RAPC u rutinskom radu mora biti osjetljiv i specifičan kako bi se smanjio broj molekularnih analiza za utvrđivanje prisutnosti FVL. U testu STA-STACLOT APC-R (Dijagnostika Stago, Asnieres, Francuska) uzorci se analiziraju samo u prisutnosti aktiviranog proteina C (APC), a rezultati se izražavaju kao vrijeme zgrušavanja u sekundama. Cilj ovoga rada bio je prilagoditi izvorni test STA-STACLOT APC-R za koagulacijski analizator BCT (Dade Behring), uz istodobno analiziranje uzoraka s i bez APC, te ispitati može li izražavanje rezultata kao omjer RAPC omogućiti bolje razlikovanje osoba s FVL od onih bez mutacije. U istraživanje je uključeno 353 uzoraka plazme ispitanika koji su bili upućeni u koagulacijski laboratorij zbog probiranja na trombofiliju, od kojih je bilo 171 uzoraka plazme trudnica, 20 ispitanika na oralnoj antikoagulacijskoj terapiji (INR-raspon: 1,36-4,66) i 15 ispitanika s lupus antikoagulantom. Rezultati omjera RAPC uspoređeni su s rezultatima analize DNA za FVL. Dodatno je ispitan mogući utjecaj različitih aktivnosti proteina C (12,4-218,5%), proteina S (71-193,9%) i FVIII:C (96-580%) na rezultate omjera RAPC. Analizom

platelet count (range $210-425 \times 10^9/L$) and not taking any antiaggregation drugs or drugs that can cause inhibition of platelet function. The reference intervals (0.025-0.975 percentiles) for induced aggregation were: ADP 68.6-92.3%, adrenaline 67.6-95.0%, collagen 72.1-89.0%, and ristocetin 79.8-98.7%. Accordingly, determination of induced platelet aggregation on BCT through aggregation inductors is a fully automated and high precision analysis. The analysis is performed on a small sample volume with standardized platelet count. Induced platelet aggregation is an important analysis for detecting the risk of bleeding, platelet dysfunction and effects of antiplatelet drugs on primary hemostasis.

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P3-2

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

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

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

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P3-3

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

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

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

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P3-3

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

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

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

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P3-4

Ispitivanje poremećaja primarne hemostaze uporabom analizatora funkcije trombocita PFA-100

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

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

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P3-4

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

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

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

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P3-5

Predviđanje stupnja deficita FVIII analizom reakcijskih krivulja za APTV

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

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

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P3-5

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

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

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

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P3-6

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

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

by one-stage assay. FVIII inhibitor screen was performed and inhibitor concentrations were determined. Hemophilia patients were further divided into 4 groups (H1-H4) according to FVIII activity, and into 2 groups according to the presence (I2) or absence (I1) of the inhibitor. Significant differences ($p < 0,05$) were found in DaPTT and aPTTSR values between normal and hemophilia patients as well as between H1 to H4 and I1 and I2 groups. In normal and hemophilia patients the obtained results (mean \pm SD) for DaPTT were 4.24 \pm 1.11 and 20.03 \pm 11.47, and for aPTTSR 0.16 \pm 0.03 and 0.33 \pm 0.14, respectively. Results in specific groups were as follows: group H1 (n=41, FVIII <0.01) DaPTT=29.48 \pm 8.62, aPTTSR=0.44 \pm 0.13; group H2 (n=12, FVIII=0.01-0.05) DaPTT=17.51 \pm 5.87, aPTTSR=0.29 \pm 0.09; group H3 (n=24, FVIII=0.05-0.30) DaPTT=10.25 \pm 4.18, aPTTSR=0.23 \pm 0.05; group H4 (n=10, FVIII=0.30-0.50) DaPTT=7.75 \pm 1.19, aPTTSR=0.19 \pm 0.04; group I1 (n=48) DaPTT=17.83 \pm 12.19, aPTTSR=0.30 \pm 0.14, group I2 (n=21) DaPTT=28.06 \pm 6.61, aPTTSR=0.42 \pm 0.13. Based on these results, DaPTT values >6.5 and >17.7 allowed for discrimination between normal subjects and hemophilia patients (sensitivity 94.7%, specificity 100%), and between patients with and without inhibitors (sensitivity 95.5%, specificity 64.7%).

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P3-6

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

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

II, Stratus CS D-Dimer testom (Dade Behring) na Stratus CS i D-Dimer Plus (Dade Behring) testom na Sysmex CA 500. Kalibratori i kontrolni uzorci bili su od istog proizvođača kao i reagensi.

Rezultati dobiveni pomoću VIDAS D-Dimer Exclusion: za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji bila je 2,8-6,2, a iz dana u dan 3,9-5,3.

Netočnost

Bias = 0,024 mg/L, deklarirano 0,597 mg/L.

Bias = 0,35 mg/L, deklarirano 4,51 mg/L.

NycoCard D-Dimer New test

Nepreciznost iz dana u dan (KV %) bila je 9,9, a netočnost je do 12%.

Stratus CS D-Dimer test

Za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji bila je 2,3-3,2.

D-Dimer Plus

Za nisko i visoko koncentracijsko područje nepreciznost (KV %) u seriji je 0,65–0,84, a iz dana u dan 1.0.

Netočnost

Bias = 0,018 mg/L, deklarirano 0,61 mg/L.

Bias = 0,027 mg/L, deklarirano 4,20 mg/L.

Usporedba rezultata

Obrada rezultata učinjena je pomoću statističkog programa SigmaStat. Rezultati pokazuju statistički značajan stupanj linearne korelacije do koncentracije 5,1 mg/L.

VIDAS D-Dimer Exclusion – NycoCard D-Dimer New test $r_s = 0.93$, $p < 0.001$, $n = 30$

VIDAS D-Dimer Exclusion – Stratus CS D-Dimer test $r_s = 0.91$, $p < 0.001$, $n = 30$

VIDAS D-Dimer Exclusion – D-Dimer Plus $r_s = 0.92$, $p < 0.001$, $n = 36$

Korelacija do koncentracije 2,0 mg/L se pokazala također statistički značajnom.

VIDAS D-Dimer Exclusion – NycoCard D-Dimer New test $r_s = 0.78$, $p < 0.001$, $n = 18$

VIDAS D-Dimer Exclusion – Stratus CS D-Dimer test $r_s = 0.77$, $p < 0.001$, $n = 21$

VIDAS D-Dimer Exclusion – D-Dimer Plus $r_s = 0.79$, $p < 0.001$, $n = 26$

Dobivena je dobra podudarnost rezultata različitih D-dimer testova prema referentnoj metodi, naročito u nižem koncentracijskom području, što ih čini prihvatljivim u rutinskom radu.

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(Dade Behring) on a Stratus CS and D-Dimer Plus (Dade Behring) assay on a Sysmex CA 500.

Results obtained by VIDAS D-Dimer Exclusion: reproducibility (CV%) in low and high concentration within runs 2.8–6.2 and between runs 3.9–5.3.

Accuracy

Bias = 0.024 mg/L, target value 0.597 mg/L

Bias = 0.35 mg/L, target value 4.51 mg/L

NycoCard D-Dimer New test

Reproducibility (CV%) between runs 9.9 and accuracy 12%.

Stratus CS D-Dimer test

Reproducibility (CV%) in low and high concentration within runs 2.3–3.2.

D-Dimer Plus

Reproducibility (CV%) in low and high concentration within runs 0.65–0.84 and between runs 1.0.

Accuracy

Bias = 0.018 mg/L, target value 0.61 mg/L.

Bias = 0.27 mg/L, target value 4.20 mg/L

Data comparison

Statistical analysis was done by SigmaStat statistical software.

Results showed a statistically significant level of linear correlation of up to 5.1 mg/L.

VIDAS D-Dimer Exclusion – NycoCard D-Dimer New assay $r_s = 0.93$, $p < 0.001$, $n = 30$

VIDAS D-Dimer Exclusion – Stratus CS D-Dimer assay $r_s = 0.91$, $p < 0.001$, $n = 30$

VIDAS D-Dimer Exclusion – D-Dimer Plus $r_s = 0.92$, $p < 0.001$, $n = 36$

A significant correlation was found even in lower concentrations of up to 2.0 mg/L.

VIDAS D-Dimer Exclusion – NycoCard D-Dimer New assay $r_s = 0.78$, $p < 0.001$, $n = 18$

VIDAS D-Dimer Exclusion – Stratus CS D-Dimer assay $r_s = 0.77$, $p < 0.001$, $n = 21$

VIDAS D-Dimer Exclusion – D-Dimer Plus $r_s = 0.79$, $p < 0.001$, $n = 26$

D-dimer assays demonstrated performances comparable with those of VIDAS D-Dimer as a reference method, and were found suitable for emergencies and individual determination.

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P3-7

Utjecaj niskomolekularnog heparina na globalne koagulacijske testove kod bolesnika na hemodijalizi

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

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P3-7

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

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Low molecular weight heparins (LMWH) have several advantages over unfractionated heparin (UFH) in the treatment of thrombosis. Unlike UFH, LMWHs have excellent bioavailability as the result of reduced nonspecific binding to plasma proteins and endothelium. Their pharmacokinetics is more uniform leading to a predictable response in most patients without the need of laboratory monitoring. LMWHs are cleared primarily by renal excretion. In patients with chronic renal failure, the elimination half-life may increase several-fold, thus also the risk of bleeding. The aim of the study was to evaluate the pharmacokinetics of the LMWH fragmin after a bolus dose as an anticoagulant during a 48-h period and its impact on the results of global coagulation assays PT, APT R and fibrinogen. We examined 10 patients (3 female and 7 male) on chronic hemodialysis (HD). The patients received a single bolus dose of fragmin of 40 IU/kg before HD. Blood samples were collected four times during a 48-h period: before HD, and at 2 h, 4 h and 48 h of HD. PT, APT R and fibrinogen were determined by the coagulation method, and anti-factor Xa activity was determined by the chromogenic method. Anti-factor Xa levels: before HD 0.08 IU/mL (range 0.07-0.17); at 2 h of HD 0.43 IU/mL (range 0.16-0.82); at 4 h of HD 0.20 IU/mL (range 0.08-0.60), and at 48 h of HD 0.08 IU/mL (range 0.06-0.10). At 2 h of HD, the activity of PT decreased from initial levels by 12%; APT R increased by 22.7%; fibrinogen concentration increased by 20.4% and stayed so throughout HD. PT, APT R, fibrinogen and anti-factor Xa activities returned to initial levels after 48 h of HD. The dose of fragmin used in the study was found to be safe considering the risk of bleeding during and after HD, and effective in preventing extracorporeal circuit thrombosis.

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P3-8

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

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Trudnoća je stanje u kojem je fiziološki prisutna povećana reaktivnost trombocita kao rezultat pojačanog lučenja trombocita i opće hiperkoagulabilnosti krvi. U ovom smo ispitivanju kao ispitanike ciljano odabrali trudne žene u svrhu procjene kliničke primjene PFA-100 u stanju hiperagregabilnosti trombocita. Analizator funkcije trombocita (PFA-100, Dade Behring) procjenjuje *in vitro* primarnu hemostazu u punoj krvi upotrebom test kasete obloženih agonistima trombocita: kolagen/epinefrin (KOL/EPI) ili kolagen/adenozin difosfat (KOL/ADP). Cilj je bio usporediti rezultate funkcije trombocita ispitane analizatorom PFA-100 (puna krv) i standardnom metodom agregacije trombocita prema Bornu (plazma) u trudnica. U ispitivanju je bilo uključeno 47 trudnica životne dobi 19-44 godina i gestacijske dobi 33-41 tjedan. Uz pretragu agregacije trombocita u svih trudnica određeni su: broj trombocita, APTV, PV, fibrinogen, TV, test fibrinolize. Rezultati su izraženi kao medijan i raspon. Zabilježeni su slijedeći rezultati: broj trombocita $198 \times 10^9/L$ ($134-399 \times 10^9/L$), APTV 26 s (23-35 s), PV 137% (105-150%), TV 17 s (13-19 s), fibrinogen 6,5 g/L (3,5-8,9 g/L), fibrinoliza 210 min (90-210 min). PFA-100: KOL/EPI 101,5 s (66-237 s), KOL/ADP 79 s (61-127 s). Standardna metoda agregacije trombocita: ADP 72,8% (19-88%), KOL 72,4% (23-95%), EPI 74,9% (23-86%). Podudarnost rezultata dviju metoda iznosila je 91,5% (43/47), a 8,5% (4/47) rezultata pokazalo je neslaganje. U dva od 47 rezultata pokazana je smanjena agregabilnost trombocita mjerena pomoću PFA-100 (KOL/EPI 147 s, 237 s; KOL/ADP 102 s, 127 s) uz pojačanu agregabilnost trombocita standardnom metodom agregacije (ADP 86%, 88%; KOL 86%, 88%; EPI 83%, 85%). U dva nepodudarna rezultata vrijednosti PFA-100 bile su normalne (KOL/EPI 82 s, 110 s; KOL/ADP 70 s, 76 s), a agregacija trombocita u plazmi smanjena (ADP 19%, 33%, KOL 30%, 36%, EPI 23%, 33%). Usporedba rezultata dviju metoda potvrdila je kliničku primjenjivost PFA-100 u procjeni funkcije trombocita u stanjima njihove pojačane agregabilnosti. U usporedbi sa standardnom metodom agregacije trombocita u plazmi PFA-100 je tehnički manje zahtjevna metoda, puna krv bolje odražava *in vivo* uvjete, a rezultati su dostupni u kraćem vremenu.

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P3-8

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

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Pregnancy is a state in which physiologically increased platelet reactivity is present as the result of enhanced platelet secretion and general blood hypercoagulability. In this study, pregnant women were included to evaluate the clinical utility of PFA-100 in the state of platelet hyperaggregability. Platelet function analyzer (PFA-100, Dade Behring) evaluates *in vitro* primary hemostasis in whole blood using test cartridges coated with platelet agonists: collagen/epinephrine (COL/EPI) or collagen/adenosin diphosphate (COL/ADP). The aim was to compare results of platelet function as assessed by the PFA-100 (whole blood) and standard method of platelet aggregation according to Born (plasma) in pregnant women. The study included 47 pregnant women aged 19-44, gestational age 33-41 weeks. In addition to platelet aggregation tests, platelet count, aPTT, PT, fibrinogen and fibrinolysis test were also performed. Results are expressed as median and range. The following results were recorded: platelet count $198 \times 10^9/L$ ($134-399 \times 10^9/L$), aPTT 26 s (23-35 s), PT 137% (105%-150%), TT 17 s (13-19 s), fibrinogen 6.5 g/L (3.5-8.9 g/L), fibrinolysis 210 min (90-210 min). PFA-100: COL/EPI 101.5 s (66-237 s), COL/ADP 79 s (61-127 s). Standard method of platelet aggregation: ADP 72.8% (19-88%), COL 72.4% (23-95%), EPI 74.9% (23-86%). The agreement between the two methods was 91.5% (43/47), while 8.5% (4/47) of the results showed discrepancies. Two of 47 results showed reduced platelet aggregation by PFA-100 (COL/EPI 147 s, 237 s; COL/ADP 102 s, 127 s) and increased platelet aggregation by standard method of aggregation (ADP 86%, 88%; COL 86%, 88%; EPI 83%, 85%). Two discordant results had normal PFA-100 values (COL/EPI 82 s, 110 s; COL/ADP 70 s, 76 s) and reduced standard platelet aggregation (ADP 19%, 33%; COL 30%, 36%; EPI 23%, 33%). Accordingly, comparison of the results obtained by the two methods confirmed the clinical utility of the PFA-100 in the assessment of platelet function in conditions of their increased aggregability. In comparison with standard method of platelet aggregation in plasma, PFA-100 is technically easier to perform, whole blood better reflects *in vivo* conditions, and results are accessible in a shorter period of time.

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P3-9

Vrijednost određivanja D-dimera kod bolnički liječenih bolesnika

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D-dimer je kao osjetljiv biljeg uključen u dijagnostički algoritam tromboembolija. Samo negativan rezultat D-dimera dobiven visoko ili umjereno osjetljivim testom može se uz kliničku vjerojatnost rabiti za isključenje tromboembolije, ali je specifičnost takvih testova za plućnu emboliju niska. Kako je samo negativan rezultat koristan u donošenju kliničke odluke, udio bolesnika s negativnim rezultatom u skupini kod koje je isključena tromboembolija (tj. specifičnost) određuje kliničku vrijednost testa.

Cilj rada bio je ispitati vrijednost određivanja D-dimera u bolničkoj skupini bolesnika. Ispitivanje je provedeno u okviru uobičajene obrade bolesnika koji su bili na bolničkom liječenju. Ispitali smo 76 bolesnika, 46 muškaraca i 30 žena u dobi od 22 do 85 godina. Koncentracija D-dimera određivana je Vidas D-dimer testom. Od ukupnog broja samo je 16 (21%) bolesnika imalo koncentracije D-dimera ispod granične vrijednosti (0,5 mg/L FEU), dok je kod 60 (79%) bolesnika nađena povišena koncentracija D-dimera. Tromboembolija je potvrđena kod 13 bolesnika, dok su ostali bolesnici imali povišene vrijednosti zbog maligne bolesti (n=21), pneumonije (n=14), KOPB (n=4), politraume (n=1). Najviše koncentracije zabilježene su u skupini politraume (16,5 mg/L). Kod bolesnika s embolijom raspon vrijednosti je bio od 0,96 do 12,99 mg/L, kod maligne bolesti od 0,3 do 5,72 mg/L, a kod pneumonije od 0,64-3,73 mg/L. Zamijetili smo velik broj bolesnika s lažno povišenim vrijednostima D-dimera u bolničkoj populaciji. Razlog je vjerojatno veća učestalost onih bolesti kod kojih se nalaze povišene vrijednosti, kao i neprepoznate tromboembolije. Stoga je njegova uporaba u ovoj populaciji ograničena te određivanje D-dimera ne treba rabiti kao test probiranja, odnosno upitno je njegovo određivanje kod visoko rizičnih bolesnika – starije populacije, bolesnika s malignom bolesti ili hospitaliziranih bolesnika.

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P3-9

Usefulness of D-dimer measurement in hospitalized patients

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

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P3-10**Procjena vrijednosti D-dimera u bolničkih i ambulantnih bolesnika upotrebom dviju metoda**

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

E-mail: tihav@net.hr**P3-10****Evaluation of D-dimer value in inpatients and outpatients by use of two methods**

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Plasma D-dimer tests as used for the last ten to fifteen years are noninvasive tests for diagnosing venous thromboembolism (VTE). Recent tests based on the ELISA method are a powerful tool with a high negative predictive value in the diagnostic workout for VTE. More precisely, low D-dimer levels exclude deep vein thrombosis (DVT). On the other hand, a high D-dimer concentration may cause diagnostic confusion. The study was aimed at comparing high D-dimer levels (>500 ng/mL; >246 µg/L) and patient clinicopathologic status using the following tests: VIDAS D-Dimer Exclusion Test and D-Dimer Plus (Dade Behring). Using the above tests, plasma D-dimers were measured in 37 patients (12 male and 25 female). At the time of performing D-dimer measurement, the patients' underlying disease had already been diagnosed or it was defined later upon subsequent clinical and laboratory testing. Patients suspected of DVT were referred for either color Doppler US or CT.

Among 33 patients showing high D-dimer levels, six (16%) had DVT, four (11%) had acute superficial thrombophlebitis, ten (27%) had advanced malignant disease, in six (16.2%) patients it was day 5 after operation for malignant disease, three (8.1%) were undergoing adjuvant chemo- and radiotherapy, and four (10.8%) patients underwent testing immediately after operation for a nonmalignant disease. Both methods showed low D-dimer levels in four (10.8%) patients. In one patient, one of the tests showed low D-dimers, whereas the other showed high D-dimer levels with DVT confirmed by CT. Of 33 patients showing high D-dimer levels, 22 (66%) had a malignant disease. In conclusion, low D-dimer levels may be sufficient to exclude DVT on the one hand, whereas on the other hand high D-dimer levels should primarily prompt further diagnostic steps towards a malignancy, and then towards DVT.

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P3-11

Procjena epruveta tvrtke Terumo u izvođenju pretraga zgrušavanja krviVukmir-Turković B¹, Brkljačić V², Sertić J², Kralik-Oguić S²¹Zavod za laboratorijsku dijagnostiku, KBC Rijeka, Rijeka, Hrvatska²Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb, Zagreb, Hrvatska

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

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P3-11

Evaluation of Terumo test tubes for coagulation testsVukmir-Turković B¹, Brkljačić V², Sertić J², Kralik-Oguić S²¹Department of Laboratory Diagnosis, Rijeka University Hospital Center, Rijeka, Croatia²Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia

Glass test-tubes produced by Terumo are used to collect blood samples for coagulation analyses.

The aim of the study was to compare Terumo plastic and glass test-tubes used to perform coagulation tests. Patient samples for coagulation tests were divided into four groups according to therapy administered to patients: no therapy, oral anticoagulant therapy, low-molecular heparin therapy, and high-molecular heparin therapy. Methods for PT, aPTT and heparin determination were introduced according to the manufacturer's instructions and adapted for the Instrumentation Laboratory ACL8000 analyzer. Results showed a high degree of correlation in patients on oral anticoagulant therapy (R=0.99), low-molecular- (R=0.93) and high-molecular heparin therapy (R=0.97), while subjects without therapy showed a satisfactory degree of correlation for PT (R=0.86) and aPTT (R=0.80) tests.

Based on the results of these tests and subsequent statistical processing, it was concluded that there were no significant differences between the glass and plastic test-tubes manufactured by Terumo.

Previous studies have shown the importance of the delay between blood sampling and test performance. A prolonged delay leads to a substantial difference in test results between glass and plastic tubes. In our study, the maximum delay was limited to 180 minutes.

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P3-12

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

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

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P3-12

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

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

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P3-13

Tromboelastografija i globalni koagulacijski testovi

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Tromboelastografija (TEG) je metoda koja ima mogućnost praćenja svih faza hemostatske aktivnosti iz jednog uzorka krvi. Ona prati trombodinarski status krvi, a isto tako mjeri viskoelastična i mehanička svojstva stvorenog ugruška. Tromboelastogram je mali analizator priključen na računalo koji prati električne signale. Osnovni parametri koji definiraju stvaranje ugruška su reakcijsko vrijeme (R); K vrijeme; kut alfa i maksimalna amplituda (MA). Globalni koagulacijski testovi (protrombinsko vrijeme (PV), aktivirano parcijalno tromboplastinsko vrijeme (APTV), broj trombocita, koncentracija fibrinogena) mjere izolirano pojedine sastavnice hemostaze i završavaju stvaranjem ugruška.

Cilj studije bio je pronaći korelaciju između parametara tromboelastografa i globalnih koagulacijskih testova. Obradeno je ukupno 50 bolesnika (muškaraca i žena). Za TEG je rabljena puna citratna krv, a plazma za globalne koagulacijske testove (PV, APTV, koncentracija fibrinogena). Globalni koagulacijski testovi rađeni su na uređaju BCS (Dade Behring, Njemačka), a tromboelastografija je rađena na uređaju TEG Analyzer 5000 (Haemoscope, SAD). MA, parametar tromboelastograma, značajno korelira s koncentracijom fibrinogena ($r=0,77$; $p=0$), dok ostali parametri tromboelastograma i globalni koagulacijski testovi nisu korelirali ili korelacija nije bila značajna (MA i broj trombocita ($r=0,54$; $p=0$); R i APTV ($r=0,411$; $p=0$)). Tromboelastograf rabi male količine uzorka pune krvi, mjeri interakciju trombocita s faktorima koagulacije, brzinu ugruška, čvrstoću ugruška, kao i vezivanje trombocita i fibrinogena i moguću lizu ugruška. Vrijeme, brzina, čvrstoća i stabilnost ugruška pokazuju ima li bolesnik normalan, hipokoagulabilan ili hiperkoagulabilan koagulacijski profil i prikazuje se kao graf. TEG nudi mogućnost pretrage uz bolesnika i jednostavna je i brza koagulacijska metoda.

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P3-13

Thromboelastography and global coagulation tests

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

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P4 – Srce i srčani biljezi, P4-1 (UP8-1)**Procjena analitičke i dijagnostičke vrijednosti kolorimetrijskog testa vezanja kobalta na albumin kod bolesnika sa sumnjom na akutni koronarni sindrom**

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

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P4 – Heart and cardiac markers, P4-1 (UP8-1)**Analytical and diagnostic assessment of serum albumin-cobalt binding assay in patients suspect of acute coronary syndrome**

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

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P4-2

Seroprevalencija *Helicobacter pylori* kod koronarne arterijske bolesti

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

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P4-3

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

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

P4-2

***Helicobacter pylori* seroprevalence in coronary artery disease**

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

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P4-3

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

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

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

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P4-4

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

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

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

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P4-4

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

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

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

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

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P4-5

BNP – biljeg ishemijske bolesti srca

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

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P4-5

BNP – a marker of ischemic heart failure

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

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P4-6

Dijagnostička vrijednost određivanja BNP i NT-proBNP

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

P4-6

Diagnostic value of BNP and NT-proBNP

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

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

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

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

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P4-7

Usporedba metoda za određivanje srčanog troponina IFeretić D¹, Zovko V¹, Fressl G², Rogić D²¹Medicinsko-biokemijski laboratorij, Klinika za plućne bolesti Jordanovac, Zagreb/, Hrvatska²Klinički zavod za laboratorijsku dijagnostiku Kliničkog bolničkog centra Zagreb, Zagreb, Hrvatska

U posljednje vrijeme srčani troponin I (cTnI) postao je vodeća pretraga u dijagnostici infarkta miokarda te kod procjene rizika u bolesnika s akutnim koronarnim sindromom. Nakon oštećenja miokarda cTnI se oslobađa u obliku kompleksa s troponinom C (I-C kompleks), kompleksa s troponinom T i troponinom C (I-T-C kompleks) te kao slobodni oblik. U cirkulaciji se mogu naći i drugi oblici cTnI (fosforilirani, defosforilirani, reducirani, oksidirani i/ili proteolitički razgrađeni). Cilj je bio usporediti vrijednosti cTnI dobivene dvjema različitim metodama. Analizirano je ukupno 67 usporednih uzoraka. Analize su izvršene na analizatoru Dimension RxL (Dade Behring) i na analizatoru Access (Beckman-Coulter). Na analizatoru Dimension RxL cTnI se određivao enzim-imunokemijskom metodom (EIA), a na analizatoru Access kemiluminiscentnom metodom. Dobivena je jednadžba regresije (Passing i Bablok regresija):

$$y(\text{Access}) = 0,5465 \cdot x(\text{Dimension RxL}) - 0,0019$$

uz koeficijent korelacije $r = 0,9637$.

Wilcoxon testom nađena je statistički značajna razlika između dobivenih vrijednosti ($p < 0,0001$).

Zaključeno je kako se različitim metodama dobiju različite brojčane vrijednosti za cTnI. Postoji više čimbenika koji pridonose tim razlikama:

- u testovima se rabe različita monoklonska antitijela koja ne prepoznaju iste epitope na molekuli cTnI
- različita osjetljivost metoda na oblike cTnI prisutne u cirkulaciji
- nepostojanje internacionalnog standarda za kalibraciju

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

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P4-7

Comparison of methods for determination of cardiac troponin IFeretić D¹, Zovko V¹, Fressl G², Rogić D²¹Laboratory of Medical Biochemistry, Jordanovac University Hospital for Lung Diseases, Zagreb, Croatia²Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia

Over recent years cardiac troponin I (cTnI) has become the leading assay in the diagnosis of myocardial infarction and for risk stratification of patients with acute coronary syndromes. Following myocardial damage cTnI is released as a complex with troponin C (I-C complex), with troponin T and troponin C (I-C-T complex), and as free cTnI. Other forms of cTnI (phosphorylated, dephosphorylated, reduced, oxidized and/or proteolytically degraded) may also exist in the circulation. The aim was to compare cTnI values obtained by two different cTnI methods. A total of 67 parallel samples were analyzed. Assays were performed on a Dimension RxL analyzer (Dade Behring) and on an Access analyzer (Beckman-Coulter). On Dimension RxL analyzer cTnI was determined by use of enzyme-immunoassay (EIA), while on Access analyzer chemiluminiscent immunoassay was used. Passing and Bablok regression line was:

$$(\text{Access}) = 0.5465 \cdot x(\text{Dimension RxL}) - 0.0019$$

with a correlation coefficient $r = 0.9637$. There was a statistically significant difference between the results (Wilcoxon test, $p < 0.0001$). Accordingly, different methods for cTnI yielded numerically different results. The following factors contribute to these discrepancies:

- the antibodies used in each method do not react with the same epitopes on cTnI,
- different sensitivity of the methods to the cTnI forms in the circulation, and
- the lack of international standard for calibration.

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

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P7 – Šećerna bolest, P7-1 (UP12-1)**Učinak trajanja šećerne bolesti tip 1 na katalitičnu koncentraciju N-acetil-beta-D-glukozaminidaze u mokraći djece i adolescenata**

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

E-mail: jasminka.matica@ri.t-com.hr**P7 – Diabetes mellitus, P7-1 (UP12-1)****Effect of diabetes mellitus type 1 duration on urinary N-acetyl-beta-D-glucosaminidase excretion in children and adolescents**

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N-acetyl-beta-D-glucosaminidase (NAG) (EC 3.2.1.30) is a lysosomal enzyme. Urinary NAG is of renal origin and is distributed mainly in renal proximal tubules. It is an early predictor of the development of diabetic nephropathy. It increases before the occurrence of microalbuminuria in diabetes mellitus type 1, and some studies have shown that there is a relationship between the level of urinary NAG excretion and duration of diabetes. The aim of the study was to assess urinary NAG in children and adolescents with diabetes mellitus type 1 as compared to healthy subjects and according to the disease duration. NAG levels were determined spectrophotometrically in random urine samples from 66 children and adolescents with type 1 diabetes mellitus and 68 control subjects. Diabetic patients were divided according to the duration of diabetes into four groups: group I, less than three years (n=17); group II, three to five years (n=19); group III, five to ten years (n=19); and group IV, more than ten years (n=11). To exclude the influence of urine concentration differences, urinary NAG levels were referred to the level of urinary creatinine. Urinary NAG excretion in diabetic patients was significantly increased as compared to controls ($p < 0,001$). All four groups had a significantly higher excretion of urinary NAG as compared to controls (group I $p = 0,001$, groups II and III $p < 0,001$, and group IV $p = 0,004$) but there was no significant difference among diabetic subgroups (group I vs. II $p = 0,716$; group I vs. III $p = 0,899$; group I vs. IV $p > 0,10$; group II vs. III $p = 0,549$; group II vs. IV $p > 0,10$ and group III vs. IV $p > 0,10$). There was no correlation between urinary NAG level and duration of diabetes ($r = -0,017$; $p = 0,892$). Urinary NAG excretion was significantly higher in children and adolescents with diabetes mellitus type 1 but it was not dependent of the disease duration.

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P7-2 (UP12-2)**Aktivnost alkalne fosfataze u bolesnika sa šećernom bolešću tip 1 i 2: razlike prema dobi i spolu**

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

E-mail: acausevic5@hotmail.com**P7-2 (UP12-2)****Alkaline phosphatase activity in patients with diabetes mellitus type 1 and 2: age and sex differences**

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

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P7-3

Direktna automatska imunoturbidimetrijska metoda za određivanje HbA_{1c}

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

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P7-3

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

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

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P7-4

Vrijednosti IGF-I i IGFBP3 u očnoj vodici i serumu dijabetičnih bolesnikaKrpan R¹, Kaštelan S², Bušić M², Zjačić-Rotkvić V¹¹KB Sestre milosrdnice, Zagreb, Hrvatska²OB Sveti Duh, Zagreb, Hrvatska

Cilj je bio kvantitativno odrediti IGF-I i IGFBP3 u očnoj vodici i serumu dijabetičnih bolesnika i u kontrolnoj skupini bolesnika, te odrediti povezanost tih parametara u očnoj vodici sa stupnjem dijabetične retinopatije. IGF-I mjereno je modificiranom metodom RIA, a IGFBP3 metodom RIA. U istraživanje smo uključili 24 bolesnika s dijagnozom šećerne bolesti tip 2. U kontrolnoj skupini ispitali smo 9 nedijabetičnih bolesnika. Bolesnike sa šećernom bolešću podijelili smo u 3 skupine: bolesnici bez dijabetične retinopatije (NDR, n=7); bolesnici s neproliferativnom dijabetičnom retinopatijom (NPDR, n=10); i bolesnici s proliferativnom dijabetičnom retinopatijom (PDR, n=7).

Očna vodica uzorkovana je tijekom operacije katarakte. Rezultati nisu pokazali povezanost između vrijednosti IGF-I i IGFBP3 u serumu ni u kojoj bolesničkoj skupini. Sinteza unutar oka povezana je s porastom vrijednosti IGF-I i IGFBP3 u očnoj vodici. Zaključili smo kako su IGF-I i IGFBP3 važni lokalni parametri u razvoju proliferativne dijabetične retinopatije.

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P7-5

Koncentracije ukupnih proteina i albumina u serumu dijabetičnih bolesnika

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

P7-4

Vitreous and serum levels of IGF-I and IGFBP3 in patients with diabetes mellitusKrpan R¹, Kaštelan S², Bušić M², Zjačić-Rotkvić V¹¹Sestre milosrdnice University Hospital, Zagreb, Croatia²Sveti Duh General Hospital, Zagreb, Croatia

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

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P7-5

Serum total protein and albumin concentrations in diabetic patients

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

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

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P7-6

Procjena promjena metaboličkih procesa ovisno o primjeni hipoglikemičnih lijekova

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

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

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P7-6

Assessment of changes in the metabolic pathway depending on hypoglycemic medicaments

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

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

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

Biljezi koštane pregradnje u terapiji osteoporoze selektivnim modulatorima estrogenskih receptora

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

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

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

Bone turnover markers in osteoporosis therapy with selective estrogen receptor modulators

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Osteoporosis is a metabolic bone disease characterized by decreased mineral density, microarchitectural deterioration of bone tissue, and decreased biomechanical properties of bone, which can lead to bone fractures and deformities. Osteoporosis is a big problem in postmenopausal women, where it occurs as the result of decline in estrogen concentration and unhealthy lifestyle.

Biochemical markers are very important in osteoporosis therapy because changes in their concentration or activity reflect the dynamic state of bone metabolism and can provide information on therapeutic response as early as at three months of therapy introduction. The aim of this study was to investigate therapeutic response of biochemical osteoporosis markers in postmenopausal women on therapy with selective estrogen receptor modulators. The study included 22 patients aged 52-76. Osteoporosis was diagnosed on the basis of bone mass density of the

dijagnoza osteoporoze. Uzorci krvi i druge jutarnje mokraće uzeti su prije terapije i tri mjeseca nakon terapije. Izmjerene su vrijednosti ALP (Olympus, Olympus AU 640), BAP (Metra, ETI-MAX 3000), osteokalcina (Metra, ETI-MAX 3000), beta-crosslapsa (Roche Modular E170) i TP1NP (Roche Modular E170) u serumu i DPD (Metra, ETI-MAX 3000) u drugoj jutarnjoj mokraći. Prosječno sniženje biljega bilo je: 5% za ALP, 38% za osteokalcin, 11% za beta-crosslaps i 21% za TP1NP, dok su BAP (2%) i DPD (38%) pokazali porast vrijednosti.

Osteokalcin i TP1NP su se pokazali pogodnim biljezima za praćenje odgovora na terapiju selektivnim modulatorima estrogenskih receptora u relativno kratkom vremenu, jer su pokazali statistički značajan ($p=0,006$ za osteokalcin i $p=0,005$ za TP1NP) pad vrijednosti već tri mjeseca nakon početka terapije.

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

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P8-2 (UP9-2)

Tartarat-rezistentna kisela fosfataza i PINP u dijagnostici osteoporoze

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Koštani biljezi tartarat-rezistentna kisela fosfataza (TRAP) i PINP određeni su u serumima ispitanika s verifikiranom osteoporozom i osteopenijom. S obzirom na različite podatke u literaturi, željeli smo utvrditi imaju li navedeni koštani biljezi dijagnostičko značenje. Prva skupina ispitanika ($n=60$) imala je T-score mineralne gustoće kostiju (BMD) između -0,4 i -1,7, što prema kriterijima SZO odgovara smanjenoj koštanoj masi (osteopeniji). Druga skupina ($n=60$) imala je T-score BMD ne veći od -2,3, što se prema standardima SZO definira kao osteoporoza. Katalitična aktivnost TRAP je mjerena kinetičkom metodom uz upotrebu reagensa tvrtke BioMerieux na analizatoru Mira Cobas Plus. BMD je određen ultrazvučnom tehnikom, a koncentracija PINP je određena tehnikom ECL i reagensima tvrtke Roche na analizatoru Elecsys 2010. Kod ispitanika s osteopenijom dobivene su katalitične aktivnosti TRAP u serumu od $3,51 \pm 1,14$ U/L, dok je aktivnost istoga enzima u skupini ispitanika s osteoporozom iznosila $5,49 \pm 1,63$ U/L. Utvrđene razlike su statistički značajne ($p < 0,001$). Razlike

P8-2 (UP9-2)

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

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

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

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P8-3

Analitička procjena P1NP – biokemijskog biljega koštane pregradnje

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

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

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P8-3

Analytical evaluation of P1NP – a biochemical marker of bone turnover

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

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

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P8-4

Oksidativni status u osteoporozi

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

ues at low concentrations of PINP (PC1) by 9.28%, at medium concentrations of PINP (PC2) by 6.19% and at low concentrations of PINP (PC3) by 8.53%. These values of bias from target values are acceptable in routine analysis of PINP. Our results showed that the only possible dilution is 1:2. Correlation between the measured and expected values yielded a largely proportional and constant error ($a=-19.5$ and 26.5). Accurate results could not be achieved from a dilution greater than 1:2. Interferences from bilirubin ($<610 \mu\text{mol/L}$), triglycerides ($<15 \mu\text{mol/L}$) and hemoglobin ($<1.8 \text{mmol/L}$) were undetectable. Thus, the new Roche electrochemiluminescence immunoassay for P1NP yields precise and accurate results, mostly free from interferences. P1NP could be a useful biochemical tool in assessing bone turnover in patients on hemodialysis and after bone fracture as well as a marker of therapeutic efficacy for osteoporosis.

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P8-4

Oxidative status in osteoporosis

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

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

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P8-5

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

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

oxidative potential, which may contribute to cell damage and consequently to the development of osteoporosis.

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P8-5

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

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

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

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P8-6

Uloga biokemijskih biljega u dijagnostici osteoporoze

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

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

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P8-6

The role of biochemical markers in the diagnosis of osteoporosis

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Biochemical markers of bone turnover are related primarily to bone structure and function. Although not related specific to any disease, they have significantly upgraded diagnostic potential in clinical practice. In combination with DEX, they are of great help to physician on deciding when to start therapy. The aim of this study was to investigate some biochemical markers in osteoporosis and to compare them to bone mass density (BMD) of lumbar spine (L1-L4) and hip measured with DEX method, thus evaluating their role in the diagnosis of osteoporosis. The study included 24 postmenopausal women aged 52-76. Bone mass densitometry was performed in all women. Blood samples and second morning urine were also collected. The following biochemical markers were measured in serum: osteocalcin (Metra, ETI-MAX 3000), beta-crosslaps (Roche Modular E170) and TP1NP (Roche Modular E170), while DPD (Metra, ETI-MAX 3000) was measured in urine samples.

Control group consisted of 35 healthy women aged 23-58. The mean BMD of lumbar spine (L1-L4) was 0.75; T-score -2.7; Z-score -1.1, and the mean BMD of hip was 0.79; T-score -1.2; Z-score -0.1.

Results showed a statistically significant difference between TP1NP ($p=0.026$) and beta-crosslaps ($p=0.005$) levels, so they seem to be good and sensitive markers to assess the risk of osteoporosis.

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P9 – Upala, P9-1**Plazmatske razine PMN-elastaze i neopterinu u ranom stadiju sepse**

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

E-mail: b_bogdanov@yahoo.com**P9 – Inflammation, P9-1****Plasma PMN-elastase and neopterin levels in the early stage of sepsis**

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

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P9-2

Utjecaj HDL-razgradnih proizvoda na gensku ekspresiju u endotelnim stanicamaCrnković S¹, Gauster M², Guelly C³, Frank S²¹Farmaceutsko biokemijski fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska²Zavod za molekularnu biologiju i biokemiju, Medicinski fakultet Sveučilišta u Grazu, Graz, Austrija³Centar za medicinska istraživanja, Medicinski fakultet Sveučilišta u Grazu, Graz, Austrija

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

P9-2

Effect of HDL-degradation products on gene expression in endothelial cellsCrnković S¹, Gauster M², Guelly C³, Frank S²¹School of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia²Institute for Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria³Center for Medical Research, Medical University of Graz, Graz, Austria

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

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

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P9-3

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

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

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

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P9-3

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

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

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P9-4

Glikozilacija u akutnom upalnom odgovoruGornik O¹, Rudd PM², Lauc G¹¹Farmaceutsko biokemijski fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska²Institut za glikobiologiju, Sveučilište u Oxfordu, Oxford, Velika Britanija

Akutni upalni odgovor je niz staničnih i molekularnih događaja koji se javljaju kao reakcija na različite podražaje. Iako je akutni upalni odgovor jedinstven homeostatski mehanizam, razlike u proizvodnji medijatora i tijeku samog odgovora postoje u različitim patofziološkim stanjima ovisno o naravi i mjestu upale. U našem radu ispitali smo promjene glikana otpuštenih sa serumskih glikoproteina bolesnika sa sepsom i bolesnika s akutnim pankreatitisom i usporedili ih sa zdravim pojedincem.

Serumi bolesnika uzimani su prilikom javljanja u bolnicu i zatim još tri puta tijekom prvih osam dana hospitalizacije. Kontrolni serum odgovarajuće dobi i spola uzet je samo jednom. Glikani su sa serumskih proteina otpušteni N glikozidazom F, enzimom koji specifično otpušta N vezane glikane i analizirani tekućinskom kromatografijom visoke djelotvornosti u kombinaciji s digestijom egzoglikozidazama. Prisutne glikanske strukture su identificirane, praćena je njihova promjena tijekom bolesti te su uspoređene s onima kontrolnog seruma. Naši rezultati pokazuju da se promjene serumskih glikana javljaju vrlo rano u akutnoj upali. Razine pojedinih struktura mijenjaju se na dnevnoj osnovi, neke u istom smjeru, dok neke variraju kroz dane. Ove promjene su složene i primjećujemo ih u tri- i tetrasialiniranim strukturama, manoznim strukturama, razini fukozilacije i stupnju razgranatosti. Promjene su prisutne već u prvom danu bolesti uspoređujući s kontrolnim serumom.

Složene promjene glikana tijekom akutnog upalnog odgovora nisu iznenađujuće ako uzmemo u obzir složenost akutnog upalnog odgovora te važnu ulogu glikana u mnogim procesima. Ove promjene mogu biti dio regulatornog mehanizma tijekom upale, jer postoje indikacije da manozna i fukoza sudjeluju u imunomodulaciji uz potencijalno dobrohotne učinke. Razlike pokazane u bolesnika sa sepsom u odnosu na onog s akutnim pankreatitisom vjerojatno proizlaze iz činjenice da je akutni upalni odgovor izazvan različitim stimulusom i stoga ima različit obrazac proizvodnje citokina.

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P9-4

Glycosylation in acute inflammatory responseGornik O¹, Rudd PM², Lauc G¹¹School of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia²Glycobiology Institute, University of Oxford, Oxford, Great Britain

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

These changes can be part of regulatory processes during inflammation since it has been shown that mannose and fucose participate in immunomodulation, presumably having beneficial effects. The differences found between sepsis and acute pancreatitis were probably due to the fact that the acute phase response is triggered by a different stimulus, thus having different patterns of specific cytokine production.

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P9-5**Identifikacija antinuklearnih antitijela pomoću imuno testa**

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

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P9-6**Enzim konverzije angiotenzina u sarkoidozi**

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

P9-5**Identification of antinuclear antibodies using an immunoassay system**

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

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P9-6**Angiotensin converting enzyme of sarcoidosis**

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

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

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P9-7

Biljezi upale u prijevremenom porodu

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

Trinity (Biotech) spectrophotometric method on a Cobas Mira biochemical analyzer. Statistical analysis of the results obtained on ACE activity in relation to sex ($p > 0,05$) did not lead to any significant and firm conclusion. Significantly higher levels of ACE activity were obtained in sarcoidosis patients (51.3 ± 23.25 U/L) as compared to the control group of blood donors (19.0 ± 7.42 U/L vs. 19.0 ± 7.42 U/L; $p < 0.005$). Accordingly, elevated ACE activity showed positive correlation with sarcoidosis and its progression, and is a good prognostic marker for this disease. The correlation of ACE activity and sex was not statistically significant.

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P9-7

Inflammation markers in preterm labor

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

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

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P9-8

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

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

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

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P9-8

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

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

The concentration of hsCRP was significantly higher in

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

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

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P9-9

Poremećaji unutarstaničnih signalnih putova kod bolesnika s kroničnom opstrukcijskom bolesti pluća

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

P9-9

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

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

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

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P9-10

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

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

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

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P9-10

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

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Rapid and accurate diagnosis of infectious diseases of the central nervous system (CNS) is important for proper treatment. The level of blood-brain-barrier (BBB) impairment in CNS diseases determines protein constituents in cerebrospinal fluid (CSF). High sensitivity C-reactive protein (hsCRP) and serum amyloid A protein (SAA) are acute phase proteins that are increased in bacterial and moderate in viral infectious diseases. The molecular size and structure of these proteins indicate that they could pass through the BBB and help in the differential diagnosis of meningitis. hsCRP and SAA were determined as potentially useful indicators of damaged BBB and also for the differential diagnosis of bacterial and viral meningitis. We measured the concentration of hsCRP and SAA in serum and CSF of patients with bacterial (n=11) and viral (n=13) meningitis. Albumin, IgG, IgA and IgM were determined to assess the BBB dysfunction. All analytes were determined nephelometrically. Serum concentrations of hsCRP ($p < 0,001$) and SAA ($p < 0,01$) were significantly higher in patients with bacterial than in those with viral meningitis. The correlation coefficient for hsCRP and SAA in serum was $r = 0,944$ ($p < 0,0001$). CSF hsCRP (10/13) and

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

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P10 – Cerebrovaskularne bolesti, P10-1

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

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

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

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P10 – Cerebrovascular diseases, P10-1

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

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

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

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P10-2

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

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

LDL-kolesterol mmol/L ($5,62 \pm 4,32$ vs. $2,89 \pm 1,32$) and apolipoprotein B mg/dL ($2,32 \pm 0,8$ vs. $1,36 \pm 0,32$), whereas HDL-kolesterol and apolipoprotein A-I did not significantly change from baseline. Besides its lipid lowering efficacy, hollesta has not effect on serum Lp(a) and tHcy levels. Hyperhomocysteinemia can easily be treated with vitamin supplements, which are favored for their cardio-protective properties, while Lp(a) levels are stable over time.

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P10-2

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

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

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

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

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P10-3

C-reaktivni protein kao biomarker visokog stupnja stenozе moždanih arterija

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

P10-3

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

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

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

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P11 – Bubrežne bolesti, P11-1

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

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

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

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P11 – Nephrological diseases, P11-1

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

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

ge etiologije (III. skupina) te kod zdravih ispitanika (n=18). Koncentracija velikog ET-1 u mokraći bolesnika s KBB bila je značajno povećana (13,13 pmol/L; p<0,001) u odnosu na kontrolnu skupinu (11,34 pmol/L), dok se koncentracije ET-1 u plazmi i mokraći nisu značajno razlikovale od kontrolne skupine. Koncentracija NO u serumu bila je značajno povećana u skupini bolesnika (72,55 μmol/L; p<0,001), kao i u mokraći bolesnika (141,74 μmol/L; p<0,05) u odnosu na kontrolnu skupinu. Određivanjem koncentracije velikog ET-1 u mokraći (dijagnostička osjetljivost 56,1%, dijagnostička specifičnost 88,9%) i NO u plazmi (dijagnostička osjetljivost 66,7%, dijagnostička specifičnost 83,3%) mogu se razlikovati bolesnici s KBB različitih etiologija u odnosu na zdrave ispitanike. Dijagnostička osjetljivost velikog ET-1 za dijagnostiku dijabetične nefropatije je 78,6%, a specifičnost 88,9%.

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P11-2

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

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

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

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P11-2

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

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

lesnika dijaliziranih na Polyflax 21S Gambro (HDF), skupina N2; 14 bolesnika dijaliziranih na dijalizatorima Lympha Belko (PHF), skupina N3. Srednja dob bolesnika bila je $43 \pm 2,8$ godine. Kt/v u svim skupinama bio je veći od 1,2. Svim bolesnicima na početku i na kraju dijalize određen je beta₂-mikroglobulin metodom imunoturbidimetrije na Olympusu AU 600. U statističkoj obradi rabio se je Mann-Whitney test. Razlika u koncentraciji beta₂-mikroglobulina kod bolesnika prije i poslije hemodijalize pokazala se statistički značajnom u skupinama N2 ($p < 0,05$) i N3 ($p < 0,01$), dok u skupini N1 nije bila statistički značajna ($p > 0,08$). Ispitivanje je pokazalo značajno uklanjanje serumskog beta-2-mikroglobulina kod bolesnika na HDF i PHF dijalizi u odnosu na bolesnike na HD dijalizi, što smanjuje brojne komplikacije, a sve to dovodi do smanjenja pobola i smrtnosti u bolesnika na hemodijalizi.

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P11-3

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

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

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

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P11-3

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

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

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

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P11-4

Značenje nalaza atipičnih stanica u svježoj mokraći

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

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

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P11-4

Significance of atypical cell finding in fresh urine

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

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

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P12 – Bolesti jetre i gastrointestinalnog trakta, P12-1 Mutacija gena za kationski tripsinogen (PRSS1) hereditarnog pankreatitisa: prikaz obitelji

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

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

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P12 – Liver and gastrointestinal diseases, P12-1 Hereditary pancreatitis with cationic trypsinogen gene (PRSS1) mutation: a family study

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

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

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P12-2

Dokazivanje prisutnosti *Helicobacter pylori* pomoću ureja izdisajnog testa

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Ureja izdisajni test se primjenjuje za dokazivanje prisutnosti i aktivnosti bakterije *Helicobacter pylori* na stijenci želuca. Izdisajnim testovima se analizira CO₂ iz izdahnutog zraka. Svojstvo bakterije *Helicobacter pylori* je sinteza ureaze. Kad ispitanik konzumira ureju obilježenu ugljikovim izotopom, a bakterija je prisutna na sluznici želuca, tada će ureaza koju sintetizira bakterija razgraditi testnu otopinu na amonijak koji se izlučuje urogenitalnim traktom i CO₂ s izotopom koji u određenom vremenskom razdoblju krvlju dopijeva u pluća i konačno u izdahnuti zrak. Tehnike za određivanje količine izotopa su razne: masena spektrometrija, plinska kromatografija-masena spektrometrija, optička spektroskopija i infracrvena spektroskopija. Cilj studije je bio utvrditi prisutnost bakterije *Helicobacter pylori* na stijenci želuca ispitanika. Primijenili smo metodu ureja izdisajnog testa ugljikovim izotopom C13. Tehnika određivanja omjera koncentracije ugljikovog izotopa C12 i C13 je infracrvena spektroskopija. Izvor infracrvenog zračenja emitira konstantno svjetlo. Ova radijacija prolazi kroz mjerne komore ispunjene zrakom koji se analizira, a zatim kroz detektorske komore od kojih je svaka ispunjena jednim od izotopski čistog ugljičnog dioksida. Plin u svakoj detektorskoj komori apsorbira energiju u spektru dotičnog izotopa i zagrijava se do stupnja proporcionalno valnoj radijaciji na ulasku u sustav. Intenzitet ovisi o količini apsorbirane energije u mjernoj komori, dakle, o koncentraciji i odgovarajućem parcijalnom tlaku komponente u mjernoj komori. Veličina koja se određuje jest omjer izotopa C12 i C13 u uzorku, a izražava se kao Delta. Rezultat se izražava kao DOS=Delta over baseline. U ovom testu se tako uspoređuju Delta C13 i C12 u nultom uzorku prije konzumacije testne otopine i Delta C13 i C12 u uzorku nakon određenog vremena za koje je utvrđeno da je potrebno za razgradnju supstrata i pojavu razgradnog proizvoda u izdahnutom zraku. Ureja izdisajni test je neinvazivna metoda dokazivanja prisutnosti i aktivnosti bakterije *Helicobacter pylori* na stijenci želuca ispitanika obilježena

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

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P12-2

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

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Urea breath test is used to determine the presence and activity of *Helicobacter pylori* on gastric mucosa. Breath tests analyze CO₂ from breath. *Helicobacter pylori* synthesizes urease. The patient takes urea labelled with carbon isotope and if the bacteria is present, then the urease that is synthesized breaks the test solution up to produce ammonia which is eliminated by urogenital pathway and CO₂ with isotope which will reach the lungs by blood within a certain period of time and eventually be detectable in breath. The techniques used to determine isotope concentration are: mass spectrometry, gas chromatography-mass spectrometry, optical spectroscopy and infrared spectroscopy. The aim of the study was to determine the presence of *Helicobacter pylori* on gastric mucosa. Urea breath test with C13 carbon isotope was used. Infrared spectroscopy was used for determination of C12 and C13 isotope concentration. In the measuring system, the infrared sources emit constant infrared light. This radiation passes measuring cells filled with measuring gas and then detector cells loaded with the isotopically pure gaseous component to be measured. The gas in each of detector cells absorbs energy in the spectral range of the particular component and is warmed up to the level proportional to the intensity of this radiation at its entrance window. This intensity depends on the degree of radiation absorption in the measuring cells and therefore also on the concentration or partial pressure of the particular component in these measuring cells. Diagnostic criterion of C13-urea breath test is Delta Over Baseline (DOB) value. Delta values are deviations from a certain standard expressed per million. For carbon isotope analysis, standard PDB is used as internal standard and its Delta value is 0 per million. Delta values are determined at zero time, Delta baseline, and 30 minutes after test solution intake, Delta after tracer intake. Urea breath test is a noninvasive method to determine the presence and activity of *Helicobacter pylori* on gastric mucosa, characterized by high sensitivity and

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

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P13 – Endokrinologija, P13-1

Laboratorijska potpora dijagnostici i praćenju bolesti štitnjače

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

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

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P13 – Endocrinology, P13-1

Laboratory support to thyroid disease diagnosis and follow up

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

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P13-2

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

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

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P13-2

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

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Clinical and experimental literature has documented that TSH can stimulate the growth of thyroid tumors. The administration of L-T4 will suppress TSH, thus reducing the growth of thyroid cancer. Thyroid suppression of serum TSH is currently employed for long-term medical management of patients with thyroid cancer. Current TSH methods with their enhanced sensitivity and specificity are capable of detecting low TSH values, thus the measurement of TSH is used as a therapeutic endpoint for adjusting the thyroxine suppression dose. We employed a third-generation assay, DELFIA hTSH Ultra kit, for determination of serum TSH in 160 patients with thyroid cancer. The therapeutic endpoint for adjusting the thyroxine suppression dose for each patient was judged by weighing the patient's factors such as age, clinical status including cardiac factors and cancer recurrence risk, against the potentially deleterious effects of iatrogenic mild (subclinical) hyperthyroidism on the heart and bone.

In the group of 88 patients on physiological dose of L-T4 therapy, 51 (57%) patients had TSH value in the subnormal range (0.05-0.42 mIU/L) and 34 (38.6%) patients had TSH value in the normal range (0.4-4.2 mIU/L). Three patients had undetectable TSH. Out of 72 patients on supraphysiological dose of L-T4 therapy, 61 (84.7%) patients had undetectable TSH (<0.05 mIU/L) and 7 (9.7%) patients had TSH value in the subnormal range. Four patients had normal TSH value. In conclusion, the efficacy of L-T4 suppression or substitution long-term medical therapy in patients with thyroid cancer can be monitored by measuring serum TSH.

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P13-3

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

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

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P13-3

Comparison of three fully automated immunassays for serum total HCG determination

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

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P13-4

Poremećaji funkcije štitnjače u bolesnika na terapiji amjodaronom

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Amjodaron je derivat benzofurana, lijek iz III. skupine antiaritmika, koji se upotrebljava za liječenje i prevenciju aritmija. Maseni udio joda u molekuli amjodarona iznosi oko 37%. Uzimanjem amjodarona dnevni unos joda se povećava 50 do 100 puta. Amjodaron može uzrokovati neke poremećaje funkcijskih testova, kao i manifestnu bolest štitnjače. Amjodaron u perifernim tkivima inhibira aktivnost tipa I 5' deiodinaze i inhibira ulazak hormona štitnjače u periferna tkiva, što doprinosi povećanju serumske vrijednosti tiroksina (T4) i sniženju vrijednosti trijodtrionina (T3). Podaci iz različitih dijelova svijeta pokazuju incidenciju amjodaronom uzrokovane tireotoksikoze (AIT) od 1% do 23% i amjodaronom uzrokovane hipotireoze (AIH) od 1% do 32%. Cilj studije bio je odrediti učestalost AIT i AIH u našoj populaciji bolesnika na terapiji amjodaronom i usporediti dobivene podatke s podacima iz literature. U razdoblju od 2002. do 2006. godine u bazi podataka Odsjeka za laboratorijsku dijagnostiku bolesti štitnjače ukupno je evidentirano 447 bolesnika na terapiji amjodaronom. U istraživanje je uključeno 97 bolesnika s medijanom praćenja od 12 (raspon 1-47) mjeseci. Serumske koncentracije TSH i hormona štitnjače određivane su imunometrijskim metodama: a) TSH (Immulite Third Generation TSH (DPC), referentne vrijednosti: 0,36-4,20 mU/L), b) T3 i T4 (DELFIAT3: 1,1-2,8 nmol/L, T4:65-160 nmol/L), c) FT3 i FT4 (BRAHMS (FT3: 4,9-7,9 pmol/L FT4: 12,6-20,9 pmol/L) i Immulite (DPC) (FT3: 2,3-6,3 pmol/L FT4: 10,3-24,5 pmol/L). AIH je definirana nalazom povišenih vrijednosti TSH uza snižene vrijednosti T4/FT4, a subklinička hipotireoza povišenim vrijednostima TSH uz uredne vrijednosti FT4/T4. AIT je definirana sniženim vrijednostima TSH uz povišene razine T3/FT3 ili izrazito povišene vrijednosti FT4/T4, a subklinička tireotoksikoza sniženim vrijednostima TSH uz uredne vrijednosti T3/FT3. U 59 od 97 (61%) bolesnika utvrđena je eutireoza. AIH je utvrđena u 14 (14%), subklinička hipotireoza u 9 (9%), AIT u 8 (8%), a subklinička tireotoksikoza u 7 (7%) bolesnika. U promatranoj populaciji zabilježen je značajan broj bolesnika s poremećajem funkcije štitnjače, od kojih je najveći broj bio s AIH, a manji dio s AIT, što je u skladu s podacima za područja s dostatnim unosom joda.

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P13-4

Thyroid function dysfunction in patients on amiodarone therapy

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Amiodarone is a benzofurane derivative, a class III antiarrhythmic drug used for the prevention and management of tachyarrhythmias. Quantitative proportion of iodine in the amiodarone molecule is 37%. Amiodarone treatment increases daily iodine intake approximately 50-100 times. Amiodarone has an effect on thyroid function in the spectrum from abnormal thyroid tests to overt thyroid dysfunction. It inhibits the activity of type I 5' deiodinase as well as thyroid hormone entry in peripheral tissues, which contributes to serum thyroxine (T4) level increase and triiodothyronine (T3) level decrease. Data from different parts of the world indicate the incidence of amiodarone induced thyrotoxicosis (AIT) to be 1% to 23%, and of amiodarone induced hypothyroidism (AIH) 1% to 32%. The aim of the study was to assess the prevalence of AIT and AIH on the basis of thyroid tests in our group of patients on amiodarone therapy, and to compare these findings with literature data. According to the Division of Thyroid Diseases database, in the 2002-2006 period there were 447 patients on amiodarone therapy. Only 97 patients with a follow up median of 12 (range 1-47) months were included in the study. Serum concentrations of TSH and thyroid hormones were measured by immunometric assay methods: (a) TSH (Immulite Third Generation TSH (DPC, reference range: 0.36-4.20 mU/L); (b) T3 and T4 (DELFIAT3:1.1-2.8 nmol/L, T4: 65-160 nmol/L); (c) FT3 and FT4 (BRAHMS (FT3: 4.9-7.9 pmol/L FT4: 12.6-20.9 pmol/L) and Immulite (DPC) (FT3: 2.3-6.3 pmol/L FT4: 10.3-24.5 pmol/L). AIH was defined by increased serum TSH level followed by lower value of T4/FT4. Subclinical hypothyroidism was defined by increased TSH level followed by normal FT4 and T4 values. AIT was defined by lower TSH level followed by increased T3/FT3 or extremely elevated FT4/T4 levels, and subclinical thyrotoxicosis was defined by lower TSH level followed by normal T3/FT3 value. Euthyroidism was recorded in 59 of 97 (61%), AIH in 14 (14%), subclinical hypothyroidism in 9 (9%), AIT in 8 (8%), and subclinical thyrotoxicosis in 7 (7%) patients. Accordingly, thyroid dysfunction was found in a significant number of patients. The higher incidence of AIH and lower incidence of AIT were consistent with data reported from areas with adequate iodine intake.

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P13-5

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

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

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P13-5

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

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

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P14 – Onkologija i tumorski biljezi, P14-1 (UP5-1)**Je li Ca 19-9 XR bolji?**

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

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P14-2**MTHFR u karcinomu prostate i benignoj hiperplaziji prostate**Nikolac N¹, Šimundić AM¹, Reljić A², Štefanović M¹, Topić E¹¹Klinički zavod za kemiju, KB Sestre milosrdnice, Zagreb, Hrvatska²Klinika za urologiju, KB Sestre milosrdnice, Zagreb, Hrvatska

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

P14 – Oncology and tumor markers, P14-1 (UP5-1)**Is Ca 19-9 XR better?**

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

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P14-2**MTHFR in prostatic cancer and benign prostatic hyperplasia**Nikolac N¹, Šimundić AM¹, Reljić A², Štefanović M¹, Topić E¹¹University Department of Chemistry, Sestre milosrdnice University Hospital, Zagreb, Croatia²University Department of Urology, Sestre milosrdnice University Hospital, Zagreb, Croatia

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

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

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P14-3

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

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

activity and hypomethylation. Many studies investigated C677T in patients with carcinoma and although most authors report a lower frequency of mutated T allele in cancer patients, results are inconclusive.

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

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P14-3

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

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

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

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P14-4

Tumorski biljezi S100 i MIA u praćenju bolesnika s malignim melanomom

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

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

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P14-4

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

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

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

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patients with poor prognosis who might benefit from an adjuvant therapy. Moreover, MM markers could be useful in monitoring the clinical course of disease during the treatment. So far, studies have described S100 and MIA (melanoma inhibiting activity) proteins as new suitable tumor markers for MM. Our earlier work confirmed the usefulness of determination of the melanoma markers S100 and MIA serum proteins in patients with melanoma. The aim of this study was to evaluate S100 and MIA results of MM patients according to the stage of disease during the five-year follow up period. Serum MIA and S100 protein levels were measured three or more times during the 5-year follow up period (2001-2006) in patients with MM stage I-IV (AJCC 2002 classification). Serum concentrations of S100 and MIA proteins were determined by immunoradiometric (IRMA) and immunoenzymatic (EIA) kits: Sangtec 100 IRMA (Sangtec Medical, Sweden), CanAg S100 EIA (CanAg Diagnostics, Sweden), Sangtec 100 ELISA (DiaSorin, USA) and MIA ELISA (Roche Diagnostics, Germany). χ^2 -test was used on statistical analysis. A total of 154 MM patients (96 women and 58 men, age range 21-81 and 16-85 yrs, respectively) were classified by stages: I (n=76), II (n=50), III (n=15) and IV (n=13).

Of 601 diagnostic tests performed (stage I-IV: 268, 222, 65 and 46, respectively), there were 110 (18.3%) elevated S100 levels and 137 (22.8%) elevated MIA levels. The percentage of elevated levels according to stages I-IV for S100 were 19.0%, 17.8%, 15.4% and 21.7%, and for MIA 21.3%, 22.5%, 23.1% and 32.8%, respectively. Both markers were within the normal range in 47 (30.5%) patients (stage I=14.3%, stage II=10.4%, stage III=3.2% and stage IV=2.6%). Metastases were diagnosed in 25 (16%) patients: 13 stage III and 12 stage IV. In patients with metastases, both markers were increased and within the normal range in 8 (32%) patients each. Only 2 (8%) patients showed an increased level of S100, and only 7 (28%) patients showed an increased level of MIA. Study results pointed to the following conclusions: 1) there were significant differences in serum marker levels between stage I and stages III and IV, as well as between stage II and stages III and IV; 2) there was no significant difference in the level of serum markers between stages I and II, and between stages III and IV; and 3) it remains unclear why there were an equal number of patients with both markers increased or both markers within the normal range among the patients with metastases?

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P15 – Toksikologija i TDM, P15-1 (UP10-1)**Određivanje lamotrigina u plazmi metodom tekućinske kromatografije visokog razlučivanja uz primjenu ultraljubičastog detektora**Petek-Tarnik I¹, Coce I¹, Petek M¹, Cvitanović-Šojat LJ², Kusić Z³¹Endokrinološki laboratorij, Zavod za nuklearnu medicinu i onkologiju, KB Sestre milosrdnice, Zagreb, Hrvatska²Klinika za pedijatriju, KB Sestre milosrdnice, Zagreb, Hrvatska³Zavod za nuklearnu medicinu i onkologiju, KB Sestre milosrdnice, Zagreb, Hrvatska

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

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P15 – Toxicology and TDM, P15-1 (UP10-1)**Determination of lamotrigine in plasma by high performance liquid chromatography with UV detection**Petek-Tarnik I¹, Coce I¹, Petek M¹, Cvitanović-Šojat LJ², Kusić Z³¹Laboratory of Endocrinology, Department of Nuclear Medicine and Oncology, Sestre milosrdnice University Hospital, Zagreb, Croatia²University Department of Pediatrics, Sestre milosrdnice University Hospital, Zagreb, Croatia³Department of Nuclear Medicine and Oncology, Sestre milosrdnice University Hospital, Zagreb, Croatia

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

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P15-2

Serumske koncentracije oligoelemenata u shizofrenih bolesnika i ovisnika o alkoholu

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

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P15-2

Serum oligoelement concentrations in schizophrenia patients and alcohol addicts

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

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P15-3 (UP10-2)**Naša iskustva u primjeni imunokemijskih testova u toksikološkom prosijavanju na lijekove i sredstva ovisnosti**

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

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P15-3 (UP10-2)**Our experience with immunochemistry testing on screening for drugs and abuse substances**

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

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

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P15-4

Koncentracija beta-endorfina u serumu i mozgu štakora izloženih trazodonu

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

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P15-5

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

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

P15-4

Brain and serum beta-endorphin concentration in trazodone treated rats

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

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P15-5

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

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

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

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P15-6

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

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

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

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P15-6

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

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

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

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P15-7

Farmakogenetička analiza i individualizacija terapije u liječenju epilepsije – prikaz slučaja

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

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

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P15-7

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

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

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

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

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P15-8

Fenotipizacija s dekstrometorfanom u procjeni metaboličkog kapaciteta enzima CYP2D6 i dometa interakcija lijekovaLovrić M¹, Božina N¹, Granić P¹, Mihaljević-Peleš A², Lalić Z¹¹Klinički zavod za laboratorijsku dijagnostiku, Klinički bolnički centar Zagreb, Zagreb, Hrvatska²Klinika za psihijatriju, Klinički bolnički centar Zagreb, Zagreb, Hrvatska

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

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P15-8

Dextromethorphan phenotyping in assessing CYP2D6 enzyme metabolic capacity and drug interactionLovrić M¹, Božina N¹, Granić P¹, Mihaljević-Peleš A², Lalić Z¹¹Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia²University Department of Psychiatry, Zagreb University Hospital Center, Zagreb, Croatia

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

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P15-9

Analitička validacija testa Seradyn-Innfluor za određivanje koncentracije everolimusa

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

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P15-9

Analytical validation of Seradyn-Innfluor assay for everolimus measurement

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

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P15-10**Prikaz slučaja: otrovanje etilen-glikolom**Lovrić M¹, Granić P¹, Čubrilo-Turek M², Lalić Z¹, Sertić J¹¹Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb, Zagreb, Hrvatska²Klinika za unutarnje bolesti, Opća bolnica Sveti Duh, Zagreb, Hrvatska

U jedinicu intenzivne skrbi zaprimljen je četrdesetgodišnji muškarac poslije pokušaja samoubojstva. Bolesnik je pronađen kod kuće, bez svijesti, a pokraj njega je bila otvorena boca antifrizna. Glavni sastojak antifrizna je etilen-glikol koji nije toksičan, ali su toksični njegovi metaboliti, glikolna i oksalna kiselina, jer uzrokuju oštećenja organa i smrt. Kod prijma je bolesnik bio bez svijesti, a kliničkom obradom isključena su neurološka oštećenja. Rezultati početnoga toksikološkog probiranja seruma i mokraće bili su negativni. Laboratorijski rezultati ukazivali su na metaboličku acidozu (pH 7,123; pCO₂ 2,03 kPa) s leukocitozom (L 32,0 10⁹/L). Analiza mokraće pokazala je prisutnost hematurije i amorfnih kristala. Osmolalnost i osmotski procijep nisu mjereni kod prijma bolesnika. Zbog metaboličke acidoze, anamnestičkih podataka i kliničkog stanja bolesnika započeto je liječenje etilnim alkoholom kao protulijekom te hemodijaliza, pa se bolesnikovo stanje postupno poboljšalo. Naknadna toksikološka analiza glikolne i oksalne kiseline u serumu i mokraći napravljena je ionskom kromatografijom metodom HPLC. Vrijednosti glikolne kiseline u slučajnom uzorku mokraće bile su kod prijma 28171,2, a poslije hemodijalize 3,01 mmol/mol kreatinina, a oksalne kiseline 370,5 kod prijma i 80,5 mmol/mol kreatinina nakon hemodijalize. U ultrafiltratu seruma dobivene vrijednosti za navedene kiseline bile su: glikolna kiselina 17661,0 mmol/L kod prijma, 35,2 mmol/L poslije hemodijalize; oksalna kiselina 353,5 mmol/L kod prijma, 19,5 mmol/L poslije hemodijalize. Otrovanje etilen-glikolom uzrokuje tešku metaboličku acidozu koja može izazvati smrt ako dijagnoza nije pravodobna i ako se specifično liječenje ne započne na vrijeme. Laboratorijski testovi značajno mogu pridonijeti bržem postavljanju dijagnoze, a uključuju: određivanje plinova u krvi s anionskim procijepom, povišenu osmolalnost s osmotskim procijepom, prisutnost kristala kalcijevog oksalata u mokraći i mjerenje etilen-glikola u serumu, ako je moguće. Prema preporukama NACB klinički laboratorij mora osigurati mjerenje etilen-glikola u serumu ili plazmi, a ako se rabi metoda GC, tada analiza mora uključivati i toksični metabolit glikolnu kiselinu.

*E-mail: lovricm@kbc-zagreb.hr***P15-10****Case report: ethylene glycol poisoning**Lovrić M¹, Granić P¹, Čubrilo-Turek M², Lalić Z¹, Sertić J¹¹Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia²University Department of Medicine, Sveti Duh General Hospital, Zagreb, Croatia

A 40-year-old man was admitted to the emergency department after a suicide attempt. The patient was found at home unconscious, with an open bottle of antifreeze near him. The main component of antifreeze is ethylene glycol that is not toxic but produces toxic metabolites that cause organ failure and death. The patient was in coma on admission, but was neurologically intact. Results of initial urine and serum toxicological screening tests were negative. Laboratory values indicated metabolic acidosis (pH 7.123; pCO₂ 2.03 kPa) with leukocytosis (L 32.0 10⁹/L). Urinalysis revealed hematuria and amorphous crystals. Osmolality and osmol gap were not determined at patient admission. Treatment with ethanol as an antidote and hemodialysis were started because of metabolic acidosis, history data and clinical status of the patient, and subsequently led to improvement of his condition. Further toxicological analyses of glycolic and oxalic acids in serum and urine samples were performed by the ion-chromatography HPLC method. Results of glycolic and oxalic acid tests in spot urine were 28171.2 and 370.5 mmol/mol creatinine, and 3.01 and 80.5 at admission and post-hemodialysis, respectively. The values of glycolic and oxalic acids in serum ultrafiltrate were 17661.0 and 353.5 mmol/L, and 35.2 and 19.5 mmol/L at admission and post-hemodialysis. Intoxication by ethylene glycol causes severe metabolic acidosis which may lead to death if diagnosis is delayed and specific treatment is not initiated promptly. Laboratory tests for ethylene glycol poisoning include determination of blood gases with anion gap, elevated osmolality with osmol gap, calcium oxalate in urine, and serum ethylene glycol if possible. According to NACB guideline, clinical laboratory should provide direct measurements of ethylene glycol in serum or plasma, and the GC assay should target glycolic acid in addition to parent intoxicant, ethylene glycol.

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P16 – Molekularna dijagnostika, P16-1 (UP7-1)**Polimorfizam gena za citokine IL-6, IL-10, TNF-alfa, TGF-beta1 i IF-gama u bolesnika s presađenim bubregom**Wagner J¹, Pavlinić D¹, Zibar L², Lauc G¹, Barbić J³¹DNA laboratorij, Medicinski fakultet, Osijek, Hrvatska²Interna klinika, Klinička bolnica Osijek, Osijek, Hrvatska³Medicinski fakultet Osijek, Osijek, Hrvatska

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

P16 – Molecular diagnostics, P16-1 (UP7-1)**Determination of the IL-6, IL-10, TNF-alfa, TGF-beta1 and IF-gamma cytokine gene polymorphism in the population of kidney transplanted patients from eastern Croatia – a single center study**Wagner J¹, Pavlinić D¹, Zibar L², Lauc G¹, Barbić J³¹DNA Laboratory, School of Medicine, Osijek, Croatia²University Department of Medicine, Osijek University Hospital, Osijek, Croatia³School of Medicine, Osijek, Croatia

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

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

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

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P16-2 (UP7-2)

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

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

P16-2 (UP7-2)

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

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

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

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P16-3

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

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

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

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P16-3

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

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

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

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P16-4

Mutacije gena PMM2 i ALG6: postoje li u hrvatskoj populaciji

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Urođeni poremećaji glikozilacije (CDG, engl. *congenital disorders of glycosylation*) uzrokovani su točkastim mutacijama gena koji kodiraju enzime uključene u N-glikozilacijski put te se očituju kao poremećaji sinteze glikanskog dijela glikoproteina ili drugih glikokonjugata. Najučestaliji urođeni poremećaji glikozilacije su CDG-Ia i CDG-Ic. Sindrom CDG-Ia čini 80% svih urođenih poremećaja glikozilacije, a uzrokovan je mutacijama u genu PMM2 koji kodira enzim fosfomanomutazu. Najučestaliji genotip za sindrom CDG-Ia je R141H/F119L. CDG-Ic uzrokuje mutacije u genu ALG6, čiji je proteinski proizvod enzim alfa1,3-glukozil-transferaza. Mutacija A333V je najučestalija u genu ALG6 i dosad je nađena u bolesnika europskog podrijetla. Nedavno smo započeli opsežan projekt određivanja učestalosti različitih mutacija/polimorfizama u genima PMM2 i ALG6 u hrvatskoj populaciji. Dosad u Hrvatskoj nije potvrđen niti jedan bolesnik obolio od sindroma CDG. Prikazani su rezultati pretraživanja mutacija u eksonu 5 i dijelova introna IVS4 i IVS5 PMM2 gena te eksona 11 ALG6 gena. Dosad su analizirani uzorci dobiveni od 350 dobrovoljnih davatelja krvi. Za utvrđivanje prisutnosti mutacija R141H i F119L rabili smo umnažanje lančanom reakcijom polimeraze i ispitivanje analizom polimorfizma konformacije jednonlačane DNA (PCR-SSCP analiza - 6% PAG-elektroforeza, 15 °C, 5 h, 3 W). Dvadesetčetiri fragmenta koji su pokazali različit elektroforetski obrazac od normalnoga dodatno su sekvencirani na genetskom analizatoru ABI-Prism 310. Mutacije R141H i F119L nisu pronađene u analiziranoj skupini. Međutim, otkriveno je šest heterozigota za IVS5+19T/T, tri homozigota za IVS5+19C/C i petnaest

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

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P16-4

Are there any PMM2 and ALG6 gene mutations and polymorphisms in the Croatian population

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Congenital disorders of glycosylation (CDGs) are a growing group of genetic disorders caused by a deficient assembly or processing of glycoproteins. The most common types of CDGs are CDG Ia and CDG Ic. Type Ia is caused by reduced N-glycosylation due to phosphomannomutase 2 deficiency as a consequence of mutations in PMM2 gene. The most frequent single-base mutations are present in exon 5, 422G>A and 357C>A, resulting in R141H and F119L substitution, respectively. CDG Ic is caused by mutations in ALG6 gene, encoding Man(9)GlcNAc(2)-PP-Dol alpha1,3-glucosyltransferase. The most significant mutation found in this gene, C998T, resulting in an A333V substitution, has been detected in patients of European origin. We have recently undertaken a comprehensive project to determine the frequency of various mutations/polymorphisms in PMM2 and ALG6 genes in Croatian population. Until now no patient with CDG was detected in Croatia. Here we present results of screening for mutations in exon 5 and parts of intervening sequences IVS4 and IVS5 of PMM2 gene and exon 11 of ALG6 gene. To date, we analyzed samples obtained of 350 unrelated Croats. Screening for R141H and F119L was performed using PCR-SSCP analysis (6% PAG-electrophoresis, 15 °C, 5 h, 3 W). Twenty-four fragments that showed aberrant electrophoretic patterns were additionally sequenced on ABIPrism 310 Genetic Analyzer. R141H and F119L mutations were not found in the group analyzed. However, we detected six homozygotes for IVS5+19T/T, three homozygotes for IVS5+19C/C and fifteen heterozygotes (IVS5+19T/C) for intragenic single nucleotide polymorphism IVS5+19T/C, while all 24

heterozigota IVS5+19T/C za polimorfizam IVS5+19T/C, dok su sve 24 osobe bile homozigoti za IVS5+22T/T. Jedan od heterozigota za IVS5+19T/C je bio također i heterozigot za deleciju od 3 bp (ATG) na poziciji -58 u intronu 4. Kako bismo mogli odrediti učestalost mutacije A333V u genu ALG6 optimirali smo postupak PCR-SSCP postupak: elektroforeza je izvedena na 6% PAG, na 4 °C u trajanju od 5 sati uz 6 W. U dosad analiziranim uzorcima nismo identificirali mutaciju A333V.

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P16-5

Polimorfizam gena za TNF-alfa +489G/A kod bolesnika s KOPB

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Tumorski nekrotizirajući faktor (TNF)-alfa je proupalni citokin čiji je gen smješten na 6. kromosomu u području III. glavnog kompleksa tkivne podudarnosti. Poznato je nekoliko polimorfizama ovoga gena, a za neke od njih utvrđena je povezanost s kroničnim upalnim bolestima. Kod bolesnika s kroničnom opstruktivskom plućnom bolesti (KOPB) nađene su povišene koncentracije TNF-alfa u sputumu i cirkulaciji, što ukazuje na važnost ovoga citokina u lokalnom i sistemskom upalnom procesu koji je djelatnik u KOPB. Opisani polimorfizmi gena za TNF-alfa povezuju se stoga sa sklonošću obolijevanju od ove bolesti. Analiziran je polimorfizam na mjestu +489G/A u prvom intronu gena za TNF-alfa za koji postoje literaturni podatci o povezanosti s pojavom KOPB u nizozemskoj populaciji. Postupkom PCR-RFLP taj je polimorfizam određen kod 31 bolesnika s KOPB te kod 51 zdravog ispitanika. Utvrđene su slijedeće frekvencije genotipa kod bolesnika oboljelih od KOPB: AG genotip 0,39, GG genotip 0,61 i AA genotip 0,00, te u zdravoj populaciji: AG genotip 0,53, GG genotip 0,45 i AA genotip 0,02. Usporedba alelnih i genotipskih frekvencija za +489G/A u bolesnika s KOPB i zdravih ispitanika ne ukazuje na postojanje značajnih razlika između ovih dviju ispitivanih populacija ($p=0,299$). Temeljem dobivenih rezultata može se zaključiti kako nije utvrđena povezanost između polimorfizma u prvom intronu gena za TNF-alfa na mjestu +489G/A i KOPB u hrvatskoj populaciji.

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individuals were homozygous for IVS5+22T/T. One of the heterozygotes for IVS5+19T/C was also a heterozygote for deletion of 3bp (ATG) at position -58 in intron 4. To be able to analyze the frequency of A333V mutation in ALG6 gene we optimized PCR-SSCP procedure: electrophoresis was performed on 6% PAG, at 4 °C for 5 hours using 6 W. In the samples analyzed until now A333V mutation has not been identified.

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P16-5

TNF-alpha +489G/A polymorphism and COPD

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The proinflammatory cytokine tumor necrosis factor (TNF)-alpha plays an important role in inflammatory processes. The gene coding for this cytokine is located on chromosome 6 in the class III region of the major histocompatibility complex. Several biallelic polymorphisms of this gene are known. Some of them have been reported to be associated with chronic inflammatory diseases. Chronic obstructive pulmonary disease (COPD) is characterized by an increased level of TNF-alpha in sputum and in circulation, indicating that this cytokine is involved in both local and systemic inflammation present in COPD. Analysis of the possible TNF-alpha gene polymorphisms in COPD could be related to a higher susceptibility to developing this disabling pathological condition. We analyzed TNF-alpha gene polymorphism at position +489G/A located in the first intron of the gene which has been reported to be associated with COPD in Dutch population. Polymorphism at position +489G/A of TNF-alpha gene was examined in 31 COPD patients and 51 healthy volunteers by PCR-RFLP procedure. We found the following genotype frequencies in the group of COPD patients: AG 0.39, GG 0.61 and AA 0.00; and in the control group: AG 0.53, GG 0.45 and AA 0.02. There were no differences in the allele and genotype frequency between the two groups ($p=0,299$). We could not find any significant link between the +489G/A polymorphism of TNF-alpha gene and COPD in Croatian population.

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P16-6

Analitička procjena sustava za umnažanje DNA u stvarnom vremenu Rotor-Gene 3000

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Rotor-Gene 3000 je otvoreni sustav koji omogućava uporabu različitih reagensa za umnažanje DNA u stvarnom vremenu. Programska potpora omogućava analizu podataka te apsolutnu i relativnu kvantifikaciju. Cilj studije bio je analitički procijeniti Rotor-Gene 3000 kvantifikacijom fuzijskog transkripta BCR-ABL u bolesnika s kroničnom mijeloidnom leukemijom u stvarnom vremenu i dobivene rezultate usporediti s rezultatima reakcije RT-PCR. Analizirano je 11 uzoraka RNA (cDNA). Devet uzoraka bilo je pozitivno, a dva su bila negativna na fuzijski transkript BCR-ABL, što je dokazano pomoću RT-PCR (kvalitativno) te citogenetski. Za kvantitativno određivanje količine fuzijskog transkripta BCR-ABL u navedenim uzorcima primijenjen je LightCycler t(9;22) Quantification kit (Roche Applied Science). Kit omogućava osjetljivu kvantifikaciju i određivanje relativne razine izraženosti transkripata BCR-ABL u odnosu na razinu izraženosti kontrolnog gena G6PDH. Količina G6PDH i BCR-ABL (u fg) u pojedinom uzorku određena je usporedbom vrijednosti Ct (ciklus u kojem intenzitet fluorescencije prelazi granicu osjetljivosti) nepoznatog uzorka sa standardnom krivuljom. Za fuzijski transkript BCR-ABL raspon količina u uzorcima je bio od 0,08 fg do 97,64 fg. Za transkript G6PDH raspon količina u uzorcima je bio od 14,76 fg do 666,25 fg. Normalizirana vrijednost izraženosti fuzijskog transkripta BCR-ABL kretala se od 0,00097 do 0,146555. Od 11 testiranih uzoraka 8 ih je bilo pozitivno (s mjerljivom količinom fuzijskog transkripta BCR-ABL), dok su 3 uzorka ustanovljena kao negativna (bez mjerljive količine fuzijskog transkripta BCR-ABL). Rezultati analitičke procjene dokazali su prisutnost mjerljive količine fuzijskog transkripta BCR-ABL u 8 od 9 pozitivnih uzoraka (osjetljivost 89,9%). Mogući uzrok snižene osjetljivosti možda je u tome što su uvjeti za reakciju PCR iz izvornog protokola prilagođeni instrumentu LightCycler koji rabi staklene kapilare, dok Rotor-Gene ima plastične tubice. S obzirom na različit toplinski kapacitet stakla i plastike optimiranje vremena pojedinih koraka PCR vjerojatno bi dovelo do povećanja osjetljivosti, što zahtijeva daljnje ispitivanje u okviru proširene analitičke validacije.

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P16-6

Analytical evaluation of the Rotor-Gene 3000 real-time instrument

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The Rotor-Gene real time DNA amplification system is an open chemistry platform, allowing for the use of any real time chemistry. The software therefore aims to provide support to the analysis of data obtained, and absolute and relative quantification. The aim of the study was to perform analytical evaluation of the RotorGene 3000 by quantification of BCR-ABL fusion transcripts in patients with chronic myeloid leukemia in real time and to compare the results obtained with the results of RT-PCR reactions. Eleven samples of RNA (cDNA) were analyzed. Nine samples were positive and two samples were negative for BCR-ABL fusion transcript, which was proven by RT-PCR (qualitative) and cytogenetic studies. The LightCycler t(9;22) Quantification Kit (Roche Applied Science) was used. The kit enables sensitive quantification and determination of the relative level of BCR-ABL fusion transcript expression normalized with G6PDH housekeeping gene expression. The amount of G6PDH and BCR-ABL (in fg) in each sample was determined by comparison of Ct values (number of the cycle when the signal was above the threshold) of an unknown sample with the standard curve. The amount in the study samples ranged from 0.08 fg to 97.64 fg for BCR-ABL fusion transcript, and from 14.76 fg to 666.25 fg for G6PDH control gene. Normalized values of BCR-ABL fusion transcripts expression were from 0.00097 to 0.146444. From eleven samples tested, eight were positive (with a measurable amount of BCR-ABL fusion transcript) and three were negative (without a measurable amount of BCR-ABL fusion transcript). Results of the analytical evaluation showed the presence of a measurable amount of BCR-ABL fusion transcript in eight of nine positive samples (sensitivity 89.9%). The possible cause of this lower sensitivity might lie in the specific conditions of reagent kit for PCR reaction, which were adapted for the LightCycler instrument. It has glass capillaries, whereas RotorGene has plastic tubes. Considering the different thermal capacity of glass and plastic, the optimization of some PCR steps would probably increase the sensitivity, which calls for additional studies as part of extended analytical validation.

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P16-7**Značenje međulaboratorijskih usporedba u području molekularne dijagnostike – vlastita iskustva**

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Zbog razvoja molekularne dijagnostike raste broj laboratorija koji rabe ovu metodologiju, što nameće potrebu za standardizacijom, a to znači i sudjelovanje u shemama vanjske procjene kvalitete. Prikazuju se rezultati sudjelovanja u međunarodnim programima kontrole kvalitete dobiveni uporabom standardiziranih metoda u molekularnoj dijagnostici. Ciljevi analitičke kvalitete obuhvaćaju standardizaciju postupaka: pohrane i rukovanja reagensima, postupka s uzorcima i izolaciju DNA/RNA, izvođenje reakcije PCR, analizu proizvoda PCR, unutarnju i vanjsku kontrolu. Zavod za kliničku kemiju KB Merkur potvrđen je prema međunarodnom standardu ISO:9001:2000, a od 2004. godine sudjeluje u dva programa vanjske procjene kvalitete: EQUAL-qual i EMQN. Od 2005. godine sudjeluje i u vanjskoj procjeni UKNEQAS u programu Molecular Diagnosis of Haematological Malignancies. Ovim kontrolama obuhvaćeni su svi aspekti analitičke kvalitete: izolacija DNA/RNA, određivanje čistoće i koncentracije, izvođenje reakcija PCR, analiza proizvoda, genotipiziranje te dokazivanje molekularnih biljega. Rezultati EQUAL-qual (2 ispitivanja) prikazani su u arbitrarnim jedinicama, prema analizi percentila, kao ukupan broj bodova i opis izvedbe. Odlični rezultati u oba navrata zabilježeni su u 7/25 (28%) laboratorija sudionika u oba ispitivanja. Naš rezultat: odlična izvedba oba puta. Rezultati EMQN (genotipiziranje gena HFE u 3 uzorka) izraženi u bodovima za genotipiziranje (2 boda/uzorku) i interpretaciju (2 boda/uzorku). Tako je 50/53 (94%) laboratorija sudionika ispravno genotipiziralo sva 3 uzorka, a 23/53 (43%) je dalo odgovarajuće tumačenje. Naši rezultati: genotipiziranje: 6/6 (100%), interpretacija: 0/6 (0%). Rezultati UKNEQAS izraženi su opisno: transkript BCR/ABL pozitivan (86% lab); preuredba gena IgH nije nađena (73% lab), što su i naši rezultati. Međulaboratorijske usporedbe u području molekularne dijagnostike imaju veliko značenje zbog različitosti metodologija rada od predanalitičkih, analitičkih do poslijeanalitičkih postupaka, u svrhu dobivanja točnih rezultata i pronalaženju uzroka mogućih pogrešaka. Tumačenje ima danas sve veće značenje, jer daje smjernice i pomaže kliničarima u donošenju relevantnih zaključaka i postavljanju dijagnoza na dobrobit bolesnika.

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P16-7**Role of inter-laboratory comparison in the field of molecular diagnosis – own experience**

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Major developments in molecular diagnosis in recent years have led to an increased number of laboratories using this methodology, which requires standardization and participation in external quality control schemes. Results of participation in international quality control schemes, obtained using standardized methods in molecular diagnosis, are presented. Analytical quality goals include standardization of procedures: reagent storage and set-up, sample treatment and preparation of DNA/RNA, PCR performance, PCR product analysis, internal and external control. Since 2004, Department of Clinical Chemistry, Merkur University Hospital, has been certified according to ISO:9001:2000, and has participated in two external quality assessments: EQUAL-qual and EMQN scheme. Since 2005, it has participated in UKNEQAS in Molecular Diagnosis of Haematological Malignancies Scheme. With this quality controls all aspects of analytical quality are included: isolation of DNA/RNA, quality and quantity determination, PCR performance, PCR product analysis, genotyping, and identifying molecular markers. Results of EQUAL-qual (two surveys) are clustered in arbitrary units, according to percentile analysis, and expressed as a final score value. Seven of 25 (28%) participating laboratories in both surveys had excellent results. Our result: excellent performance on both surveys. Results of EMQN (HFE gene genotyping in three samples) are expressed in score for genotyping (2 marks/sample) and interpretation and reporting (2 marks/sample). Fifty of 53 (94%) laboratories identified all three genotypes accurately, and 23 of 53 (43%) produced an appropriate interpretation. Our results: genotyping 6/6 (100%); interpretation 0/6 (0%). UKNEQAS results are descriptive: BCR/ABL fusion transcript positive (86% of labs); IgH gene rearrangements negative (73% of labs). Our results were the same. Accordingly, inter-laboratory comparison is of major importance in molecular diagnosis because of the wide range of different methodologies from preanalytical, analytical, and postanalytical procedures in getting accurate results and finding out the cause of possible errors. Interpretation of results is of great importance today because it can help clinicians draw relevant conclusions and make an accurate diagnosis to patient's benefit.

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P16-8

Usporedba osjetljivosti ugnježđenog PCR i kvantitativnog PCR u određivanju Bcr/Abl p210 transkripta kronične mijeloične leukemijeMarušić-Vrsalović M¹, Kušec R², Pejša V³, Romić Ž¹¹Klinički zavod za laboratorijsku dijagnostiku, KB Dubrava, Zagreb, Hrvatska²Klinički zavod za laboratorijsku dijagnostiku i Interna klinika, KB Dubrava, Zagreb, Hrvatska³Odjel za hematologiju, Interna klinika, KB Dubrava, Zagreb, Hrvatska

Za otkrivanje minimalne ostatne bolesti (MRD) kod bolesnika s kroničnom mijeloičnom leukemijom (CML) koji su postigli potpunu kliničku remisiju i potpun citogenetički odgovor rabe se metode ugnježdene PCR (nested PCR) i kvantitativne PCR (Q-PCR). Budući da je cilj terapije postizanje molekularne remisije, od iznimne je kliničke važnosti postići što bolju osjetljivost molekularnog testiranja. Cilj studije bio je usporediti razinu osjetljivosti semikvantitativne, ugnježdene PCR s kvantitativnom PCR u dokazivanju transkripta Bcr/Abl p210 u pokusu razrjeđenja stanica Filadelfija-kromosom pozitivne stanične linije K562. Za određivanje razine osjetljivosti napravljena su serijska razrjeđenja stanica stanične linije K562 (Bcr/Abl pozitivna) sa stanicama stanične linije NB4 (Bcr/Abl negativna) u rasponu 10⁻³ (1:1000) – 10⁻⁷ (1:10 mil). Izolirana RNA iz pojedinih staničnih omjera prevedena je u cDNA te su uzorci testirani na p210 transkript metodama ugnježdene PCR (Biomed1, Leukemia 1999:13) i Q-PCR-Taqman (EAC, Leukemia 2003:17). Također su istim metodama usporedno testirani uzorci koštane srži (KS) i periferne krvi (PK) dvaju bolesnika s CML na terapiji Imatinib mesilatom. U dilucijskom testu metoda ugnježdene PCR pokazala je osjetljivost dokazivanja fuzijskog transkripta Bcr/Abl p210 od 10⁻⁴, dok je metoda Q-PCR pokazala osjetljivost 10⁻⁶. Obje metode pokazale su prisutnost transkripta p210 u koštanoj srži bolesnika. Metodom Q-PCR otkriven je transkript p210 i u uzorcima periferne krvi bolesnika sa značajno manjom količinom transkripta u odnosu na uzorke koštane srži. U ovom smo pokusu pokušali odrediti razine osjetljivosti dviju molekularnih metoda za dokazivanje minimalne ostatne bolesti kronične mijeloične leukemije. Za to smo rabili model rastuće dilucije onkogen-pozitivnih i negativnih stanica. Iako se često izvještava kako su ugnježdene metode za oko 1 log osjetljivije od metoda Q-PCR, u našem pokusu je Q-PCR prema protokolu Europe Against Cancer Program postigao višu osjetljivost dokazivanja tumorske stanice. Ovaj ćemo nalaz dodatno komentirati, a zanimljivo je da je i u kliničkim uzorcima bolesnika u remisiji bolesti Q-PCR bila u prednosti za dokazivanje MRD.

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P16-8

Comparison of sensitivity of nested PCR and quantitative PCR in Bcr/Abl p210 transcript detection in chronic myelogenous leukemiaMarušić-Vrsalović M¹, Kušec R², Pejša V³, Romić Ž¹¹Clinical Institute of Laboratory Diagnosis, Dubrava University Hospital, Zagreb, Croatia²Clinical Institute of Laboratory Diagnosis and University Department of Medicine, Dubrava University Hospital, Zagreb, Croatia³Department of Hematology, University Department of Medicine, Dubrava University Hospital, Zagreb, Croatia

Nested PCR and quantitative real-time PCR (Q-PCR) can be used to detect minimal residual disease (MRD) in chronic myelogenous leukemia (CML) patients who have achieved complete clinical remission and complete cytogenetic response. Achieving molecular remission is the goal of therapy, so it is of critical importance for a clinical utility to attain high sensitivity of molecular testing. The aim of the study was to compare the level of sensitivity of nested PCR and Q-PCR in the detection of Bcr/Abl p210 transcripts in Bcr/Abl-positive cell dilution model. Serial dilutions of K562 cell line (Bcr/Abl-positive) in NB4 cell line (Bcr/Abl-negative) were made (range of positive cell dilution: 10⁻³ – 10⁻⁷) to determine the level of sensitivity. Isolated RNA samples were transcribed into cDNA and tested for p210 transcript by nested PCR (Biomed1, Leukemia 1999:13) and Q-PCR-Taqman (EAC, Leukemia 2003:17). Bone marrow and peripheral blood samples of two CML patients on Imatinib mesylate therapy were also tested by both methods. In the cell dilution test, nested PCR showed a sensitivity for detecting Bcr/Abl positive cell of 10⁻⁴, and Q-PCR showed a sensitivity of 10⁻⁶. Both methods detected p210 transcripts in bone marrow samples of CML patients. However, Q-PCR also detected transcripts in peripheral blood samples, with a significantly lower level of transcription in comparison to bone marrow samples. Although nested-PCR has been frequently reported as being by approximately 1 log more sensitive than Q-PCR, in our marker-positive cell line K562 dilution experiment we documented higher sensitivity for standardized Europe Against Cancer Program Q-PCR method.

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P16-9

Mutacija gena JAK2 u kroničnim mijeloproliferativnim bolestima

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Nedavno otkrivena mutacija gena JAK2 za tirozin kinazu staničnih receptora za citokine i faktore rasta čini se patogenom za skupinu kroničnih mijeloproliferativnih bolesti. To je prva značajna genska mutacija nakon otkrića Filadelfija kromosoma i preuredbe Bcr/Abl onkogena. Posljedica točkaste mutacije G/T ili V617F konstitucijski aktivira ovu kinazu, što ima za posljedicu staničnu proliferaciju. Ovdje prikazujemo učestalost JAK2V617F mutacije u velikoj skupni bolesnika s kroničnim mijeloproliferativnim bolestima: policitemija vera (PV), esencijalna trombocitemija (ET), primarna mijelofibroza (IMF) te njezinu diferencijalno dijagnostičku ulogu u obradi poliglobulija-eritrocitoza. Mutacija smo određivali alel specifičnom PCR prema Baxter *et al.* (Lancet 2005;365), koja je modificirana i za određivanje mutacije na molekulama RNA. DNA i RNA izolirane su iz nukleiranih stanica krvi. Osjetljivost metode za dokazivanje mutiranog alela je oko 1%. U skupni od 85 bolesnika s PV (prema kriterijima WHO) 6 ih nije imalo mutaciju (93% pozitivnih). Od 22 bolesnika s ET 10 ih je bilo negativnih (55% pozitivnih). Od 15 bolesnika s IMF 54% ih je bilo pozitivnih. Od tri CMML bolesnika jedan je imao mutaciju, dok ju u skupini od 5 MDS-RA bolesnika nismo našli. Zanimljivo je da je od 10 bolesnika koji su obrađivani zbog nalaza eritrocitoze samo jedan bio pozitivan, dok je negativan test za druge značio usmjerenje dijagnostike ka traženju razloga sekundarne poliglobulije. Molekularna dijagnostika JAK2V617F danas je postavljena na sam početak algoritma obrade poliglobulija i drugih kroničnih mijeloproliferacija.

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P16-9

JAK2 gene mutation in chronic myeloproliferative disorders

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Mutation in the JAK2 tyrosine kinase gene has recently been shown as the major molecular genetic defect in non-Philadelphia chronic myeloproliferative disorders. G/T point mutation in the autoinhibitory region of the gene constitutively activates its kinase activity, which leads to cell proliferation. We sought to investigate the frequency of this mutation in a large cohort of chronic myeloproliferative patients with particular interest in its role in guiding differential diagnosis of polyglobulia – erythrocytosis patients. We used allele specific PCR test as described by Baxter *et al.* (Lancet 2005;365), which we also modified for amplification of mutated RNA molecules of JAK2 gene. Of 85 PV patients 6 were negative for the mutation (7% (PV diagnosis according to WHO criteria). Of 22 ET patients, 45% were negative and so were 7/15 (46.6%) IMF patients. We also found one of three CMML patients positive for mutation, but did not detect it in any of the five MDS-RA patients. In the work-up of erythrocytosis patients, the test was positive in only one of 10 patients, which fitted well their later diagnosis of secondary polycythemia. Today, V617F JAK2 mutation search has been considered the first test in the differential diagnosis of patients with increased hematocrit and red blood cell count.

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P16-10**Pankreatitis i genske varijante lipoprotein lipaze u ispitanika hrvatskoga podrijetla**Pašalić D¹, Bilić-Zulle L², Ferenčak G¹, Stavljenić-Rukavina A¹¹Zavod za kemiju i biokemiju, Medicinski fakultet, Zagreb, Hrvatska²Zavod za laboratorijsku dijagnostiku, Klinički bolnički centar Rijeka i Katedra za medicinsku informatiku, Medicinski fakultet, Rijeka, Hrvatska

Lipoprotein lipaza (LPL) je ključni enzim metabolizma triacilglicerolom bogatih lipoproteina. Polimorfizmi gena za LPL mogu utjecati na patogenezu nekih bolesti kao što je pankreatitis. Cilj studije je bio pretražiti mutacije i polimorfizme u genu za LPL te ispitati utjecaj različitih polimorfizama na razvoj pankreatitisa. U studiju je bilo uključeno 77 odraslih ispitanika s pankreatitisom različite etiologije i 98 kontrolnih ispitanika. Petogodišnji dječak s hilomikronemijom i njegovi roditelji prikazani su kao slučaj. Pretraživanje dijelova gena za LPL koji se prepisuju načinjeno je pomoću PCR-SSCP. Polimorfizmi LPL-gena D9N, N291S, S447X i rijetka mutacija W86R potvrđeni su analizom nukleotidnog slijeda i metodom RFLP. Polimorfizmi dijelova gena za LPL koji se ne prepisuju analizirani su metodom RFLP. U ispitanika s pankreatitisom učestalost X alela genske varijante LPL-S447X bila je značajno manja u odnosu na kontrolnu skupinu ($p < 0,05$). Omjer rizika za povezanost istog polimorfizma s pankreatitisom iznosio je 0,39 (95% CI 0,176-0,866; $p < 0,05$). U skupini s pankreatitisom koncentracije HDL-kolesterola su bile značajno više u nositelja 447X alela ($p < 0,05$). Koncentracije triacilglicerola su bile znatno više u nositelja LPL-Pvu II P+ alela ($p = 0,05$) u ispitanika s pankreatitisom. Petogodišnji dječak i njegovi roditelji su nositelji rijetke mutacije W86R. Polimorfizam LPL-S447X bi mogao imati zaštitnu ulogu od pankreatitisa. Kod osoba koje boluju od pankreatitisa polimorfizmi LPL-Pvu II i Hind III pokazuju značajan utjecaj na lipidni profil. Genske varijante LPL, a osobito rijetka mutacija W86R mogu biti od velike važnosti u razumijevanju genski uvjetovane sklonosti za obolijevanje od pankreatitisa različite etiologije.

E-mail: daria.pasalic@mef.hr**P16-10****Pancreatitis and lipoprotein lipase gene variants in subjects of Croatian descent**Pašalić D¹, Bilić-Zulle L², Ferenčak G¹, Stavljenić-Rukavina A¹¹Department of Chemistry and Biochemistry, School of Medicine, Zagreb, Croatia²Department of Laboratory Diagnosis, Rijeka University Hospital Center and Department of Medical Informatics, School of Medicine, Rijeka, Croatia

Lipoprotein lipase (LPL) is a key enzyme involved in lipoprotein triglyceride-rich lipoproteins. LPL gene polymorphisms may contribute to the pathogenesis of some diseases such as pancreatitis. The aim of the study was to screen for the mutations and polymorphisms of the LPL gene as well as to investigate the influence of different polymorphisms on the development of pancreatitis. The study included 77 adults with pancreatitis of different etiology and 98 control subjects. A 5-year-old male with chylomicronemia and his parents were also included in the study as a case report. The screening of the coding region was performed by PCR-SSCP. LPL gene polymorphisms D9N, N291S, S447X, and the rare W86R mutation were confirmed by DNA sequence analysis and RFLP. Intronic polymorphisms Hind III and Pvu II were analyzed by RFLP. The X allele of the LPL S447X genetic variant was less frequent in the individuals with pancreatitis ($p < 0.05$). Odds ratio for the association between LPL-S447X and pancreatitis was 0.39 (95% CI 0.176-0.866; $p < 0.05$). In the patient group, HDL-C levels were significantly higher in carriers of 447-X allele ($p < 0.05$). TG levels were significantly higher in carriers of LPL-Pvu II P+ allele ($p = 0.05$) in the patient group. The 5-year-old male and his parents were carriers of the W86R mutation. Accordingly, LPL-S447X polymorphism may have a protective role against pancreatitis. LPL-Pvu II and Hind III polymorphisms can contribute to the lipid profile differences in individuals with pancreatitis. LPL genetic variations, especially the rare W86R mutation, may be of considerable importance for the understanding of the genetic predisposition to pancreatitis of different etiology.

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P16-11**Učestalost mutacije V617F u genu za JAK2 u mijeloproliferativnim bolestima**

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Mijeloproliferativne bolesti obuhvaćaju skupinu poremećaja obilježenih sličnim kliničkim slikama te klonalnom proliferacijom jedne ili više staničnih loza, što je posljedica transformacije na razini pluripotentne matične stanice. Toj skupini pripadaju i policitemija vera (PV), esencijalna trombocitemija (ET) i idiopatska mijelofibroza (IM), za koje je tek odnedavno poznata molekularna podloga bolesti. Točkasta mutacija gvanina u timin u genu za Janus kinazu 2 (JAK2), koja kodira zamjenu valina fenilalaninom na poziciji 617 (V617F), javlja se u značajnom postotku bolesnika s dijagnozom PV, ET i IM. Mutacija V617F smještena je u domeni JH2 gena za JAK2, a uključena je u autoinhibiciju vlastite tirozin kinazne aktivnosti. Mutirani protein se neprekidno iznova fosforilira aktivirajući signalne putove, a stanice postaju neovisne ili preosjetljive na citokine te pojačano proliferiraju. Cilj ovoga istraživanja bio je odrediti učestalost mutacije V617F u genu za JAK2 kod bolesnika s dijagnozom PV, ET ili IM. Obradeno je ukupno 47 bolesnika, od kojih 17 s uputnom dijagnozom PV (skupina PV), 20 s dijagnozom ET (skupina ET) te 10 s uputnom dijagnozom IM (skupina IM). Iz stanica periferne krvi ili koštane srži izolirana je DNA. Za dokazivanje točkaste mutacije V617F u genu za JAK2 primijenjena je metoda alel-specifične PCR (prema Baxter i sur., Lancet 2005.). Svim bolesnicima napravljena je i analiza PCR na fuzijski gen BCR/ABL. U skupini PV dokazana je učestalost mutacije V617F u genu za JAK2 od 59%, u skupini ET 70%, a u skupini IM 30%. Prisutnost fuzijskog gena BCR/ABL nije dokazana ni u jednom analiziranom uzorku. Nadalje, skupine PV i ET podijeljene su svaka na dvije podskupine s obzirom na prisutnost mutacije V617F u genu za JAK2 te su u svakoj podskupini analizirani parametri krvne slike. Utvrđeno je da ne postoji statistički značajna razlika u prosječnom povišenju broja eritrocita i hematokrita u skupini PV s obzirom na prisutnost mutacije. U skupini ET nije bilo statistički značajne razlike u povišenju broja trombocita kod bolesnika s dokazanom mutacijom V617F, ali je dokazana statistički značajna razlika u povišenju broja leukocita ($p=0,007$) i hematokrita ($p<0,005$). U zaključku, učestalost točkaste mutacije V617F u genu za JAK2 značajna je u PV i ET, a njeno dokazivanje metodom alel-specifične PCR pomaže pri dijagnozi bolesti i praćenju terapije na molekularnoj razini.

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P16-11**The prevalence of JAK2 V617F mutation in myeloproliferative diseases**

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Myeloproliferative disorders include a group of diseases with clinical and biological similarities sharing the major common feature of being clonal hematopoietic disorders arising from the transformation of pluripotent hematopoietic progenitor cell. This group, among others, includes polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM). Recently discovered guanine-to-thymine point mutation encoding valine-to-phenylalanine substitution at position 617 (V617F) in the autoinhibitory JH2 domain of JAK2 gene is present in most patients with PV, ET and IM. The mutant protein is constitutively phosphorylated, activating its downstream signaling pathways in the absence of any cytokine. Cells expressing JAK2 V617F become independent and hypersensitive to cytokines causing abnormal cell proliferation. The aim of this study was to determine the prevalence of JAK2 V617F mutation in patients with PV, ET and IM. The study included 47 patients: 17 patients with PV (PV group), 20 patients with ET (ET group) and 10 patients with IM (IM group). DNA was isolated from bone marrow cells or peripheral blood cells. The detection of JAK2 V617F was performed by allele specific PCR (published in Baxter *et al.*, Lancet 2005). All patients were also analyzed for BCR/ABL fusion gene. The presence of JAK2 V617F mutation was detected in 59% of patients in PV group, 70% in ET group, and 30% in IM group. All samples analyzed were negative for BCR/ABL fusion gene. Additionally, PV and ET groups were each divided in two subgroups. The presence or absence of mutation was correlated to the erythrocyte count and hematocrit value in PV subgroups, and to platelet and leukocyte count and hematocrit value in ET subgroups. No statistically significant difference was found between PV subgroups in the mean increment in erythrocyte count and hematocrit value. In ET subgroups there was no statistically significant difference in platelet count while a statistically significant difference was found in the mean increment in leukocyte count ($p=0.007$) and hematocrit value ($p<0.005$) in patients with JAK2 V617F mutation. The prevalence of JAK2 V617F is significant in patients with PV, ET and IM. Detection of this point mutation can be easily performed by allele specific PCR at diagnosis and used for therapy monitoring at molecular level.

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P16-12**Probiranje za mutacije u genu GALT kod zdrave hrvatske populacije**

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Gen GALT kodira enzim galaktoza-1-fosfat uridil-transferazu (GALT) koji katalizira pretvorbu galaktoze-1-fosfat u uridil difosfat (UDP)-galaktozu. Do danas su otkrivene brojne mutacije u genu GALT koje na različite načine utječu na aktivnost GALT i mogu uzrokovati galaktozemiju. Kod ovoga poremećaja prisutna je značajna heterogenost alela kod različitih populacija i etničkih skupina. Dvije najučestalije mutacije kod pripadnika bijele rase su Q188R i K285N. Osobe koje imaju ove mutacije na oba alela oboljevaju od tzv. klasične galaktozemije obilježene potpunim gubitkom enzimske aktivnosti i različitim teškim simptomima. Blaži oblik galaktozemije, poznat kao varijanta Duarte, često je povezan s mutacijom N314D u genu GALT, ali i s drugim genetičkim promjenama poput varijacije u slijedu introna V (IVS5-24G<A) (Duarte-2) ili tihe mutacije L218L (varijanta Duarte-1 ili Los Angeles). Homozigoti s Duarte-2 imaju oko 50%, a heterozigoti oko 75% ostatne aktivnosti GALT, dok Duarte-1 obilježava povećana aktivnost GALT. Iako heterozigoti za klasičnu galaktozemiju nemaju izraženih simptoma pri rođenju, a Duarte galaktozemija se čini dosta bezazlenom, neka su istraživanja ukazala na to da kod osoba s ovim poremećajima postoji povećani rizik za razvoj određenih bolesti tijekom života. Cilj je ovoga istraživanja bio utvrditi prisutnost i učestalost pojedinih mutacija u genu GALT (Q188R, K285N, IVS5-24G<A i N314D) kod zdrave hrvatske populacije. DNA smo izolirali iz pune krvi prikupljene od 166 zdravih dobrovoljaca, umnožili smo određene fragmente DNA lančanom polimeraznom reakcijom, digestirali smo ih pomoću specifičnih restrikcijskih endonukleaza (PCR-RFLP) i razdvajali smo dobivene proizvode na 4%-tnom agaroznom gelu. Učestalost alela s mutacijom Q188R, K285N, IVS5-24G<A ili N314D bila je 0%, 0%, 3,6% i 6,6%. Među osobama kod kojih smo utvrdili prisutnost mutacija IVS5-24G<A i/ili N314D bilo je 30,3% varijanta Duarte-2. Dobiveni rezultati ukazuju na to da je mutacija N314D najučestalija kod zdrave hrvatske populacije. Naši rezultati dobro koreliraju s onima objavljenim za zdravu slovensku populaciju.

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P16-12**Screening for GALT gene mutations in a healthy Croatian population**

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The GALT gene codes for the galactose-1-phosphate uridyl transferase (GALT) enzyme that catalyzes the conversion of galactose-1-phosphate to uridyl diphosphate (UDP)-galactose. Numerous mutations in the GALT gene have been found to impair GALT activity to a varying extent, causing galactosemia. This disorder exhibits considerable allelic heterogeneity in different populations and ethnic groups. Two most common mutations among Caucasians are Q188R and K285N. Individuals homoallelic for these mutations have a severe phenotype, named classic galactosemia, with complete loss of enzyme activity. A milder form of galactosemia, known as Duarte variant, is caused by the N314D mutation in the GALT gene. Along with the N314D mutation, Duarte variants of galactosemia depend on other genetic changes such as intronic sequence variation G1391A in intron V (IVS5-24G<A) (Duarte-2) or silent mutation L218L (Duarte-1 or Los Angeles variant). In Duarte-2 variant, homozygotes have approximately 50% and heterozygotes 75% of residual GALT activity, whereas Duarte-1 is characterized by increased GALT activity. Although heterozygotes for classic galactosemia are asymptomatic at birth and Duarte galactosemia appears to be quite benign, there are some indications that these disorders can increase the risk of developing certain diseases later in life. The aim of our study was to analyze a healthy Croatian population for the frequencies of Q188R, K285N, IVS5-24G<A and N314D mutations within GALT gene. DNA samples from 166 healthy individuals were analyzed for all four mutations by polymerase chain reaction and digestion with restriction enzymes (PCR-RFLP). Allele frequencies for Q188R, K285N, IVS5-24G<A and N314D were found to be 0%, 0%, 3.6% and 6.6%, respectively. There were 30.3% of Duarte-2 variant among the persons carrying IVS5-24G<A and/or N314D mutations. Study results showed N314 to be the most frequent mutation in the healthy Croatian population. Our results correlate well with those reported for a healthy Slovenian population.

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P16-13**Molekularna dijagnostika mitohondrijskih bolesti – MELAS**Sinković M¹, Zrinski Topić R¹, Barišić N², Barić I², Sertić J¹,¹Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb, Zagreb, Hrvatska²Klinika za pedijatriju, KBC Zagreb, Zagreb, Hrvatska

MELAS, mitohondrijska encefalomiopatija, laktoacidoza s epizodama nalik apopleksiji je sindrom uzrokovan mutacijama u mitohondrijskom genu MTTL1 koji kodira tRNA za leucin (tRNA^{Leu}). Opisano je više točkastih mutacija ovoga gena od kojih je A3243G utvrđena u 80%, a T3271C u 10% bolesnika. Molekularne promjene u genu za MTTL1 imaju za posljedicu smanjenu sintezu mitohondrijskih enzima te smanjenu proizvodnju energije potrebne za normalno odvijanje staničnih procesa. Kako svaka stanica ima nekoliko mitohondrija u kojima se nalazi veći broj kopija mitohondrijske DNA (mtDNA), broj mutiranih kopija unutar stanice ili organa može biti varijabilan (heteroplazija), o čemu ovisi i klinička slika. Simptomi se javljaju najčešće već u dječjoj dobi u organima koji najviše troše energiju: mozak, skeletni i srčani mišić. Cilj je bio postaviti metodu lančane reakcije polimeraze (PCR) za određivanje mutacije T3271C te ispitati prisutnost mutacija A3243G i T3271C u bolesnika sa sumnjom na MELAS. Ispitivanjem je obuhvaćeno 41 dijete sa simptomima koji upućuju na poremećeno stvaranje stanične energije. mtDNA je izolirana iz leukocita periferne krvi. Pretraživanje na mutacije A3243G i T3271C je provedeno metodom PCR uz cijepanje umnoženih ulomaka s restriktivnim enzimima (PCR/RFLP). Čimbenici reakcije PCR/RFLP za mutaciju T3271C su optimirani i provjereni uz pozitivni kontrolni uzorak. Mutacije A3243G i T3271C nisu utvrđene u ispitivanih bolesnika. Zaključuje se kako određivanje molekularnih promjena u mtDNA može pomoći u diferencijalnoj dijagnostici mitohondrijskih bolesti, a zbog heteroplazije bi mtDNA trebalo analizirati i u uzorcima zahvaćenih tkiva.

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P16-13**Molecular diagnosis of mitochondrial diseases – MELAS**Sinković M¹, Zrinski Topić R¹, Barišić N², Barić I², Sertić J¹,¹Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia²University Department of Pediatrics, Zagreb University Hospital Center, Zagreb, Croatia

MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, is a syndrome caused by mutations in mitochondrial MTTL1 gene coding tRNA for leucine (tRNA^{Leu}). A number of point mutations of this gene have been described, of which A3243G and T3271C were established in 80% and 10% of patients, respectively. Molecular changes in MTTL1 gene lead to reduction in both mitochondrial enzyme synthesis and production of energy required for normal development of cellular processes. As each cell has several mitochondria with many copies of mitochondrial DNA (mtDNA), the number of mutated copies within a cell or an organ may vary (heteroplasia) and thus affect the clinical picture. Most frequently, the disease symptoms occur already during childhood in organs that are the highest energy consumers: the brain, skeletal and cardiac muscle. The aim was to establish a method of polymerase chain reaction (PCR) to determine T3271C mutation and to examine the presence of A3243G and T3271C mutations in patients with suspected MELAS. The study included 41 children with symptoms indicating impaired cellular energy production. mtDNA was isolated from peripheral blood leukocytes. Screening for A3243G and T3271C mutations was conducted by the PCR-restriction fragment length polymorphism methods (PCR-RFLP). Factors of PCR/RFLP reaction for T3271C mutation were optimized and verified against a positive control sample. A3243G and T3271C mutations were not detected in the patients observed. Determination of molecular changes in mtDNA may aid in the differential diagnosis of mitochondrial diseases. Due to heteroplasia, mtDNA analysis should also include affected tissue samples.

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P16-14

Neinvazivno određivanje spola fetusa iz majčine plazme – usporedba metoda multipleks PCR i PCR u stvarnom vremenu

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Pouzdanost utvrđivanje spola fetusa tijekom rane trudnoće zahtijeva uzorkovanje fetalnih stanica invazivnim metodama (amniocenteza, biopsija korionskih resica itd.), koje sa sobom nose i određeni rizik kako za majku tako i za fetus. U obiteljima s X-vezanim nasljednim bolestima osobito je važno rano utvrditi spol fetusa. Zbog svega navedenog nastoje se razviti neinvazivne metode prenatalne dijagnostike. Svrha ovoga rada bila je usporediti pouzdanost metoda multipleks PCR i PCR u stvarnom vremenu za rano dokazivanje fetalnog Y kromosoma (*cell free fetal DNA*) u majčinoj plazmi.

Cell free DNA je izolirana iz 80 uzoraka majčine plazme uporabom QIAamp® DNA Blood Midi Kit, a zatim je: A) umnožena pomoću AmpFI STR® Identifier™ kita (15 STR lokusa I amelogenin) i nakon toga analizirana kapilarnom elektroforezom na genetičkom analizatoru ABI PRISM 310; B) amplificirana i analizirana uporabom Quantifiler™ Y Human Male DNA Quantification kita (SRY gen) na uređaju ABI PRISM 7000 Sequence Detection System. Spol fetusa je potvrđen citogenetičkom analizom. Amplifikacija paternalnih STR alela trebala je poslužiti kao pozitivna kontrola prisutnosti fetalne DNA u majčinoj plazmi, osobito u slučajevima ženskih fetusa. No, u većini slučajeva amelogenin je bio jedini umnoženi fetalni lokus. Od ukupno 41 muškog fetusa prisutnost Y kromosoma u majčinoj plazmi je dokazana u 37 (90%) uporabom metode multipleks PCR, dok je primjenom PCR u stvarnom vremenu dokazano u 39 (95%) uzoraka. Nije bilo lažno pozitivnih uzoraka. Rezultati su u nekim slučajevima bili neuvjerljivi, jer je postojala mogućnost da je uzrok izostanka amelogeninskog/SRY gena bila neodgovarajuća količina fetalne DNA u uzorku ili u izolatu DNA. Daljnja ispitivanja na ovu temu trebala bi se usredotočiti na pronalaženje novih biljega koji bi bili sigurniji pokazatelji prisutnosti fetalne DNA u majčinoj plazmi.

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P16-14

Noninvasive determination of fetal sex from maternal plasma – comparison of multiplex PCR and real-time PCR

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Reliable determination of fetal gender in early pregnancy generally requires sampling of fetal cells by invasive procedures that are associated with low but definite risks for both the fetus and the mother. In families with X-linked recessive genetic disorders it is important to early determine the sex of the fetus, thus the development of safer methods is needed. The purpose of this study was to compare the reliability of multiplex PCR and real-time PCR methods for the determination of the presence of fetal Y-chromosome DNA in maternal plasma. Cell-free DNA was extracted from 80 samples of maternal plasma using QIAamp® DNA Blood Midi Kit. The extracted cell free fetal DNA was: (a) amplified using AmpFI STR® Identifier™ kit (15 STR loci and sex-specific amelogenin) and subsequently analyzed on a capillary electrophoresis system ABI PRISM 310 Genetic Analyzer; and (b) amplified and analyzed using Quantifiler™ Y Human Male DNA Quantification kit (SRY gene) on ABI PRISM 7000 Sequence Detection System. The sex of fetuses was confirmed by cytogenetic analysis. Amplification of paternal STR alleles was expected to serve as a positive control for the presence of fetal DNA in maternal plasma in case of female fetuses, but unfortunately in a vast majority of samples amelogenin was the only fetal locus that was successfully amplified. Out of 41 male fetuses, the presence of Y-chromosome DNA in maternal plasma was successfully determined in 37 (90%) using multiplex PCR method and in 39 of 41 (95%) using real time PCR method. There were no falsely positive detected Y-chromosomes in female fetuses, but the results were partly inconclusive because we were not able to eliminate the possibility that the lack of male amelogenin/SRY gene was simply due to inadequate amount of fetal DNA. Further work on this method should consider the use of different markers that will be more confirmative for the presence of fetal DNA in maternal plasma.

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P16-15**Molekularna dijagnostika nasljednih ataksija**Zrinski-Topić R¹, Mitrović Z², Barišić N³, Sertić J¹¹Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb, Zagreb, Hrvatska²Klinika za neurologiju, KBC Zagreb, Zagreb, Hrvatska³Klinika za pedijatriju, KBC Zagreb, Zagreb, Hrvatska

Nasljedne ataksije su heterogena skupina neurodegenerativnih bolesti kod kojih prevladava poremećaj koordinacije pokreta. Atrofija malog mozga i/ili njegovih veza s drugim strukturama živčanog sustava razvija se kao posljedica molekularnih promjena u više od 40 različitih gena. Osnovni genski poremećaj u većine ovih gena je povećanje broja ponavljajućih slijedova nukleotida (CAG, CTG, CGG, GAA ili ATTCT). Produljenje slijeda u kodirajućoj regiji gena rezultira sintezom nefunkcionalnog proteina, dok produljeni nukleotidni slijed u nekodirajućoj domeni gena uzrokuje promjenu regulacije transkripcije gena ili pogrešno izrezivanje molekula mRNA, što u konačnici završava aktivacijom apoptoze živčanih stanica. Prema načinu nasljeđivanja razlikujemo autosomno dominantne (spinocerebelarne ataksije, SCA), autosomno recesivne, spolno (X-vezane) te ataksije vezane uz mitohondrijske bolesti. Cilj studije bio je proširiti diferencijalnu dijagnostiku ataksija molekularnom analizom te ispitati učestalost pojedinih oblika nasljednih ataksija u našoj populaciji bolesnika. Ispitana su 73 bolesnika u kojih su isključeni stečeni oblici ataksije. Određivanje broja tripleta CAG u genu za ataksin-1 (SCA 1), ataksin-2 (SCA 2) i ataksin-3 (SCA 3) te broja tripleta GAA u genu za frataksin (Friedreichova ataksija, FRDA) provedeno je metodom lančane reakcije polimerazom (PCR). Proizvodi PCR su analizirani kvalitativno na 2%-tnoj agarozu i kvantitativno na 6%-tnom poliakrilamidnom gelu. Od 13 bolesnika sa sumnjom na autosomno recesivni oblik ataksije FRDA je potvrđena u 3 (23%) bolesnika. U preostalim bolesnika otkrivene su dvije autosomno dominantne ataksije, podtipa SCA2 i SCA3. Nasljedne ataksije su potvrđene u samo 5 (7%) bolesnika. Uvedene metode za FRDA, SCA1, SCA2 i SCA3 su tek prva faza molekularne analize nasljednih ataksija kojima se potvrđuju najučestaliji oblici. U idućem razdoblju predviđa se proširenje dijagnostičkog slijeda.

E-mail: renata.zrinski-topic@zg.t-com.hr**P16-15****Molecular diagnosis of hereditary ataxias**Zrinski-Topić R¹, Mitrović Z², Barišić N³, Sertić J¹¹Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia²University Department of Neurology, Zagreb University Hospital Center, Zagreb, Croatia³University Department of Pediatrics, Zagreb University Hospital Center, Zagreb, Croatia

Hereditary ataxias are a heterogeneous group of neurodegenerative diseases characterized by movement coordination disorder. Atrophy of the cerebellum and/or its connections with other nervous system structures develops as a consequence of molecular changes in more than 40 different genes. The basic gene disorder in most of these genes is an increased number of nucleotide repeats (CAG, CTG, CGG, GAA or ATTCT). Expansion of repeats in gene encoding region results in the synthesis of nonfunctional protein, while expanded nucleotide repeat in non-coding gene domain causes a change in gene transcription regulation or defective mRNA splicing, eventually leading to activated apoptosis of neuronal cells. Ataxias may be distinguished according to the manner of inheritance: autosomal dominant (spinocerebellar ataxias, SCA), autosomal recessive, X-linked, and ataxias related to mitochondrial diseases. The aim was to expand the differential diagnosis of ataxias by molecular analysis and to investigate the frequency of individual types of hereditary ataxias in our patient population. The study included 73 patients with the acquired types of ataxia excluded. Determination of the CAG triplet number in the frataxin gene (Friedreich's ataxia, FRDA) was performed by the method of polymerase chain reaction (PCR). PCR products were analyzed qualitatively on 2% agarose and quantitatively on 6% polyacrylamide gel. Of 13 patients suspected of autosomal recessive type of ataxia, FRDA was confirmed in 3 (23%) patients. Two autosomal dominant ataxias were detected in the remaining patients, i.e. subtypes SCA2 and SCA3. Hereditary ataxias were confirmed in only five (7%) patients. The methods introduced for FRDA, SCA1, SCA2 and SCA3 are only the first stage of the molecular analysis of hereditary ataxias used to confirm the most frequent types. Expansion of diagnostic range is anticipated in the forthcoming period.

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P16-16

Asocijacijska studija porasta tjelesne težine i terapijskog odgovora na olanzapin s polimorfizmom gena SERT u shizofrenih bolesnica

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U usporedbi s tipičnim antipsihoticima druga generacija antipsihotika (DGA) ima prihvatljiviji profil nuspojava, kao i moguću širu učinkovitost glede simptoma. Porast tjelesne težine kao jedan od najčešćih nepovoljnih učinaka liječenja atipičnim antipsihoticima, poglavito klopazinom i olanzapinom, povezan je, međutim, s brojnim medicinskim problemima, tj. dijabetesom, kardiovaskularnim i malignim bolestima, nepridržavanjem terapije, čime značajno ometa učinkovitost liječenja shizofrenije. Veći afinitet DGA prema serotoninom sustavu u odnosu na uobičajene antipsihotike dovodi do zaključka da bi taj sustav mogao biti uključen kako u terapijskim učincima tako i u opsegu nuspojava. Transporter serotonina (SERT) regulira čitav serotoninergični sustav kroz prilagodbu izvanstaničnih koncentracija serotonina. Polimorfne alelske varijacije SERT čine se značajnima za izražaj i funkciju transportera serotonina. Karakterizirane su dvije polimorfne regije gena SERT, tj. insercijski/delecijski polimorfizam s 44 para baza u promotorskoj regiji (SERTPR) i promjenjivi broj motiva ponavljanja u drugom intronu (SERT-in2). Cilj studije bio je ispitati odnose između genetičkih varijanata promotora L/S (SERTPR) i introna 2 l/s polimorfizama gena SERT i porasta tjelesne težine potaknutog olanzapinom te odgovora na terapiju u 94 shizofrenične bolesnice liječene olanzapinom do 3 mjeseca. Genotipizacija SERT provedena je metodom PCR. Indeks tjelesne mase (BMI) je izračunat za svaku bolesnicu prije liječenja olanzapinom te tri mjeseca kasnije. Kako bi se procijenilo poboljšanje kliničkih psihotičnih simptoma i terapijski odgovor na antipsihotik, sve su bolesnice ocijenjene prema ljestvici za pozitivne i negativne sindrome (engl. *positive and negative syndrome scale*, PANSS). Općenito je prisutnost alelske varijante S, SERTPR i genotipa SS povezana sa značajno višim porastom težine u bolesnika koji nisu bili pretili kod prijma ($p=0,04$). Prisutnost varijante L SERTPR je povezana sa značajno boljim odgovorom na terapiju mjerenim prema ukupnoj kao i općoj podljestvici PANSS ($p<0,05$), dok je prisutnost varijante I, SERTin2 uzrokovala bolji tera-

P16-16

Association study of olanzapine-induced weight gain and therapeutic response with SERT gene polymorphisms in female schizophrenic patients

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Compared to typical antipsychotics, second generation antipsychotics (SGA) have more acceptable side effect profiles, and possibly broader symptom efficacy. However, weight gain as one of the most common adverse effects of treatment with atypical antipsychotics, especially clozapine and olanzapine, is associated with numerous medical problems, i.e. diabetes, cardiovascular disease, malignant disease, and noncompliance, thus significantly obstructing the therapeutic efficacy for schizophrenia. The higher SGA affinity for serotonin system as compared to typical antipsychotics leads to a conclusion that serotonin system could be implicated in both therapeutic effects and side effect spectrum. Serotonin transporter (SERT) regulates the entire serotoninergic system through modulation of extracellular serotonin concentrations. SERT polymorphic allelic variations seem to be significant for the SERT expression and function. Two polymorphic regions of SERT gene: a 44-base-pair (bp) insertion/deletion polymorphism in the promoter region (SERTPR), and a variable number of tandem repeats in second intron (SERT-in2) have been characterized. The aim of this study was to investigate the relationships between L/S promoter (SERTPR) and l/s intron2 (SERTin2) genetic variants of SERT gene polymorphisms with olanzapine-induced weight gain and treatment response in 94 female schizophrenic patients treated with olanzapine for up to 3 months. SERT genotyping was performed by PCR method. Body mass index (BMI) was calculated in each patient prior to olanzapine administration and three months afterwards. To assess and evaluate improvement of clinical psychotic symptoms and therapeutic response to the antipsychotic, all patients were rated using the Positive and Negative Syndrome Scale (PANSS). Overall, the presence of S SERTPR allelic variant and SS genotype was associated with a significantly greater weight gain in subjects who were nonobese at the time of admission ($p=0.04$). The presence of L SERTPR variant was associated with a significantly better treatment response measured with

pijski odgovor za samo neke simptome. Rezultati ukazuju na genetičke čimbenike povezane s porastom težine potaknutim olanzapinom i odgovorom na terapiju u shizofreničnih bolesnika.

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P16-17

Frekvencija polimorfizma S311C gena PON2 u bolesnika s kroničnim bubrežnim zatajenjem

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Genska obitelj za paraoksonazu (PON) smještena je na dugom kraku kromosoma 7q21.3-22.1 i predstavljena je s tri gena (pon1, pon2 i pon3) koji kodiraju enzime djelatne u zaštiti organizma od oksidativnog stresa. Paraoksonaza 2 (PON2) prisutna je u gotovo svim ljudskim stanicama. Smatra se da PON2 djeluje kao stanični antioksidant te mu se s tim u svezi pripisuje zaštitni učinak naspram razvoja ateroskleroze. Najčešći uočeni polimorfizam kodirajuće regije pon2 gena je S311C kod kojega dolazi do zamjene serina cisteinom na poziciji 311. Taj se polimorfizam povezuje s povećanim rizikom od nastanka ateroskleroze. Utvrđena je pojavnost polimorfizma S311C u populaciji bolesnika s kroničnim zatajenjem bubrega koji su podvrgnuti hemodijalizi (n=160) i zdravih ispitanika (n=167) te su dobiveni rezultati međusobno uspoređeni. Prisutnost polimorfizma S311C utvrđena je standardnim postupkom: Millerovom metodom izolirana je DNA iz pune krvi ispitanika, a analiza genotipa provedena je postupkom PCR-RFLP. Analiza polimorfizma gena pon2 kod bolesnika s kroničnim bubrežnim zatajenjem pokazala je slijedeće frekvencije: 88 (0,55) SS, 63 (0,39) CS, 9 (0,06) CC. U kontrolnoj skupini dobili smo vrlo slične frekvencije: 83 (0,50) SS, 80 (0,48) CS, 4 (0,02) CC. Frekvencije alela kod bolesnika s kroničnim bubrežnim zatajenjem bile su 239 (0,75) za alel S i 81 (0,25) za alel C, a u kontrolnoj skupini 246 (0,74) za alel S alel i 88 (0,26) za alel C. Na temelju rezultata χ^2 -testa zaključeno je da ne postoji statistički značajna razlika u distribuciji genotipa S311C ($p=0,139$) i frekvenciji alela ($p=0,831$) između bolesnika s kroničnim zatajenjem bubrega i zdravih dobrovoljaca.

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total PANSS and general PANSS subscale ($p<0.05$), while the presence of I SERTin2 variant was associated with better treatment response only in several items. These findings identify genetic factors associated with olanzapine-induced weight gain and treatment response in female schizophrenic patients.

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P16-17

Frequency of PON2 gene S311C polymorphism in patients with chronic renal failure

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The paraoxonase (PON) gene family, located on the long arm of chromosome 7q21.3-22.1, includes three members (pon1, pon 2, pon 3) that code for enzymes involved in the protection against oxidative stress. Paraoxonase 2 (PON2) is ubiquitously expressed in nearly all human tissues and acts as a cellular antioxidant. Due to the reduction of oxidative stress PON2 may also act as an antiatherogenic enzyme. Common polymorphism of pon2 gene related to the serine/cysteine substitution at position 311 (S311C) has been reported to be associated with the high risk of atherosclerosis. We determined S311C polymorphism of pon2 gene in patients with chronic renal failure undergoing hemodialysis (n=160) that have an increased risk of developing atherosclerosis, and compared the results obtained with a control group (n=167). For S311C polymorphism detection we used standard procedure: Miller's method for DNA isolation from whole blood, and PCR-RFLP method for polymorphism analysis. The analysis of pon2 S311C gene polymorphism in patients with chronic renal failure showed the following frequencies: 88 (0.55) SS, 63 (0.39) CS and 9 (0.06) CC. In the control group we found rather similar frequencies: 83 (0.50) SS, 80 (0.48) CS and 4 (0.02) CC. Allele frequencies in patients were 239 (0.75) for S allele and 81 (0.25) for C allele. In control group we found the following allele frequencies: 246 (0.74) for S allele and 88 (0.26) for C allele. According to statistical evaluation by χ^2 -test we concluded that there was no significant difference in the distribution of S311C genotype ($p=0.139$) and allele frequency ($p=0.831$) between chronic renal failure patients and healthy controls.

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P17 – Imunologija, P17-1 (UP11-1)**Analiza stanica bronhoalveolarnog lavata protočnom citometrijom u sarkoidozi**

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Limfocitozu u uzorku bronhoalveolarnog lavata (BAL) nalazimo u virusnim infekcijama, alveolitisu, sarkoidozi i idiopatskoj plućnoj fibrozi (IPF). Analiza limfocitnih T-staničnih subpopulacija može pomoći u razgraničavanju sarkoidoze od ostalih bolesti. U većini slučajeva sarkoidoze glavnu T staničnu populaciju čine CD4+ stanice i povećan je omjer CD4/CD8 te se smatra da ovakav nalaz ima dijagnostičko značenje za plućnu sarkoidozu. Limfociti u BAL su analizirani protočnom citometrijom u svrhu određivanja preciznog imunofenotipa, tj. površinskih biljega (CD3, CD4, CD8) i omjera CD4/CD8 u populaciji bolesnika sa sarkoidozom (n=26). Radi usporedbe nalaza dobivenih u sarkoidozi analizirali smo i skupinu bolesnika s IPF (n=14). Panel za imunofenotipizaciju limfocita sastojao se od FITC-CD3/PE-CD19, FITC-CD3/PE-CD4, FITC-CD3/PE-CD8, FITC-CD45/PE-CD14 i negativne kontrole FITC-IgG1/PE-IgG2a. Primijenjena je direktna imunofluorescentna metoda dvostrukog bojenja po standardnom protokolu. Obojene stanice smo analizirali na protočnom citometru (FACSCalibur, BD). Analizirano je 10000 stanica po uzorku. Rezultati su izraženi kao postotak pozitivnih limfocita unutar limfocitne populacije. U uzorcima bolesnika sa sarkoidozom zabilježili smo značajan ($p < 0,05$) porast CD3 i CD4 uz pad CD8. IPF pokazuje suprotnu tendenciju. Više vrijednosti omjera CD4/CD8 ($8,89 \pm 7,06$) su zabilježene kod bolesnika sa sarkoidozom u usporedbi s bolesnicima s IPF ($0,86 \pm 0,75$). Imunofenotipizacija limfocita u BAL od velikog je značenja za diferencijalnu dijagnostiku plućnih bolesti kao što su sarkoidoza i idiopatska plućna fibroza.

*E-mail: dobrosevic.blazenka@kbo.hr***P17 – Immunology, P17-1 (UP11-1)****Flow cytometry analysis of bronchoalveolar lavage cells in sarcoidosis**

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Bronchoalveolar lavage (BAL) hyperlymphocytosis, observed in viral infections, alveolitis, sarcoidosis and idiopathic pulmonary fibrosis (IPF), may involve different T cell subsets. CD4+ cells represent the major T cell subset in most cases of sarcoidosis, and demonstration of an increased CD4/CD8 ratio has been proposed as a diagnostic tool for pulmonary sarcoidosis. BAL lymphocytes were analyzed by flow cytometry to determine cell surface markers (CD3, CD4, CD8) and CD4/CD8 ratio in a population of patients with sarcoidosis (n=26). A group of IPF patients (n=14) were included to compare the findings in sarcoidosis. The staining panel for lymphocyte typing included FITC-CD3/ PE-CD19, FITC-CD3/ PE-CD4, FITC-CD3/ PE-CD8, FITC-CD45/ PE-CD14 and negative control FITC-IgG1/ PE-IgG2a (BD). An two-color direct immunofluorescence assay was performed using a standard protocol. Stained cells were analyzed on a flow cytometer (FACSCalibur, BD). A total of 10000 cells *per* sample were analyzed. Results were expressed as percentage of positive lymphocytes within the lymphocyte population. Both CD3 and CD4 were significantly ($p < 0.05$) increased with decreasing CD8 in sarcoidosis, while IPF showed a reverse tendency. A higher CD4/CD8 ratio (8.89 ± 7.06) was detected in patients with sarcoidosis than in those with IPF (0.86 ± 0.75). The phenotyping of lymphocytes in BAL fluid is of special value in the differential diagnosis of lung disorders such as sarcoidosis and IPF.

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P17-2

Stanični biljezi u sinusnom ispirku astmatičnih i neastmatičnih bolesnika

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Velik broj bolesnika s kroničnim rinosinuzitisom (CRS) pati od respiracijskih alergija. Aktivacija eozinofilne, mastocitne i neutrofilne stanične aktivnosti dokazana je u mukozi sinusa kod CRS unatoč tome što se radi o gornjim dišnim putovima. Eozinofilna upala je u sinusnoj sluznici najizraženija kada postoji bolest jedinstvenih gornjih i donjih dišnih putova (*united airways disease*). Namjera je bila usporediti stanične biljege eozinofila (ECP), mastocita (triptaze) i neutrofila (MPO) u sinusnom ispirku kod bolesnika sa CRS i onih s astmom + CRS prije i nakon endosinusne terapije antibiotikom i steroidom. Skupinu astmatičara činilo je 19 bolesnika s astmom + CRS, a skupinu neastmatičara 17 bolesnika sa CRS bez astme (alergičari i nealergičari uključeni u skupinu). Bolesnici su bili podvrgnuti 7-dnevnoj terapiji 40 mg gentamicina i 2 mg deksametazona po sinus. Terapija i uzimanje sinusnog ispirka (5 mL fiziološke otopine) provedeni su kroz polietilensku cjevčicu umetnutu antralnom punkcijom u lokalnoj anesteziji. Sinusni ispirci uzeti sinuskopski 2 sata su stajali na sobnoj temperaturi, centrifugirani 10 min na 1000 G te smrznuti na -20°C . Mjerenje ECP, MPO i triptaze izvršeno je fluoroenzim imuno metodom, prema standardnim uputama za serumsku detekciju (ImmunoCAP, Phadia, Švedska). Nakon terapije astmatičara ECP se značajno smanjuje, sa $64\pm 72,22$ na $19,04\pm 32,67$ $\mu\text{g/L}$, ali se u skupini CRS povisuje s $25,36\pm 61,77$ na $48,34\pm 72,2$ $\mu\text{g/L}$. Vrijednosti triptaze kod astmatičara značajno se smanjuju s $15,64\pm 30,89$ na $2,31\pm 2,66$ $\mu\text{g/L}$, ali i u skupini CRS s $11,07$ na $9,02\pm 24,58$ $\mu\text{g/L}$. MPO se mijenja s $456,0\pm 488,0$ na $277,08\pm 95,12$ $\mu\text{g/L}$ kod astmatičara, te s $364,0\pm 802,0$ na $488,59\pm 741,83$ u bolesnika s CRS. ECP i MPO su bili značajno viši u sinusnom ispirku astmatičara prije terapije, dok triptaze ne pokazuju značajnu razliku između skupina. Astmatičari sa CRS pokazuju značajno višu aktivaciju eozinofila i mastocita u sinusnoj mukozi od neastmatičara sa CRS. Endosinusna terapija steroidima i antibioticima učinkovita smanjuje lokalnu eozinofilnu i mastocitnu aktivaciju kod astmatičara, te mastocitnu staničnu aktivaciju jedino kod neastmatičara sa CRS. Endosinusna terapija nema učinka na neutrofilnu aktivnost.

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P17-2

Cellular markers in sinus fluid of asthmatic and non-asthmatic patients

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Significant proportions of patients with chronic rhinosinuzitis (CRS) suffer from respiratory allergy. Activation of eosinophils, mastocytes and neutrophils has been demonstrated in sinus mucosa in patients with CRS, despite evidence of lower airways disease. Eosinophilic inflammation in sinus mucosa is most pronounced in the presence of upper and lower airways disease (*united airways disease*). The study was so designed as to compare cellular markers of eosinophils (ECP), mastocytes (tryptase), and neutrophils (MPO) in sinus fluid between patients with CRS and those with asthma + CRS before and after endosinus treatment with antibiotic and steroid for 7 days. Asthma group consisted of 19 asthmatics with asthma + CRS, and the group of non-asthmatics consisted of 17 patients with CRS without asthma (allergic and nonallergic included in both groups). Patients received 7-day treatment with 40 mg gentamicin and 2 mg dexamethasone *per sinus*. Therapy was administered and sinus lavage (5 mL of saline) performed through polyethylene tubes inserted by antral puncture under local anesthesia. Sinus samples were obtained by sinuscopy, stored at room temperature for 2 hours, centrifuged for 10 minutes at 1000 G, and stored at -20°C . Samples were analyzed according to standard instructions for serum samples by fluoroenzyme immunoassay (ImmunoCAP, Phadia, Sweden). After the treatment, ECP decreased significantly from 64 ± 72.22 to 19.04 ± 32.67 $\mu\text{g/L}$ in asthmatics, but increased insignificantly from baseline 25.36 ± 61.77 to 48.34 ± 72.20 $\mu\text{g/L}$ in CRS group. Tryptase levels decreased significantly from 15.64 ± 30.89 to 2.31 ± 2.66 $\mu\text{g/L}$ in asthmatics and from 11.07 to 9.02 ± 24.58 $\mu\text{g/L}$ in CRS group. MPO changed from 456.0 ± 488.0 to 277.08 ± 95.12 $\mu\text{g/L}$ in asthmatics and from 364.0 ± 802.0 to 488.59 ± 741.83 $\mu\text{g/L}$ in CRS patients. ECP ($p<0.0001$) and MPO ($p<0.01$) at baseline were significantly higher in the asthma group, while tryptase showed no significant between-group difference. In both groups, ECP and MPO demonstrated significant correlation at baseline. Accordingly, asthmatics with CRS show a significantly higher activation of eosinophils and mastocytes in sinus mucosa than non-asthmatics with CRS. Endosinus treatment with steroid and antibiotic is effective in reducing local eosinophil and mastocyte activation in asthmatics, but influence mast cells only in CRS non-asthmatics. Endosinus treatment has no effect on neutrophils.

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P17-3

Interleukin-8 (IL-8) i interleukin-10 (IL-10) u bronhoalveolarnom lavatu (BAL) u bolesnika sa sarkoidozom: korelacija s kliničkim parametrimaFijačko M¹, Fijačko V², Dobrošević B¹, Pavela J¹, Cetina N¹¹Odjel za medicinsku biokemiju, Klinička bolnica Osijek, Osijek, Hrvatska²Odjel za plućne bolesti, Klinička bolnica Osijek, Osijek, Hrvatska

Sarkoidoza je sistemska granulomatозна bolest obilježena nakupljanjem T stanica i makrofaga. Razni citokini mogu imati ključnu ulogu u aktivaciji T stanica i makrofaga, a time i u stvaranju granuloma. Cilj rada bio je istražiti koncentracije proupalnih interleukina-8 (IL-8) i antiupalnih interleukina-10 (IL-10) u bronhoalveolarnom lavatu (BAL) bolesnika s plućnom sarkoidozom (PS) i korelirati ih s kliničkim parametrima. U rad je bilo uključeno 17 bolesnika s plućnom sarkoidozom (3 muškarca i 14 žena, dob 34–61 godina). Koncentracije IL-8 i IL-10 izmjerene su metodom ELISA (R&D Systems). BAL je učinjen pomoću 4 x 50 mL sterilne fiziološke otopine. Svi su bolesnici imali limfocitozu u BAL (36,1±21,2%) i porast omjera CD4/CD8 (8,42±7,13). IL-8 i IL-10 izmjereni su u svih bolesnika. Nađena je statistički značajna korelacija između ovih dvaju citokina i biljega oštećenja tkiva: za IL-8 pozitivna s alkalnom fosfatazom na razini statističke značajnosti $p < 0,05$, a za IL-10 negativna s albuminom na razini statističke značajnosti $p < 0,05$. Statistički značajna korelacija na razini statističke značajnosti $p < 0,01$ nađena je između koncentracije IL-8 i interleukina-1 beta (IL-1 beta), kao i tumor nekrotskog faktora alfa (TNF alfa). Međutim, statistički značajna negativna korelacija na razini statističke značajnosti $p < 0,05$ nađena je između IL-10 i angiotenzin konvertirajućeg enzima (ACE). Rezultati pokazuju da lokalno stvoreni IL-8 i IL-10 u plućnoj sarkoidozi koreliraju s aktivnošću ove granulomatозne plućne bolesti.

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P17-3

Interleukin-8 (IL-8) and interleukin-10 (IL-10) in bronchoalveolar lavage (BAL) of patients with sarcoidosis: correlation with clinical parametersFijačko M¹, Fijačko V², Dobrošević B¹, Pavela J¹, Cetina N¹¹Department of Medical Biochemistry, Osijek University Hospital, Osijek, Croatia²Department of Lung Diseases, Osijek University Hospital, Osijek, Croatia

Sarcoidosis is a systemic granulomatous disease characterized by accumulation of T cells and macrophages. Various cytokines may play crucial roles in the activation of T cells and macrophages, and thereby in the formation of granulomas. The aim of the study was to investigate the levels of proinflammatory interleukin-8 (IL-8) and anti-inflammatory interleukin-10 (IL-10) in bronchoalveolar lavage (BAL) of patients with pulmonary sarcoidosis (PS), and to correlate them with clinical parameters. The study included 17 patients with pulmonary sarcoidosis (PS; 3 male and 14 female, age 34–61). The concentrations of IL-8 and IL-10 were measured by ELISA method (R&D Systems). BALF was performed with 4 x 50 mL sterile saline solution. All patients presented BAL lymphocytosis (36.1±21.2%) and an increase of CD4/CD8 ratio (8.42±7.13). IL-8 and IL-10 were detected in all patients. These cytokines correlated with the markers of tissue damage: IL-8 positively ($p < 0.05$) with alkaline phosphatase, and IL-10 negatively ($p < 0.05$) with albumin. A strong correlation ($p < 0.01$) was found between the concentration of IL-8 and interleukin-1 beta (IL-1 beta) as well as tumor necrosis factor alpha (TNF alpha). Moreover, IL-10 showed negative correlation ($p < 0.05$) with ACE. These results suggested that locally derived IL-8 and IL-10 in PS correlated with the activity of this granulomatous lung disease.

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P17-4

Imunofenotipizacija stanica citološkog punktata metodom protočne citometrije

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Citološki punktati limfnog čvora predstavljaju suspenziju stanica, ali i nerutinski, specifičan heterogeni analitički uzorak čija je primjena u dijagnostici limfadenopatija najčešće ograničena nedostatnim brojem stanica uzorka. Cilj je bio analitički i dijagnostički evaluirati metodu protočne citometrije, odrediti prikladnost citološkog punktata limfnog čvora za analizu, ocijeniti učinkovitost literaturnih graničnih vrijednosti omjera lakih lanaca, a moguće latentne povezanosti prikazati matematičkim modelom. Citološki punktati limfnog čvora (n=245) dobiveni aspiracijom analizirani su metodom protočne citometrije u Zavodu za kliničku kemiju KB Merkur potvrđenom prema međunarodnom standardu ISO:9001:2000. Analitičkom procjenom (prema preporukama Instituta za kliničko-laboratorijske standarde NCCLS) je određena nepreciznost, netočnost, linearnost i donja granica detekcije. Dijagnostička osjetljivost i specifičnost, pozitivna (PPV) i negativna (NPV) prediktivna vrijednost metode protočne citometrije su određene eksplorativnom statistikom neuronske mreže programske potpore Statistica Version 6. Klasifikacijom podataka metodom stabla odlučivanja izgrađen je matematički model predviđanja dijagnoze. Kontrola ispravnosti optičkog i protočnog sustava citometra, kvalitete reagensa, monoklonskih protutijela i specifičnih analitičkih uvjeta bila je dio unutarnje kontrola kvalitete. Za kontrolu pouzdanosti analitičkih rezultata primijenjena je međunarodna nezavisna procjena rezultata UKNEQAS. Prema kriteriju odgovarajućeg broja leukocita ($0,35 \times 10^9/L$) iz analize su izuzeta 72 (22%) citološka punktata. Nepreciznost mjernog instrumenta je iznosila do 7%, a netočnost do 10%. Određena je dijagnostička osjetljivost (82%), specifičnost (72%), PPV (93%) i NPV (48%) metode protočne citometrije. Primjena jačeg kriterija određivanja klonalnosti limfocita B smanjila je osjetljivost, a povećala specifičnost metode. Pouzdanost analitičkih rezultata potvrđena je zadovoljenjem svih međunarodnih kriterija prihvatljivosti. Zaključeno je kako standardizacija metode protočne citometrije prema radnim protokolima osigurava pouzdanost analitičkih rezultata u citološkom punktu limfnog čvora. Potencijalna primjena i učinkovitost

P17-4

Immunophenotyping of fine needle aspirate cells by flow cytometry method

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Fine-needle aspirate (FNA) represents a suspension of lymph node cells but also a nonroutine, specific and heterogeneous analytical specimen the use of which is usually limited by the inadequate number of cells for the flow cytometry method (FCM). The aim of the study was to perform critical analytical and diagnostic evaluation of FCM, to assess the adequacy of FNA for FCM analysis, to evaluate the usefulness of the light chain ratio borderline values reported in the literature, and to present the possible associations as a mathematical model. Upon collection, 245 FNA specimens were analyzed by FCM at Department of Clinical Chemistry, Merkur University Hospital, certified according to ISO:9001:2000. Brief analytical evaluation (imprecision, inaccuracy, linearity and lower detection limit) was done according to the evaluation guides of the Clinical and Laboratory Standards Institute (NCCLS). Diagnostic specificity and sensitivity as well as positive (PPV) and negative (NPV) predictive value of the FCM were determined using explorative statistics of the neural network (Statistica Version 6). Using the classification tree method for the classification of input data, a mathematical model of diagnosis prediction was generated. The instrument quality control assurance of the optic and fluid system, reagents and monoclonal antibodies as well as specific analytical control were performed. The reliability of our analytical results was evaluated by UKNEQAS independent international external quality control. According to the criteria of FNA leukocyte count ($0.35 \times 10^9/L$), 72 (22%) FNA specimens were excluded from further analysis. For all parameters of the instrument (Coulter EPICS-XL), imprecision and inaccuracy were 7% and 10%, respectively. Diagnostic sensitivity, specificity, PPV and NPV for FCM were 82%, 72%, 93% and 48%, respectively. The use of the literature light chain ratio borderline values reduced diagnostic sensitivity but increased diagnostic specificity of FCM. Standardization of FCM in nonroutine, specific and heterogeneous analytical specimens like FNA is possible due to the sample preparation protocols and instrument quality control assurance. In routine classification of new data, the use of the gener-

izgrađenog matematičkog modela u klasifikaciji novih podataka i svakodnevnom rutinskom radu predstavljala bi krajnji rezultat istraživanja i unaprjeđenje klasifikacije limfadenopatija.

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P17-5

Empirijska metoda konverzije rezultata dvaju dijagnostičkih testova za anti-CMV IgG protutijela

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Određivanje citomegalovirus imunoglobulina G (IgG) ima za cilj praćenje imunog statusa bolesnika, dok praćenje promjene aktivnosti titra IgG protutijela tijekom bolesti značajno doprinosi postavljanju dijagnoze infekcije ovim virusom. Zbog nepostojanja međunarodnog standarda teško je uskladiti i usporediti rezultate različitih proizvođača, pa je tako otežana i longitudinalna procjena nalaza, što je od iznimne važnosti u serodijagnostici. Neusporedivost rezultata dvaju proizvođača koji izražavaju rezultate u različitim jedinicama (metode Abbott AxSYM CMV IgG i Behring Enzygnost AntiCMV/IgG) predstavlja ozbiljnu prepreku u racionalnoj dijagnostici infekcije ovim virusom i postavljanju dijagnoze. Razlog za prelazak s jednog dijagnostičkog sustava na drugi može biti financijske naravi, zbog prestanka proizvodnje pojedinog reagensa, parni serum za usporednu analizu nije dostupan ili pak treba usporediti rezultate različitih laboratorija. Predlažemo dvije empirijske formule utemeljene na linearnoj regresiji, prema kojima je moguća pretvorba vrijednosti metoda i međusobna usporedba rezultata sa zadovoljavajućom preciznošću. Analizirali smo 38 seropozitivnih uzoraka metodama Abbott AxSYM CMV IgG i Behring Enzygnost AntiCMV/IgG. Nakon logaritamske transformacije na osnovi 10 izvornih vrijednosti provedene su dvije linearne regresijske analize uz izračun jednadžba pravca regresije i visine Pearsonovog koeficijenta korelacije. Medijan i raspon vrijednosti u jedinicama AU/mL prema metodi Abbotta iznosili su: M=183,5 (30-821), a vrijednosti titra prema metodi Behringa iznosili su: M=7486 (352-67675). Vrijednost korelacijskog koeficijenta između transformiranih vrijednosti metoda iznosila je 0,87 (95%CI: 0,76-0,93), $p < 0,001$. Obrazac za preračunavanje vrijednosti titra do-

ated mathematical model may present the latest research result in the classification of lymphadenopathies.

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P17-5

Empirical method for conversion of results obtained by two anti-CMV IgG diagnostic tests

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Determination of cytomegalovirus (CMV) immunoglobulin G (IgG) serves for monitoring patient immune status, whereas monitoring of changes in IgG titer activity during the course of disease helps in making the diagnosis of CMV infection. Because international standards are lacking, comparison of the results of diagnostic tests from different manufacturers is very difficult or impossible. Longitudinal assessment, which is of paramount importance in serodiagnosis, is thus greatly hampered. The impossibility to compare the results obtained by the methods developed by different manufacturers (Abbott AxSYM CMV IgG and Behring Enzygnost AntiCMV/IgG method) presents a serious obstacle for rational diagnosis of CMV infection. There are many potential reasons for switching diagnostic systems in use, e.g., inadequate resources, the manufacture of a particular reagent may be stopped, paired serum may be missing, or one may simply need to compare results from different laboratories. We propose two empirical formulas for conversion between values obtained by the two methods, based on linear regression technique and with a satisfactory level of conversion precision. We analyzed 38 seropositive samples with both Abbott AxSYM CMV IgG and Behring Enzygnost AntiCMV/IgG methods. After the original results had been log(10)-transformed, two separate linear regression analyses were performed. The corresponding regression line equations and Pearson correlation coefficient were calculated. The median and range of values in AU/mL obtained by Abbott method were: M=183.5 (30-821), and titer values obtained by Behring method were: M=7486 (352-67675). Correlation coefficient between the log-transformed values was $r=0.87$ (95%CI: 0.76-0.93),

bivenih metodom Behringa u približne vrijednosti AU/mL prema metodi Abbotta glasi $10^{(1,255+1,136*\log(10)X)}$. Obrnuti obrazac za izračun vrijednosti titra iz AU/mL glasi $10^{(-0,282+0,664*\log(10)X)}$. Visoki koeficijent korelacije ukazuje na jaku statističku povezanost vrijednosti dobivenih u različitim analitičkim sustavima. Predložene formule za konverziju omogućuju longitudinalnu procjenu nalaza u slučajevima kada serum nije moguće usporedno analizirati istom metodom. Bez obzira na širinu granica regresije, klinički je značajno najmanje dvostruko povećanje aktivnosti protutijela, što izlazi iz granica pogreške regresije.

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P17-6

Usporedba dviju metoda određivanja imunoglobulina

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Određivanje koncentracije imunoglobulina daje važnu informaciju o humoralnom imunom statusu.

Cilj ovoga rada bio je usporediti rezultate dobivene usporednim određivanjem imunoglobulina G, imunoglobulina A i imunoglobulina M dvjema imunokemijskim metodama: imunonefelometrijski i imunoturbidimetrijski. Koncentracije imunoglobulina odredili smo u istim uzorcima seruma nasumce odabranih bolesnika (n=45) reagensima dvaju proizvođača: imunonefelometrijski (Dade Behring, nefelometar ProSpec) i imunoturbidimetrijski (Abbott, biokemijski analizator Architect c8000). Ispitivanje je obuhvatilo nepreciznost iz dana u dan, netočnost i usporedna određivanja. Mjerene su koncentracije imunoglobulina G, imunoglobulina A i imunoglobulina M. Za određivanje nepreciznosti iz dana u dan i netočnosti primijenjeni su kontrolni serum (kod imunonefelometrijskog određivanja NIT Protein Control M tvrtke Dade Behring i kod imunoturbidimetrijskog Precinom U tvrtke Roche). Dobiveni rezultati usporednih određivanja statistički su obrađeni neparametrijskom linearnom regresijskom analizom po Passingu i Babloku. Nepreciznost iz dana u dan određena je mjerenjem imunoglobulina u kontrolnom serumu kroz 25 dana. Koeficijent varijacije dobiven imunonefelometrijskim mjerenjem iznosi za IgG 3,9%, IgA 4,1%, IgM 4,3%, a imunoturbidimetrijskim mjerenjem za IgG 4,4%, IgA 4,2%, IgM 5,1%. Određivanjem netočnosti s kontrolnim

$p < 0.001$. The formula for converting the values of titer derived by Behring method into approximate values of AU/mL is: $10^{(1.255+1.136*\log(10)X)}$, where X may be any value within the range. The inverse formula for converting AU/mL to titer is: $10^{(-0.282+0.664*\log(10)X)}$. The high correlation coefficient indicates strong statistical correspondence between transformed values across two different analytical systems and different units. The proposed conversion formula allows for longitudinal assessment in cases when serum analysis in parallel with the same method is not possible. Regardless of the regression uncertainty limits, at least twofold increase of antibody activity is clinically relevant, which is beyond the regression confidence intervals.

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P17-6

Comparison of two methods of immunoglobulin determination

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Quantitative determination of immunoglobulins provides important information on the humoral immune status. The aim of the study was to compare the results obtained by immunoglobulin G, immunoglobulin A and immunoglobulin M determination by two immunochemistry methods, immunonephelometric and immunoturbidimetric methods. Immunoglobulin concentrations were determined in the same serum samples of randomly selected patients (n=45) using two assays for determination of immunoglobulins in serum: immunonephelometric (Dade Behring, Nephelometer Pro Spec) and immunoturbidimetric (Abbott, Architect c8000 biochemistry analyzer). Day-to-day imprecision, inaccuracy and correlation were determined. Immunoglobulin G, immunoglobulin A and immunoglobulin M concentrations were also measured. Day-to-day imprecision and inaccuracy were measured using control serum (immunonephelometrically by N/T Protein Control M, Dade Behring, and immunoturbidimetrically by Precinorm U, Roche). Results of these parallel determinations were statistically processed by use of nonparametric linear regression Passing-Bablok analysis. Day-to-day imprecision was determined by measuring immunoglobulins in control serum over 25 days. The coefficients of variation yielded by immunonephelometric determination were for IgG 3.9%, IgA 4.1% and IgM 4.3%, whereas those recorded

serumima dobivene su vrijednosti kod nefelometrijskog određivanja za IgG 6,4%, IgA 1,9%, IgM 1,24%, a kod imunoturbidimetrijskog određivanja za IgG 4,3%, IgA 1,3%, IgM 2,9%. Usporedbom rezultata dobivenih na nefelometru i biokemijskom analizatoru dobiveni su koeficijenti korelacije za IgG $r=0,9882$; za IgA $r=0,9953$; za IgM $r=0,9933$. Određivanjem imunoglobulina imunonefelometrijski i imunoturbidimetrijski dobili smo rezultate koji su usporedivi i ukazuju da su obje metode prihvatljive za rutinski rad.

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P17-7

Dokazivanje plazmatskih proupalnih citokina i čimbenika rasta na mikročipu

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Citokini su važni biološki čimbenici u regulaciji imunoaktivnosti i patofiziologiji raznih infektivnih bolesti, te bolesti uzrokovanih upalom, poremećajem staničnog rasta i stvaranja novih krvnih žila. Mjerenjem pojedinačnih citokina enzimoimunotestom (ELISA) opažena je povišena koncentracija različitih proupalnih citokina u plazmi bolesnika s teškim infekcijama, autoimunim bolestima i nekim karcinomima. Otuda i zanimanje za ispitivanje moguće dijagnostičke i prognostičke vrijednosti proupalnih citokina u raznim bolestima. Nove tehnologije, kao što su mikrokuglice ili mikročipovi s monoklonskim antitijelima specifičnim za razne citokine, omogućuju istodobno dokazivanje više različitih citokina u istom uzorku plazme. U istraživanju smo rabili mikročip s dvanaest različitih citokina i čimbenika rasta. Koncentraciju citokina u plazmi odredili smo u 11 novorođenčadi s različitim bakterijskim infekcijama, 7 novorođenčadi s raznim urođenim grješ-kama bez popratne infekcije, te u 7 odraslih bolesnika s raznim histološkim tipovima i lokalizacijama karcinoma. Razinu proupalnih citokina odredili smo mikročipom koji omogućuje istodobno mjerenje IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-gama, TNF-alfa, IL-1 alfa, IL-1 beta, MCP-1 i EGF. Koncentracija kemokina IL-8 i MCP-1 povišena je u svih ispitanika. Povišena je i koncentracija IL-6 i TNF-alfa u 75%

by immunoturbidimetric determination were IgG 4.4%, IgA 4.2% and IgM 5.1%. Determination of inaccuracy with control serum by immunonephelometric method yielded the following values: IgG 6.4%, IgA 1.9% and IgM 1.24%, and with immunoturbidimetric method IgG 4.3%, IgA 1.3% and IgM 2.9%. Comparison of the results obtained by these two analyses (nephelometer and biochemistry analyzer) produced the following correlation coefficients: IgG $r=0.9882$, IgA $r=0.9953$ and IgM $r=0.9933$. The results obtained by immunonephelometric and immunoturbidimetric assays show that both methods are comparable and acceptable for routine work.

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P17-7

Detection of plasma proinflammatory cytokines and growth factors by biochip array

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Cytokines are major biologic factors in the modulation of immune responses and in the pathophysiology of various infections and diseases related to inflammation, growth deregulation and vascular remodeling. Determination of a single cytokine by ELISA has shown elevated concentrations of various proinflammatory cytokines in plasma of patients with serious infections, autoimmune diseases and cancer. These data motivated further investigations to determine the possible diagnostic and prognostic value of proinflammatory cytokine measurement. New technologies such as microbeads or biochip array platform with monoclonals specific for various cytokines allow for simultaneous detection of multiple cytokines in the same plasma sample. In this study, we used a biochip array platform for proinflammatory cytokine determination in the plasma of newborn infants and adults with cancer. Plasma cytokine levels were determined in 11 newborns with various bacterial infections, 7 infants without infection, and 7 adults with different histologic types and localization of cancer. Plasma cytokine levels were evaluated by biochip array for simultaneous detection of IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-gamma, TNF-alfa, IL-1 alfa, IL-1 beta, MCP-1 and EGF. Plasma levels of IL-8 and MCP-1 chemokines were elevated in all patients.

ispitanika. Nasuprot tome, razina IL-1 alfa i IL-1 beta nije bitno povišena (0-28%). Koncentracija ostalih citokina (IL-4, IL-10, VEGF, EGF, IFN-gama) sporadično je povišena samo u nekih ispitanika (0-40%), s iznimkom IL-2 koncentracija kojega je povišena u oko 85% ispitanika. Navedeni rezultati pokazuju kako istodobno dokazivanje brojnih citokina može biti vrlo informativno, ali interpretativno vrlo složeno.

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Elevated concentrations of IL-6 and TNF-alfa were found in 75% of infants and adults. At the same time, the concentrations of IL-1 alfa and IL-1 beta were not significantly elevated (0-28%). Plasma levels of other cytokines (IL-4, IL-10, VEGF, EGF, IFN-gamma) were only sporadically elevated (0-40%), with the exception of IL-2. Plasma levels of this cytokine were elevated in 85% of patients. These data suggest that simultaneous determination of multiple proinflammatory cytokines and growth factors by biochip array may be very informative but very complex in terms of interpretation.

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P17-8

Uspoređivanje testa AtheNA Multi-Lyte ANA za određivanje autoantitijela s indirektnom imunofluorescencijom i testom ELISA

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Laboratorijski testovi koji se rabe u obradi bolesnika s autoimunim bolestima služe za dijagnosticiranje bolesti, kliničko praćenje bolesnika i procjenu uspješnosti liječenja. Test AtheNA Multi-Lyte ANA rabi metodu homogene fluoroimunokemije gdje su polistirenske kuglice obložene s više različitih antigena. Stoga ovaj test služi za polukvantitativno dokazivanje IgG klase antitijela za SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, centromera i histona, te za kvantitativno dokazivanje IgG klase antitijela na dsDNA i kvalitativno dokazivanje IgG klase antitijela na ANA. Cilj rada je bio usporediti test AtheNA Multi-Lyte ANA s konvencionalnim metodama indirektno imunofluorescencije (IIF) i enzimmunoanalize (ELISA) koje rabimo u našem svakodnevnom radu. Analizirali smo 152 uzorka seruma zaprimljenih u laboratorij sa zahtjevima za određivanje slijedećih antitijela: 97 ANA, 59 SS-A, 56 SS-B, 11 Sm, 9 Sm/RNP, 39 Scl-70, 17 Jo-1, 39 dsDNA, 16 histona i 19 centromera bez obzira na dijagnozu. Svi uzorci usporedno su određivani i mjereni testom AtheNA Multi-Lyte ANA (Zeus Scientific, Inc.) na instrumentu Luminex 100 IS, komercijalnim testom ANA Hep-2 (BioSystems) na fluorescentnom mikroskopu, komercijalnim testom ELISA za dokazivanje ENA antitijela (SS-A, SS-B, Sm, Sm/RNP, Scl-70, Jo-1), dsDNA (Hycor test) te histona i centromera (DiaSorin) na automatskom analizatoru miniBos, Biomedica. Svaki pojedinačni rezultat

P17-8

Comparative analysis of Multiplex AtheNA Multi-Lyte ANA system with conventional laboratory methods of autoantibody detection

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Measurement of autoantibodies provides supporting evidence in the diagnosis and monitoring of systemic autoimmune diseases. The AtheNA Multi-Lyte ANA Test System (Zeus Scientific, Inc.) is a homogeneous, multiplexed, fluorescence-based microparticle immunoassay intended for semi-quantitative detection of IgG class antibody to 8 separate analytes (SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, centromere B and histone), quantitative detection of IgG class antibody to dsDNA, and qualitative detection of IgG class antibody to ANA in human serum. The objective of this study was to determine the performance of the AtheNA Multi-Lyte ANA test relative to the established, commercial indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) currently in use in our laboratory. The study population consisted of 152 serum specimens referred to our laboratory for autoimmune testing: 97 ANA requests, 59 SS-A, 56 SS-B, 11 Sm, 9 Sm/RNP, 39 Scl-70, 17 Jo-1, 39 dsDNA, 16 histone and 19 centromere. Diagnosis was not included in the study. All specimens were tested on the AtheNA Multi-Lyte ANA Test System and a multitude of conventional immunoassays including commercial ANA Hep-2 test systems (BioSystems), commercial ELISA test systems for detection of antibodies to ENA (SS-A, SS-B, Sm, Sm/RNP, Scl-70, Jo-1), dsDNA (Hycor test, miniBos analyzer,

dobiven testom AtheNA Multi-Lyte ANA uspoređivan je s rezultatima dobivenim određivanjem IIF ANA Hep-2 i s rezultatima dobivenim metodom ELISA. Ovim metodama postotak usporedivih pozitivnih rezultata bio je za: ANA 83%, SS-A 96%, SS-B 98%, Sm 97%, Sm/RNP 85%, Scl-70 87%, Jo-1 67%, dsDNA 88%, histone 79%, centromere 89%. Postotak negativnih rezultata bio je za: ANA 86%, SS-A 98%, SS-B 94%, Sm 96%, Sm/RNP 85%, Scl-70 85%, Jo-1 67%, dsDNA 79%, histone 84%, centromere 87%. Rezultati koji se nisu slagali provjereni su ponovljenim određivanjem. Iz dobivenih rezultata možemo zaključiti da rezultati dobiveni testom AtheNA Multi-Lyte ANA daju u kratkom vremenu pouzdane rezultate s devet različitih antitijela, što ubrzava put do postavljanja dijagnoze.

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P17-9

Učestalost antikardiolipinskih, antinuklearnih i anti-beta2 glikoproteinskih protutijela u djece s epilepsijom

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Visoka učestalost epilepsije u specifičnim imunim bolestima upućuje na to da imuni sustav može igrati ulogu u patogenezi epilepsije ili može biti povezan s tim. Ispitali smo učestalost antikardiolipinskih protutijela (aCL), antinuklearnih protutijela (ANA) i anti-beta2-glikoproteinskih protutijela (anti-beta2-GPI) kod 40 djece s epilepsijom i u kontrolnoj skupini opće populacije bez kliničkih dokaza za imune poremećaje ili akutnu infekciju, kako bismo procijenili prisutnost aCL, ANA i anti-beta2-GPI te njihovu povezanost sa spolom, vrstom epilepsije, težinom i trajanjem epilepsije. Studija je provedena u Klinici za pedijatriju, Klinička bolnica Split. Uključeno je 40 bolesnika u dobi do do 18 godina s dijagnosticiranom epilepsijom, bez znakova i simptoma sukladnih kliničkim poremećajima imunog sustava, vezivnog tkiva ili reumatskih bolesti te akutnih infekcija. Kontrolna skupina se sastojala od 38 zdrave djece. U svakog bolesnika su zasebno provedena enzimimuno mjerenja za protutijela aCL i anti-beta2-GPI. Uzorci bolesnika s optičkom gustoćom većom od prijelomne vrijednosti kontrole smatrali su se pozitivnima. ANA smo određivali indirektnom imunofluorescencijom. Kad je fluorescencija bila u omjeru 1/40 ili veća, smatrali smo

Biomedica), histone and centromere (DiaSorin, miniBos analyzer, Biomedica). Each individual reportable result from AtheNA Multi-Lyte ANA test was compared with the established methods. For most analytes evaluated, positive agreement was high: ANA 83%, SS-A 96%, SS-B 98%, Sm 97%, Sm/RNP 85%, Scl-70 87%, Jo-1 67%, dsDNA 88%, histone 79%, centromere 89%. For negative results agreement was: ANA 86%, SS-A 98%, SS-B 94%, Sm 96%, Sm/RNP 85%, Scl-70 85%, Jo-1 67%, dsDNS 79%, histone 84%, centromere 87%. When results were discrepant, analysis were repeated. The AtheNA Multi-Lyte ANA technology provides a fast, flexible and cost effective tool for measuring multiple disease markers from a single sample.

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P17-9

Frequency of anticardiolipin, antinuclear and anti-beta2 glycoprotein antibodies in children with epilepsy

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The high prevalence of epilepsies in specific immune diseases suggests that immune system may play a role in the pathogenesis of epilepsy or might be associated with it. We studied the frequency of anticardiolipin antibodies (aCL), antinuclear antibodies (ANA) and anti-beta2-glycoprotein antibodies (anti-beta2-GPI) in 40 consecutive children with epilepsy and in matched control subjects from the general population without any clinical evidence of immune disorder or acute infection, in order to evaluate the presence of aCL, ANA and anti-beta2-GPI, and their association with various factors including sex, type of epilepsy, severity and duration of epileosy, and anticonvulsants. The study was conducted at Department of Pediatric Neurology, University Department of Pediatrics, Split University Hospital. The study included patients with the diagnosis of epilepsy, no signs or symptoms consistent with clinical immune system disorders, connective tissue or rheumatic disease, acute infection, and aged 18 years or younger. Forty consecutive patients with these criteria were enrolled. Control group consisted of 38 healthy children. Commercially available immunometric enzyme immunoassays were used for measurement of

da su antinuklearna protutijela pozitivna. Prema učestalosti protutijela aCL i ANA kod djece s epilepsijom (44% i 16%) i kod zdrave djece (10% i 0%) izračunali smo da se kod 40 bolesnika trebala otkriti razlika s 90%-tnom točnošću i 5% značajnih nivoa. Upotrijebili smo Fisherov test za usporedbu razlika između bolesničke i kontrolne skupine. Statistička značajnost je utvrđena na razini $p < 0,05$. Nismo našli značajne statističke povezanosti s godinama starosti, spolom, godinom početka epilepsije, trajanjem epilepsije i vrstom epilepsije, učestalosti epileptičnih izbijanja ili specifičnih antiepileptičnih lijekova s prisutnošću bilo kojeg izmjerenog protutijela.

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P17-10

Intolerancija na hranu

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Hrana koju svakodnevno uzimamo sadrži različite spojeve, od kojih neki mogu uzrokovati neželjene posljedice na zdravlje. Nepoželjne reakcije na hranu mogu se podijeliti u 2 skupine: toksične i netoksične reakcije. Toksične reakcije posljedica su uzimanja hrane zaražene bakterijama ili toksinima. Netoksične reakcije javljaju se u preosjetljivih osoba kao alergijska reakcija i intolerancija na hranu. U slučaju alergije alergen (specifični protein iz hrane) izaziva specifični imuni odgovor organizma, povećavajući razinu specifičnog IgE antitijela. Pri alergijskoj reakciji nastaje brza uzročno-posljedična reakcija unutar nekoliko sati od unošenja određene hrane, s izraženim kliničkim simptomima. Drugi proces, intoleranciju hrane, teže je otkriti. Kod nje izostaje brza uzročno-posljedična reakcija, a kliničke manifestacije su često manje jasne, neprimjetne, ponekad teško uočljive i nepredvidive. Antigeni iz hrane koji mogu izazvati štetne reakcije su proteini ili glukoproteini manje molekularne mase, otporni na želučanu kiselinu i aktivnost probavnih enzima. Denaturaliziraju se uslijed toplote. Bivaju obuhvaćene Peyerovim pločicama M stanica gornjeg sloja sluznice crijeva i fagocitiraju se pomoću makrofaga. U određenim slučajevima pojavljuje se imuna osjetljivost koja rezultira stvaranjem IgA antitijela u prvoj fazi, odnosno IgG antitijela nakon višestruke stimulacije. Hrana koja potiče proizvodnju specifičnih IgG antitijela

aCL and anti-beta2-GPI antibodies. Patient samples exhibiting optical densities higher than the optical density of the cut-off control were considered positive. ANA were detected by the standard indirect immunofluorescence method. Serum showing nuclear fluorescence at 1/40 or higher was considered positive. With the reported prevalence of aCL and ANA antibodies in children with epilepsy (44% and 16%) and in healthy children (10% and 0%), we calculated that a difference with 90% certainty and 5% significance should have been recorded in 40 patients. Fisher's exact test was used to compare differences between the study and control groups. Statistical significance was set at $p < 0.05$. There was no statistically significant correlation of age, sex, age at onset of epilepsy, duration of epilepsy, type of epilepsy, seizure frequency or specific antiepileptic medications with the presence of any antibody determined.

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P17-10

Food intolerance

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The food taken daily is made of various compounds, some of which may cause undesirable effects on human health. Undesirable reactions to food can be divided into two groups: toxic and nontoxic reactions. Toxic reactions are caused by the consumption of food contaminated by bacteria or toxins. Nontoxic reactions occur in hypersensitive persons in the form of allergic reactions and food intolerance. In case of allergy, the allergen (a specific food protein) induces specific immune response of the body, thus raising the concentration of the specific IgE antibody. Allergic reaction is characterized by an immediate cause and effect reaction, within only a few hours of the specific food consumption, with visible clinical symptoms. The other process, food intolerance, is more difficult to detect. Unlike allergies, food intolerance does not provoke an immediate cause and effect reaction, and clinical manifestations are often less clear, obscure, hard to notice and unpredictable. Food antigens that can provoke adverse consequences are food proteins or glucoproteins that have smaller molecular weight, and are resistant to gastric juice and digestive enzyme activity. They are denatured at high temperatures. These molecules are taken up by Peyer's patches of M cells of the upper layer of intestinal epithelium and are phagocytosed by macrophages. In some cases, immune susceptibility develops,

u pojedinim slučajevima izaziva probavne tegobe, dok je u drugim slučajevima manifestacija neprimjetna i vrlo je teško povezati ju s hranom, jer dolazi do promjena u patologiji. Cilj je bio utvrditi koja hrana ili više vrsta hrane štete zdravlju bolesnika uzrokujući probavne tegobe, dermatološke procese, neurološke smetnje, respiracijske tegobe, psihološke smetnje, artritis, pretilost. Provedeno je mjerenje koncentracije IgG antitijela na 93 različita antigena u serumu metodom ELISA. Koncentracije IgG antitijela na 93 različite vrste namirnica određene su u serumu 400 osoba različite dobi, spola i zdravstvenog stanja. Kao najčešći uzročnici intolerancije pokazale su se žitarice, mliječni proizvodi, jaja, soja, ananas i Coca Cola. Ukidanjem jedne ili više vrsta namirnica s povećanom koncentracijom IgG dobivene testom intolerancije na hranu u više od dvije trećine slučajeva došlo je do poboljšanja između 20 i 60 dana primjene odgovarajuće dijeta.

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P17-11

Dijagnostička točnost protutijela na mutirani citrulinirani vimentin (anti-MCV) za reumatoidni artritis

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Novi terapijski pristup u reumatoidnom artritisu (RA) podrazumijeva primjenu antireumatske terapije prije ireverzibilnih oštećenja zglobova kada još nisu prisutne sve znakovite kliničke manifestacije. Stoga je neophodan specifičan i osjetljiv biljeg koji će pomoći u diferencijalnoj dijagnozi. Protutijela na citrulinirane peptide (CP) pokazala su se izrazito specifičnim serološkim biljegom RA. Citrulinirani vimentin, prisutan u reumatoidnom sinovijskom tkivu, jedan je od potencijalnih autoantigena u RA. Cilj je bio ispitati dijagnostičku točnost novoga testa ELISA (Orgentec Diagnostica, Njemačka) za otkrivanje protutijela na mutirani citrulinirani vimentin (anti-MCV) za RA i usporediti ju s dijagnostičkom točnošću testa anti-CCP ELISA dru-

resulting in the formation of IgA antibodies in the first phase, and of IgG antibodies upon multiple stimulations. The food that provokes the production of specific IgG antibodies in some cases induces gastrointestinal disorders, whereas in other cases manifestations are invisible and it is very difficult to connect them with food, while altering the pathology. The aim was to determine which food, or kinds of food, are detrimental to patient health, causing gastrointestinal disorders, dermatologic processes, neurologic disorders, respiratory problems, psychological disorders, arthritis, and overweight. Serum concentration of IgG antibodies to 93 different antigens from food was measured by ELISA in 400 patients of different age, sex and health condition. Cereals, dairy products, eggs, soy, pineapple and Coca Cola proved to be the most common causes of food intolerance. By excluding one or several kinds of food provoking an increase in IgG concentration measured by the food intolerance test, improvement was observed in more than two-thirds of cases within 20-60 days of introducing a diet.

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P17-11

Diagnostic accuracy of antibodies against mutated citrullinated vimentin (anti-MCV) for rheumatoid arthritis

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According to the new therapeutic approach in rheumatoid arthritis (RA), antirheumatic therapy should be initiated before irreversible joint damage has set in. Since in this early stage symptoms are equivocal, a highly specific and sensitive marker is needed for differential diagnosis. Antibodies against citrullinated peptides (CP) have emerged as a highly specific serologic marker of RA. Citrullinated vimentin, present in rheumatoid synovial tissue, is a potential autoantigen in RA. The aim of the study was to evaluate diagnostic accuracy of the new ELISA (Orgentec Diagnostica, Germany) for detection of antibodies against mutated citrullinated vimentin (anti-MCV) for RA and to compare it with diagnostic accuracy of the second

ge generacije. Protutijela anti-MCV i anti-CCP određena su u serumima 264 ispitanika: 92 s RA i 172 ispitanika kontrolne skupine (38 s degenerativnim ili drugim upalnim bolestima zglobova, 27 s bolestima vezivnog tkiva ili vaskulitisom i 107 zdravih osoba). ROC analizom rezultata za anti-MCV utvrđena je AUC=0,874 (95% CI=0,828-0,911). Za graničnu vrijednost >20 U/mL, koju predlaže proizvođač, osjetljivost je bila 63,0% a specifičnost 97,1%. Za anti-CCP utvrđena je AUC=0,897 (95% CI=0,854-0,931), a osjetljivost i specifičnost za preporučenu graničnu vrijednost >5 RU/mL 70,6% odnosno 97,7%. AUC za anti-MCV i anti-CCP nisu se statistički značajno razlikovale ($p=0,298$). U skupini RA 11 uzoraka je bilo negativno na MCV (3 s visokom koncentracijom anti-CCP), dok su 4 uzorka bila negativna na CCP (1 s visokom koncentracijom anti-MCV). U kontrolnoj skupini 2 uzorka su imala visoke koncentracije obaju protutijela, 3 uzorka su bila pozitivna samo na MCV (1 s visokom koncentracijom), dok su 2 uzorka bila pozitivna samo na CCP (1 s visokom koncentracijom). Optimalna granična vrijednost za MCV dobivena ROC analizom je >10 U/mL uz osjetljivost od 71,7% i specifičnost od 95,3%. Rezultati naše studije pokazali su da test anti-MCV ima podjednaku dijagnostičku točnost za RA kao i anti-CCP, iako je osjetljivost nešto niža. Primjenom optimalne granične vrijednosti koju smo dobili ROC analizom osjetljivost se značajno povećava zadržavajući još uvijek visoku specifičnost.

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P18 – Hitna laboratorijska dijagnostika, P18-1
Spektrofotometrijsko određivanje
karboksihemoglobina – usporedba
vrijednosti dobivenih na analizatoru NOVA
CO-oksometar i spektrofotometrijskom
metodom po Bruckneru

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Karboksihemoglobin (COHb) nastaje reverzibilnim vezanjem ugljičnog monoksida na hemoglobin. Određivanjem udjela COHb u stanjima akutnog trovanja ugljičnim monoksidom (CO) procjenjuje se izloženost bolesnika i jačina intoksikacije. Referentna metoda mjerenja COHb je plinska kromatografija, dok se u rutinskom radu zbog analitičke jednostavnosti i brzine rabi spektrofotometrijska metoda. Analizom uzoraka krvi bolesnika nepuša-

generation anti-CCP ELISA. Anti-MCV and anti-CCP antibodies were determined in 264 serum samples: 92 from RA patients and 172 from control patients including 38 with degenerative and other inflammatory joint diseases, 27 with connective tissue diseases or vasculitis, and 107 healthy subjects. ROC analysis of anti-MCV results yielded AUC=0.874 (95% CI=0.828-0.911). For the cut off >20 U/mL, recommended by the manufacturer, the sensitivity was 63.0% and specificity 97.1%. Anti-CCP revealed AUC=0.897 (95% CI=0.854-0.931) with a sensitivity of 70.6% and specificity of 97.7% for the recommended cut off >5 RU/mL. The AUC for anti-MCV and anti-CCP were not significantly different ($p=0.298$). In the RA group 11 samples were anti-MCV negative (3 with high anti-CCP concentration) and 4 were anti-CCP negative (1 with high anti-MCV concentration). Among controls, 2 samples had high antibody concentrations in both assays, 3 samples were only anti-MCV positive (1 with high concentration) and 2 were only anti-CCP positive (1 with high concentration). The optimal cut-off for anti-MCV yielded by ROC analysis was >10 U/mL with a sensitivity of 71.7% and specificity of 95.3%. According to our results, the anti-MCV assay has a comparable diagnostic accuracy as anti-CCP assay for RA, although the sensitivity was somewhat lower. Using optimal cut-off yielded by ROC analysis, the sensitivity became significantly higher without a considerable decrease of specificity.

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P18 – Emergency clinical chemistry, P18-1
Spectrophotometric carboxyhemoglobin
determination – comparison of values
obtained on a NOVA CO-oximeter and by
spectrophotometric method according to
Bruckner

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Carboxyhemoglobin (COHb) is produced by reversible carbon monoxide binding to hemoglobin. Determination of COHb values in conditions of acute poisoning by carbon monoxide (CO) is used to assess patient exposure and the degree of intoxication. The reference method for COHb measurement is gas chromatography, while spectrophotometric method is used in routine practice due to analytical simplicity and rapidity. The values rang-

ča koji nisu bili u kontaktu s izvorima CO na analizatoru NOVA CO-oksometar dobivane su vrijednosti od oko 3%-5% ukupnog hemoglobina. Kako su postojeće referentne vrijednosti ispod navedenih granica (<1,5% nepušači, <5% pušači), cilj istraživanja bio je ispitati osjetljivost analizatora NOVA CO-oksometar, usporediti vrijednosti s ručnom spektrofotometrijskom metodom i ustanoviti primjenjivost postojećih referentnih intervala. Analiziran je ukupno 81 uzorak pune krvi u duplikatu. Ispitanici su podijeljeni u 3 skupine: akutno intoksicirani (I.), bez kontakta s izvorima CO (II.) i pušači (III.). Srednje izmjerene vrijednosti COHb dobivene na instrumentu NOVA i "ručnim" postupkom za pojedinu skupinu ispitanika bile su ($\chi \pm SD$): za I. skupinu $25 \pm 11\%$ i $14 \pm 10\%$, za II. skupinu $5 \pm 1\%$ i $0 \pm 0\%$, te za III. skupinu $8 \pm 2\%$ i $1 \pm 1\%$. Koeficijenti korelacije bili su: za I. skupinu 0,998, II. skupinu 0,973 i III. skupinu 0,423. Referentni interval za COHb uz primjenu NOVA CO-oksometra nešto je viši od intervala koji se uobičajeno nalaze u literaturi, te iznosi <5% za nepušače, odnosno <10% za pušače. U kliničkom otkrivanju akutnog trovanja ugljičnim monoksidom (>20% ukupnog hemoglobina) oba spektrofotometrijska postupka mogu se primijeniti s podjednakom pouzdanošću.

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P18-2

Analitička procjena semikvantitativnog određivanja psihoaktivnih supstancija analizatorom Olympus AU 640

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Uporaba mokraće kao biološkog uzorka izbora pri pretraživanju na psihoaktivne supstancije (droge) ima ograničenu vrijednost zbog velikih intra- i inter-individualnih varijacija samog uzorka, ali i zbog ograničene specifičnosti imunokemijskih metoda pretraživanja, pa se rezultati izražavaju kvalitativno (pozitivan/nije dokazan). Novija generacija imunokemijskog određivanja reagensima Olympus omogućuje izdavanje semikvantitativnog (brojčanog) rezultata. Cilj rada bila je analitička procjena nove generacije reagensa za određivanje koncentracije droga (benzodiazepini, barbiturati, amfetamini, kokain, opijati, THC i metadon), mjerena spektrofotometrijskom metodom na analizatoru Olympus AU 640. Načelo metode se

ing from 3% to 5% of total hemoglobin were obtained by blood sample analysis of non-smoking patients not in contact with the sources of CO, performed on a NOVA CO-oximeter. As these results were above the current reference values (<1.5% non-smokers, <5% smokers), the aim of the study was to investigate the sensitivity of the NOVA CO-oximeter, to compare it with manual spectrophotometric method, and to establish applicability of the current reference intervals. A total of 81 whole blood samples were analyzed in duplicate. Subjects were divided into three groups: acutely intoxicated (I), without contact with CO sources (II), and smokers (III). The mean COHb values determined on the NOVA instrument and by manual procedure were ($\chi \pm SD$): $25 \pm 11\%$ and $14 \pm 10\%$ in group I, $5 \pm 1\%$ and 0 ± 0 in group II, and $8 \pm 2\%$ and $1 \pm 1\%$ in group III, respectively. Correlation coefficients were 0.998, 0.973 and 0.423 in groups I-III, respectively. Accordingly, the reference intervals of COHb obtained by using NOVA CO-oximeter were higher than the intervals commonly found in the literature, i.e. <5% and <10% for nonsmokers and smokers, respectively. Both spectrophotometric procedures are equally reliable for clinical detection of acute carbon monoxide intoxication (>20% of total hemoglobin).

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P18-2

Analytical evaluation of semi-quantitative determination of psychoactive substances on Olympus AU 640 analyzer

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The use of urine as a biological sample of choice on screening for psychoactive substances (drugs of abuse) is of limited value because of great intra- and inter-individual variation within the sample as well as for limited specificity of the immunochemistry methods of screening, with qualitatively expressed results (positive/not detected). The new generation of immunochemistry determination by use of Olympus reagents allows for generation of semi-quantitative (numerical) result. The aim of the study was to perform analytical evaluation of the new generation of reagents for determination of drug concentration (benzodiazepines, barbiturates, amphetamines, cocaine, opiates, THC, and methadone)

zasniva na bakterijskoj beta-galaktozidazi koja je sintetizirana u dva inaktivna fragmenta, čijim spontanom spajanjem nastaje aktivan enzim koji cijepa supstrat (ciljnu drogu), pri čemu se spektrofotometrijski mjeri promjena boje supstrata. Analitička procjena novog semikvantitativnog određivanja droga prema ECCL obuhvaća nepreciznost u seriji određivanjem svake droge u mokraći deset puta tijekom dva dana, nepreciznost iz dana u dan određivanjem svake droge u mokraći tri puta tijekom deset dana, dok se netočnost određivala u uzorcima svih droga u mokraći s niskim, srednjim i visokim koncentracijama tri puta. Kao uzorci mokraće rabljeni su komercijalni kontrolni uzorci tvrtke Olympus, kao i nasumični pozitivni uzorci ovisnika. Rezultati analitičke procjene prema ECCL: nepreciznost u seriji iznosila je za THC, barbiturate, amfetamine/metamfetamine, benzodiazepine, kokain, opijate i metadon 6,7%, 3,6%, 3,2%, 3,4%, 5,4%, 2,2% i 2,4%, dok je reproducibilnost bila 2,3%, 2,1%, 3,6%, 3,8%, 2,7%, 3,6% i 2,4%, a netočnost 1,5%, 2,0%, 1,5%, 2,2%, 1,0%, 2,2% i 2,1%. Na temelju dobivenih rezultata zaključak je kako reagensi za određivanje droga zadovoljavaju gore navedene kriterije pretraživanja mokraće brzinom, jednostavnošću izvedbe i većom točnošću u odnosu na stariju generaciju imunokemijskog određivanja.

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P18-3

Analitička procjena aparata GEM Premier 3000

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Poremećaji acido-bazične ravnoteže i elektrolita česti su u kliničkoj praksi. Brza i točna dijagnoza, te odgovarajuće liječenje takvih poremećaja od velikog su značenja. Razvoj tehnologije, a naročito senzorske tehnologije, doveo je do izrade analizatora koji iz uzorka pune krvi brzo i jednostavno određuju spomenute analite ne samo u laboratoriju nego i uz bolesnika (POCT). GEM Premier 3000 je prijenosni aparat za brzu analizu uzoraka pune krvi uz bolesničku postelju (POCT). Aparat određuje vrijednosti pH, pCO₂, pO₂, Na⁺, K⁺, Ca⁺⁺, glukoze, laktata i hematokrita. Cilj rada bila je analitička procjena aparata GEM Premier

by spectrophotometric method on an Olympus AU 640 analyzer. The principle of the method is based on bacterial beta-galactosidase that is synthesized in two inactive fragments, their spontaneous coupling resulting in the production of active enzyme which splits the substrate (target drug), whereby the change in the substrate color is being spectrophotometrically measured. Analytical evaluation of the new semi-quantitative drug determination according to ECCL includes serial imprecision by determination of each drug in urine on ten occasions during two days, day to day imprecision by determination of each drug in urine on three occasions during ten days, and inaccuracy by determination of all drugs tested in urine samples with low, intermediate and high concentrations on three occasions. Commercial control samples provided by Olympus and random positive samples from drug addicts were used as urine samples. Analytical evaluation according to ECCL produced the following results: serial imprecision 3.4%, 5.4%, 2.2% and 2.4%; reproducibility 2.3%, 2.1%, 3.6%, 3.8%, 2.7%, 3.6% and 2.4%; and inaccuracy 1.5%, 2.0%, 1.5%, 2.2%, 1.0%, 2.2% and 2.1% for THC, barbiturates, amphetamines/metamphetamines, benzodiazepines, cocaine, opiates and methadone, respectively. Study results indicated the reagents for drug determination to meet the above criteria for urine screening, offering a rapid, simple to perform and more accurate tool as compared with the previous generation of immunochemistry drug determination.

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P18-3

Analytical evaluation of the GEM Premier 3000 instrument

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Acid-base balance and electrolyte impairments are frequently encountered in clinical practice. Rapid and accurate diagnosis followed by appropriate treatment for these disturbances are of utmost importance. Technological advancement, in sensor technology in particular, has led to the advent of analyzers for fast and simple determination of these analytes from whole blood samples not only in a laboratory but also as the point-of-care testing (POCT). GEM Premier 3000 is a portable instrument for rapid POCT analysis of whole blood samples. The following analytes can be determined on the instrument: pH, pCO₂,

3000. Ispitivanja su provedena u skladu s preporukama ECCLS. Analitička procjena obuhvatila je nepreciznost u seriji, nepreciznost iz dana u dan, netočnost i usporedna uspoređivanja u uzorcima bolesnika. Nepreciznost u seriji i netočnost ispitane su višekratnim određivanjem (20 puta) analita u jednom danu u kontrolnim uzorcima Critical Care QC Control 9 (Level 1, 2 i 3) proizvođača Instrumentation Laboratory. Nepreciznost iz dana u dan ispitana je određivanjem analita tijekom 20 dana u kontrolnim uzorcima Rapid QC Complete (Level 1, 2 i 3) proizvođača Bayer HealthCare. Analizirano je 160 hepariniziranih uzoraka pune krvi na ispitivanom aparatu i referentnom aparatu Ciba Corning 865 (Bayer HealthCare) koji je uključen u program vanjske procjene kvalitete rada, ali ne može određivati glukozu, laktat i hematokrit. Maksimalne vrijednosti koeficijenta korelacije (%) za pojedini analit za sve tri razine za nepreciznost u seriji iznosile su: pH 0,03; pCO₂ 1,87; pO₂ 2,47; Na 1,01; K 1,83; Ca⁺⁺ 1,09; glukoza 1,89 i laktat 3,46.

Maksimalne vrijednosti koeficijenta korelacije (%) za pojedini analit za sve tri razine za nepreciznost iz dana u dan iznosile su: pH 0,07; pCO₂ 3,80; pO₂ 4,65; Na 0,54; K 0,72; Ca⁺⁺ 1,03; glukoza 2,70 i laktat 9,35. Rezultati ispitivanja netočnosti (R%, maksimalna vrijednost): pH -0,14; pCO₂ 4,19; pO₂ -4,16; Na 0,59; K -2,24; Ca⁺⁺ 2,92; glukoza 4,53 i laktat 13,59. Rezultati usporednih određivanja uzoraka bolesnika obrađeni su linearnom regresijskom analizom (Passing Bablok). Izračunati su koeficijenti korelacije za svaki analit i iznosili su: pH 0,991; pCO₂ 0,985; pO₂ 0,988; Na 0,921; K 0,992; Ca⁺⁺ 0,973. Statistička obrada dobivenih podataka pokazala je zadovoljavajuću nepreciznost u seriji za sve analite (KV <3,46%), dok je nepreciznost iz dana u dan bila zadovoljavajuća za sve analite (KV <4,65%) osim za laktat (KV=9,35%). Rezultati ispitivanja netočnosti bili su zadovoljavajući za sve analite (R <4,53%) osim za laktat (R=13,59%). Koeficijenti korelacije pokazali su visoku podudarnost rezultata svih određivanih analita (r=0,921-0,992). Temeljem dobivenih rezultata zaključujemo da je GEM Premier 3000 jednostavan i pouzdan analizator (osim analize laktata). Visoka podudarnost sa standardnom metodom čini ga pogodnim za rad na bolničkom odjelu, jer omogućava brz i siguran rad.

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pO₂, Na⁺, K⁺, Ca⁺⁺, glucose, lactate and hematocrit. The aim of the study was analytical evaluation of the GEM Premier 3000 device. Testing was performed according to ECCLS recommendations. Analytical evaluation included serial imprecision, day to day imprecision, inaccuracy and parallel comparisons in patient samples. Serial imprecision and inaccuracy were tested by multiple determinations (20 times) of the analytes during a day in the control samples Critical Care QC Control 9 (Level 1, 2 and 3) manufactured by Instrumentation Laboratory. Day to day imprecision was tested by analyte determination for 20 days in the control samples Rapid QC Complete (Level 1, 2 and 3) manufactured by Bayer HealthCare. A total of 160 heparinized whole blood samples were analyzed on the instrument evaluated and on the reference device Ciba Corning 865 (Bayer HealthCare), included in the External Quality Assessment program; glucose, lactate and hematocrit cannot be determined on the latter. The maximal correlation coefficients (%) for serial imprecision at all three levels for particular analytes were as follows: pH 0.03; pCO₂ 1.87; pO₂ 2.47; Na 1.01; K 1.83; Ca⁺⁺ 1.09; glucose 1.89; and lactate 3.46. The maximal correlation coefficients (%) for day to day imprecision at all three levels for particular analytes were as follows: pH 0.07; pCO₂ 3.80; pO₂ 4.65; Na 0.54; K 0.72; Ca⁺⁺ 1.03; glucose 2.70; and lactate 9.35. Results of testing for inaccuracy (R%, maximal value): pH -0.14; pCO₂ 4.19; pO₂ -4.16; Na 0.59; K -2.24; Ca⁺⁺ 2.92; glucose 4.53; and lactate 13.59. Results of parallel determinations in patient samples were processed by linear regression analysis (Passing Bablok). The calculated coefficients of correlation for particular analytes were as follows: pH 0.991; pCO₂ 0.985; pO₂ 0.988; Na 0.921; K 0.992; Ca⁺⁺ 0.973. Statistical analysis of the data obtained yielded a satisfactory serial imprecision for all analytes (CV <3.46%), whereas day to day imprecision was satisfactory for all analytes (CV <4.65%) except for lactate (CV=9.35%). Testing for inaccuracy yielded satisfactory results for all analytes (R <4.53%) except for lactate (R=13.59%). Coefficients of correlation revealed a high result concordance for all the analytes determined (r=0.921-0.992). Study results have indicated that GEM premier 3000 is a simple and reliable analyzer (with the exception of lactate). The high concordance with the standard method makes it suitable for work at hospital ward for its rapid and reliable performance.

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P19 – Pedijatrijska laboratorijska dijagnostika, P19-1 (UP6-1)

Enzimске aktivnosti AP, AST, ALT, LDH i GGT u novorođenčadi i babinjača

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Cilj studije je bio procijeniti enzimsku aktivnost alkalne fosfataze (AP), aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), laktat dehidrogenaze (LDH) i gama-glutamil transpeptidaze (GGT) u babinjača i njihove novorođenčadi. U studiju smo uključili skupinu babinjača (n=93) i skupinu novorođenčadi (n=94; uzorci krvi uzeti su iz pupčane vene) na Klinici za ginekologiju i porodništvo u Skopju, te kontrolnu skupinu zdravih studenata u dobi od 18-22 godine (n=77). Enzimsku aktivnost AP, AST, ALT, LDH i GGT određivali smo standardnim kinetičkim metodama prema IFCC na biokemijskom analizatoru Cobas Mira Plus.

	AP U/L	AST U/L	ALT U/L	LDH U/L	GGT U/L
Kontrole (n=77)	61,31 ±18,45	24,89 ±6,38	16,57 ±4,99	185,83 ±49,84	10,91 ±5,09
Babinjače (n=93)	153,19 ±53,89	24,23 ±21,18	18,84 ±19,22	199,40 ±61,81	64,75± 42,42
Novo- rođenčad (n=94)	132,03 ±51,06	35,06 ±15,13	17,67 ±5,55	347,19 ±107,25	64,75 ±42,42

Sve skupine ispitanika bile su negativne na HBsAg i anti HCV. Rezultati su prikazani u tablici ($\chi \pm SD$):

Statistička analiza enzimske aktivnosti pokazala je značajno povišenje aktivnosti AP i GGT ($p=0,000$ oboje) u skupini babinjača u usporedbi s kontrolnom skupinom. Tome je razlog fiziološka kolestaza tijekom trudnoće. Iako statistički povišene, enzimske aktivnosti u skupini novorođenčadi nisu bile usporedive s onima u kontrolnoj skupini. Povišenje enzimskih aktivnosti u ovoj skupini vjerojatno su bile uzrokovane prilagodbom na novu životnu sredinu.

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P19 – Pediatric laboratory diagnostics, P19-1 (UP6-1)

Enzyme activities of AP, AST, ALT, LDH and GGT in newborns and puerperae

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The aim of the study was to estimate enzyme activities of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and gamma- glutamyltranspeptidase (GGT) in women in puerperium and their newborns. The study included a group of puerperae (n=93) and a group of newborns from Skopje University Department of Gynecology and Obstetrics (n=94) (blood was taken from umbilical vein); and a control group of healthy students aged 18-22 (n=77). Enzyme activities of AP, AST, ALT, LDH and GGT were determined by standard kinetic methods according to IFCC (International Federation of Clinical Chemistry) on a Cobas Mira Plus biochemistry analyzer. All study groups were HBsAg and anti HCV negative. Results are presented in table ($\chi \pm SD$):

	AP U/L	AST U/L	ALT U/L	LDH U/L	GGT U/L
Controls (n=77)	61.31 ±18.45	24.89 ±6.38	16.57 ±4.99	185.83 ±49.84	10.91 ±5.09
Puerperae (n=93)	153.19 ±53.89	24.23 ±21.18	18.84 ±19.22	199.40 ±61.81	64.75± 42.42
Newborns (n=94)	132.03 ±51.06	35.06 ±15.13	17.67 ±5.55	347.19 ±107.25	64.75 ±42.42

Statistical evaluation of enzyme activities showed a significant elevation in the activities of AP and GGT ($p=0.000$ both) in the group of puerperae as compared with control group. This is due to physiological cholestasis during pregnancy. Although statistically elevated, enzyme activities in the group of newborns were not comparable with the control group. The elevation of enzyme activities in this group was probably caused by adaptation to the new life environment.

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P19-2

Određivanje koncentracije kalprotektina u stolici u diferencijalnoj dijagnostici kroničnih upalnih crijevnih bolesti kod djece

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Kalprotektin je S100 protein koji kod upale nastaje iz aktiviranih ili odumrlih neutrofilnih granulocita, monocita, makrofaga i drugih epitelnih stanica. Brojna istraživanja potvrđuju da je koncentracija kalprotektina u stolici značajno povišena kod kroničnih upalnih crijevnih bolesti (KUCB) i kolorektalnog karcinoma, kod upotrebe određenih lijekova (NSAID, PPI) te kod drugih bolesti (karcinom želuca, polipi kolona, akutni enterokolitis, ciroza jetre). Kalprotektin je vrlo koristan novi parametar u dijagnostici KUCB. Određivanje koncentracije kalprotektina u stolici uvodi se kao rutinska pretraga. To je neinvazivan test za određivanje upale u donjem dijelu probavnog sustava kod djece i odraslih. Granična vrijednost za djecu staru od 4 do 17 godina je 50 µg/g stolice. Cilj našega istraživanja je bio utvrditi ulogu testa Calprest: 1) u diferencijalnoj dijagnostici KUCB kod djece s gastrointestinalnim simptomima i 2) u razlikovanju Crohnove bolesti (CB) od ulceroznog kolitisa (UK). Koncentracija kalprotektina u stolici određena je metodom ELISA (Calprest, Eurospital, Italija). U ispitivanje smo uključili djecu s endoskopski i histološki potvrđenom KUCB (n=25), od toga 18 s CB i 7 s UK, te kontrolnu skupinu djece bez KUCB (n=18). Rezultati su prikazani u tablici:

	KUCB	Kontrolna skupina	p
Koncentracija u stolici (µg/g)	138,7 (9,4-452,5)	27,4 (9,4-371,3)	0,000
	CB	UK	p
	275,4 (12,2-452,5)	100,6 (9,4-452,3)	0,717

Vrijednosti su izražene kao medijan (raspon), uz razinu vjerojatnosti $p < 0,05$. Razlika u koncentraciji kalprotektina između KUCB i kontrolne skupine bila je statistički značajna. Rezultati su pokazali da test Calprest za određivanje KUCB kod djece s probavnim simptomima ima osjetljivost 92%, specifičnost 66%, pozitivnu prognostičnu vrijednost 79%, negativnu prognostičnu vrijednost 86% i točnost 81%. Kod djece sa CB i UK nismo dobili statistički značajne razlike u koncentraciji kalprotektina. Test Calprest je

P19-2

Fecal calprotectin in the differential diagnosis of childhood inflammatory bowel disease

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Calprotectin is an S100 protein which is released in inflamed tissues by activation or lysis of neutrophils, monocytes, macrophages and some epithelial cells. Increased fecal calprotectin levels have been found in inflammatory bowel disease (IBD) and colorectal cancer as well as in patients using drugs like NSAID and PPI or being diagnosed with gastric cancer, colonic polyps, infectious gastroenteritis, liver cirrhosis and some other diseases. Calprotectin is a highly useful new parameter in the diagnosis of IBD, and determination of fecal calprotectin has been introduced as a routine test. It is a noninvasive test to indicate inflammation in lower gastrointestinal tract in children and adults. The cut-off level in children aged 4 to 17 years is 50 µg/g feces. The aim of this study was to evaluate the use of Calprest in (1) differential diagnosis of IBD in children with gastrointestinal symptoms; and (2) distinguishing between Crohn's disease (CD) and ulcerative colitis (UC). Stool samples were obtained from children who underwent endoscopy and had histopathologic findings suggesting IBD (n=25; CD: n=18, UC: n=7) and from a control group of children without IBD (n=19). Fecal calprotectin was measured using a simple ELISA test, Calprest (Eurospital, Italy). Results are presented in table below:

	IBD	Controls	p
Fecal calprotectin (µg/g)	138.7 (9.4-452.5)	27.4 (9.4-371.3)	0.000
	CB	UC	p
	275.4 (12.2-452.5)	100.6 (9.4-452.3)	0.717

Values are expressed as median (range); $p < 0.05$ = statistically significant difference.

The difference in median fecal calprotectin concentrations between IBD and control group was highly significant. According to study results, Calprest test has a 92% sensitivity 68% specificity, 79% positive predictive value, 86% negative predictive value and 81% accuracy to detect IBD in children with gastrointestinal symptoms. There was no statistically significant difference between

obilježen velikom osjetljivošću i niskom specifičnošću za određivanje KUCB kod djece. Pozitivan rezultat (>50 µg/g stolice) kod djece s gastrointestinalnim simptomima upućuje na dodatnu endoskopsku dijagnostičku obradu. Razlikovanje između CB i UK ovim testom nije moguće.

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P19-3

Vrijednosti kolesterola u djece predškolske dobi

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Nepravilna prehrana u najranijoj dobi udružena s nasljednim čimbenicima uvelike povećava rizik za razvoj nekih kroničnih bolesti. Cilj istraživanja bila je usporedba izmjenjenih vrijednosti kolesterola kao značajnog rizičnog čimbenika s preporučenim vrijednostima za dobnu skupinu predškolske djece (manje od 4,7 mmol/L), te usporedba s indeksom tjelesne mase. Ispitivanjem je obuhvaćena skupina od 458 djece (243 dječaka i 215 djevojčica) rođenih 1999. i 2000. godine, s područja grada Pule i okolice, koja su u laboratorij upućena radi redovnog sistematskog pregleda za upis u prvi razred osnovne škole. Nakon izrade KKS iz uzoraka plazme izmjerene su vrijednosti ukupnog kolesterola na automatskom analizatoru Architect c8000 (Abbott, SAD). U slučajevima kada su vrijednosti kolesterola bile iznad preporučenih (4,7 mmol/L) određene su vrijednosti HDL i LDL kolesterola. Izmjerene vrijednosti statistički su obrađene i iskazane srednjom vrijednosti (SV), medijanom (m), standardnom devijacijom (SD), koeficijentom varijacije (KV). Odnos prema preporučenim vrijednostima iskazan je značajnošću razlike (p). Medijan vrijednosti kolesterola u cijeloj skupini djece iznosio je 4,16 mmol/L; djevojčice su imale vrijednost kolesterola 4,29 mmol/L, a dječaci 4,09 mmol/L. Kolesterol viši od preporučenih 4,7 mmol/L imalo je 22% djece kod kojih je medijan za HDL iznosio 1,81 mmol/L, a za LDL 3,38 mmol/L; 11,5% djece imalo je kolesterol viši od preporučenih vrijednosti za odrasle, uz HDL 1,80 mmol/L i LDL 3,41 mmol/L. Indeks tjelesne mase u skupini djece s povišenim vrijednostima kolesterola pokazao je visok koeficijent korelacije ($r=0,966$). Iako su vrijednosti kolesterola u plazmi za oko 3% niže nego u serumu, relativno velik postotak djece ove ispitne skupine s povišenim vrijednostima kolesterola i indeksom tjelesne mase ukazuje na pojačanu potrebu re-

the values in CD and UC. In conclusion, Calprest is a test of high sensitivity and low specificity for detecting childhood IBD. A positive test (>50 µg/g) in a child with gastrointestinal symptoms will facilitate the decision to proceed with additional studies including endoscopy. The test cannot be used to distinguish between CD and UC.

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P19-3

Cholesterol values in preschool children

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Unhealthy nutrition in the earliest age combined with inherited factors may increase the risk for the development of some chronic diseases. The purpose of the study was to compare the measured values of cholesterol (as a significant risk factor) with recommended values for preschool children (less than 4.7 mmol/L) and to compare it with body mass index. The study included 458 children (243 boys and 215 girls) born in Pula and its surroundings in 1999 and 2000. The children were referred to the laboratory as part of the regular medical check-up prior to school enrolment. Upon complete blood count, total cholesterol values were measured in plasma samples on an Architect c8000 (Abbott, USA) autoanalyzer. In cases when cholesterol values were above the recommended level (4.7 mmol/L), HDL and LDL cholesterol values were also measured. Measured values were statistically processed and expressed as mean (M), median (m), standard deviation (SD), and coefficient of variation (CV). Relation to the recommended values was shown by the significance of difference (p). The median for cholesterol values in the whole group was 4.16 mmol/L; cholesterol value was 4.29 mmol/L in girls and 4.09 mmol/L in boys; 22% of the children had cholesterol higher than the recommended level of 4.7 mmol/L. In this group, the median for HDL was 1.81 mmol/L and for LDL 3.38 mmol/L; 11.5% of the children had cholesterol higher than the recommended level for adults, with HDL 1.80 mmol/L and LDL 3.41 mmol/L. There was a high correlation coefficient ($r=0.966$) for body mass index in children with high cholesterol values. Although plasma cholesterol values are by some 3% lower than in those in serum, a relatively large percentage of study children had high cholesterol values and high body mass index. Study results pointed to the need of regular control

dovite kontrole ovih parametara već u najranijoj životnoj dobi, zajedno sa sticanjem navika pravilne prehrane.

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P19-4

Kondenzat izdaha u djece – neinvazivna metoda za praćenje biomarkera u djece s respiracijskim bolestima

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Cilj je bio odrediti biomarkere u kondenzatu izdaha (KI) i usporediti ih s FENO te plućnom funkcijom u djece s astmom i/ili gastroezofagusnim refluksom (GER). Ispitivanje je provedeno u djece s astmom i/ili GER. KI je uzorkovan uređajem ECoScreen, Jaeger. U KI su izmjereni pH, pCO₂, koncentracija CO₂, koncentracija željeza. FENO je mjeren uređajem NIOX, Aerocrine AB, a ventilacijski parametri spirometrom SanoScope, Schiller. Ventilacija pluća bila je u rasponu od urednih vrijednosti do srednje teških opstruktivno-restriktivnih smetnja. U 54% ispitanika dokazan je patološki GER. Utvrđena je statistički značajna korelacija između pH u KI i FENO u izdah, te koncentracije željeza u KI i parametara plućne funkcije PEF, MEF75, MEF50. Kondenzat izdaha uzorkuje se neinvazivno, može se ponavljati, a prikladan je i za dječju dob. Nakon postupaka optimiranja i standardizacije, kondenzat izdaha mogao bi postati metodom izbora u dijagnostici i praćenju plućnih bolesti u djece.

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of these parameters in the earliest age and of upgrading the general awareness of the healthy and balanced diet.

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P19-4

Exhaled breath condensate in children – a noninvasive method for monitoring biomarkers in children with respiratory diseases

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The aim was to determine biomarkers in exhaled breath condensate (EBC) and to compare them with FENO and lung function tests in children with asthma and gastroesophageal reflux (GER). Children with asthma and GER were included in the study. EBC was sampled by ECoScreen, Jaeger. pH, pCO₂, CO₂ concentration and iron concentration were measured in EBC. Breath FENO was determined by use of a NIOX, Aerocrine AB. Lung function was evaluated using a SanoScope spirometer, Schiller. GER was found in 54% of children. Results of lung function tests ranged from normal to mild obstructive-restrictive changes. There was a statistically significant correlation between both EBC pH and breath FENO, and between EBC iron concentration and lung function tests PEF, MEF75 and MEF50. EBC is a noninvasively obtained sample, it is reproducible and thus applicable in childhood. Upon optimization and standardization, the analysis of EBC could be the method of choice in the diagnosis and monitoring of lung diseases in children.

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P19-5

Dijagnostička točnost C-reaktivnog proteina, ukupnog broja leukocita, broja trombocita i diferencijalne krvne slike u sepsi novorođenčadi

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Određivanje koncentracije C-reaktivnog proteina (CRP), ukupnog broja leukocita, broja trombocita i diferencijalne krvne slike (DKS) predstavljaju dijagnostički "sepsa-screening" pristup pri kliničkoj sumnji na novorođenačku sepsu. Cilj rada bio je ispitati dijagnostičku točnost navedenih parametara u novorođenčadi sa sepsom. U ispitivanje je bilo uključeno 58 novorođenčadi sa sepsom i 328 zdrave novorođenčadi. Koncentracija CRP određena je imunoturbidimetrijskom metodom povećane osjetljivosti (Olympus AU2700). Kompletna krvna slika određena je na automatskom hematološkom brojaču (Cell-Dyn 3200, Abbott Diagnostics). DKS određena je mikroskopskom metodom u obojenom razmazu periferne krvi. Dijagnostička točnost ispitana je analizom ROC (engl. *receiver operating characteristic*). Analiza ROC pokazala je izvrsnu diskriminacijsku učinkovitost CRP (AUC=0,970) uz optimalnu graničnu vrijednost (*cut-off*) 11,5 mg/L kojom se postiže dijagnostička osjetljivost (Os) 89,7% i specifičnost (Sp) 96,0%. Među parametrima DKS prihvatljivu dijagnostičku točnost pokazali su omjer nesegmentiranih i ukupnih neutrofilnih granulocita (I/T, AUC=0,789, Os=77,2%, Sp=73,2%, *cut-off* >0,11), omjer nesegmentiranih i segmentiranih neutrofilnih granulocita (I/M, AUC=0,774, Os=75%, Sp=73,6%, *cut-off* >0,13) te postotak nesegmentiranih neutrofilnih granulocita (AUC=0,784, Os=82,8%, Sp=66,2% *cut-off* >5%). Apsolutni broj neutrofilnih granulocita (AUC=0,607, Os=54,7%, Sp=66,1%) i limfocita (AUC=0,546, Os=45,6%, Sp=68%), postotak segmentiranih neutrofilnih granulocita (AUC=0,499, Os=51,7%, Sp=54,7%) i limfocita (AUC=0,659, Os=82,5%, Sp=47,9%), kao i ukupni broj leukocita (AUC=0,629, Os=65,5%, Sp=56,9%) i broj trombocita (AUC=0,542, Os=62,1% Sp=49,2%) pokazali su nezadovoljavajuću diskriminacijsku učinkovitost u dijagnostici novorođenačke sepse. Zaključuje se kako je koncentracija CRP parametar najveće dijagnostičke točnosti u sepsi novorođenčadi. Prihvatljivu diskriminacijsku učinkovitost u dijagnostici novorođenačke sepse pokazali su omjeri I/T i I/M te postotak nesegmentiranih neutrofilnih granulocita. Ukupni broj leukocita i broj trombocita nisu pouzdani dijagnostički pokazatelji novorođenačke sepse.

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P19-5

Diagnostic accuracy of C-reactive protein, total leukocyte count, platelet count and differential blood count in newborn sepsis

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Determination of C-reactive protein (CRP), total leukocyte count, platelet count and differential blood count represents a "sepsa-screen" diagnostic approach in newborns clinically suspected of sepsis. The aim of the study was to assess diagnostic accuracy of these parameters in newborns with sepsis. The study included 58 newborns with sepsis and 328 healthy newborns. CRP concentration was determined by particle-enhanced immunoturbidimetric assay (Olympus AU2700). Complete blood count was determined on an automated blood counter (Cell-Dyn 3200, Abbott Diagnostics). Differential blood count was determined by microscopic method in stained peripheral blood smear. Diagnostic accuracy was evaluated using the receiver operating characteristic (ROC) analysis. ROC analysis showed excellent discriminating power of CRP (AUC=0.970) at optimal cut-off point of 11.5 mg/L, which achieves diagnostic sensitivity (Se) of 89.7% and specificity (Sp) of 96.0%. Among the parameters of differential blood count, acceptable diagnostic accuracy was obtained for immature to total neutrophil ratio (I/T ratio, AUC=0.789, Se=77.2%, Sp=73.2%, cut-off >0.11), immature to mature neutrophil ratio (I/M ratio, AUC=0.774, Se=75%, Sp=73.6%, cut-off >0.13) and percentage of band cells (AUC=0.784, Se=82.8%, Sp=66.2%, cut-off >5%). Absolute neutrophil count (AUC=0.607, Se=54.7%, Sp=66.1%) and lymphocyte count (AUC=0.659, Se=82.5%, Sp=47.9%), percentages of mature neutrophils (AUC=0.499, Se=51.7%, Sp=54.7%) and lymphocytes (AUC=0.659, Se=82.5%, Sp=47.9%) as well as total leukocyte count (AUC=0.629, Se=65.5%, Sp=56.9%) and platelet count (AUC=0.542, Se=62.1% Sp=49.2%) showed unacceptable discriminating power in the diagnosis of neonatal sepsis. Thus, the concentration of CRP showed the best diagnostic accuracy in the diagnosis of neonatal sepsis. Acceptable discriminating power in the diagnosis of neonatal sepsis showed I/T and I/M ratios and percentage of band cells. Total leukocyte count and platelet count are not reliable diagnostic indicators of neonatal sepsis.

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P19-6

Dijagnostička vrijednost "klasičnih" biljega neonatalne sepse

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Neonatalna sepsa ili bakteremija novorođenčadi, uz neusuglašenu terminologiju za sistemski odgovor na bakterijski infekt u novorođenčadi zasad nema ni savršene laboratorijske dijagnostičke pretrage temeljem koje će se i kod nesimptomatske novorođenčadi brzo uvesti antibiotska terapija. Potvrda infekcije nalazom hemokulture predugo traje, tako da se u sadašnjoj dijagnostici neonatalne sepse i u KB Sestre milosrdnice na Klinici za ginekologiju i porodništvo primjenjuju "klasični" biljezi. Oni obuhvaćaju određivanje kompletne krvne slike, omjera nesegmentiranih i segmentiranih granulocita (I/T) i CRP u sve novorođenčadi u prvih 24 sata života. U primjeni je i protokol SNA-P II za procjenu fizioloških funkcija novorođenčadi. Cilj je bio utvrditi dijagnostičku i prognostičku vrijednost standardnih pretraga koje se rutinski rade kroz 24 sata za svu novorođenčad, nalazi kojih su bitni neonatolozima u svakodnevnom odlučivanju o pravodobnom uvođenju antibiotske terapije. U 69 novorođenčadi rođenih kroz mjesec dana na Klinici za ginekologiju i porodništvo određena je kompletna krvna slika na hematološkim analizatorima Beckman Coulter HmX i Micro Diff II. Omjer nesegmentiranih i segmentiranih granulocita određen je iz krvnog razmaza koji su pregledali medicinski biokemičari s iskustvom iz neonatalne hematologije. Koncentracija CRP određena je na analizatoru Olympus AU 640 uz uporabu visoko osjetljivog reagensa (HSCRP). U tumačenju i obradi rezultata slijedili smo važeći referentni raspon za zdravu novorođenčad u prvih 24 sata za sve određivane pretrage. Koncentraciju CRP iznad gornje granice (preporuka za bakteremiju je 10 mg/L) imalo je 83,7% novorođenčadi, 71,4% ih je imalo povišene leukocite ($>17,8 \times 10^9/L$). Samo je 2,3% novorođenčadi imalo povišenu vrijednost omjera I/T. Na osnovi izmijenjenih fizioloških funkcija i laboratorijskih pretraga kod osmoro novorođenčadi učinjena je hemokultura, a pozitivan nalaz je zabilježen kod dvoje djece. U zaključku, "klasične" pretrage sepse, CRP i broj leukocita te omjer I/T, imaju vrlo dobru osjetljivost i visoku negativnu prediktivnu vrijednost u dijagnostici neonatalne sepse. Naši rezultati potvrđuju promišljanja da bi se za pravilnu interpretaciju graničnih vrijednosti za određivane biljege trebalo postaviti vrijednosti koje slijede fiziološku dinamiku njihovih promjena unutar prvih 48 sati života.

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P19-6

Diagnostic value of "classic" markers of neonatal sepsis

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There is an urgent need to know whether the newborn has developed sepsis, in order to introduce appropriate treatment as early as possible. Confirmation of the diagnosis (blood culture) may take time, and diagnostic tests are used to obtain quick indication of the infection status. These tests are not perfect. Some real cases of infection will produce negative test results, whereas some children free from infection will test positive. The potential usefulness of the test will depend on the clinical condition of the child. Current diagnostic approach in our hospital includes determination of "classic" markers of sepsis in all newborns: total leukocyte count, absolute neutrophil count, immature/total neutrophil count ratio (I/T) and C-reactive protein (CRP) within 24 hours of birth, and SNAP II physiology scores. The aim of the present study was to investigate the sensitivity and specificity of standard diagnostic tests for bacterial infection-sepsis of newborns that may be important for clinicians in their daily work at Department of Neonatology, Sestre milosrdnice University Hospital. We determined complete blood count (CBC) on the Cell Dyn Beckman Coulter HmX and Micro Diff II analyzers. The immature to total neutrophil ratio (I/T) was determined by a hematology technologist. CRP concentration was measured by the HSCRP (high sensitive CRP) reagent on an Olympus AU 640 analyzer. The analysis was performed within the first 24 hours of life in all neonates born during a one-month period (n=69) at our hospital. On result interpretation, we followed reports on the upper marker limits within the first 24 h of life in healthy newborns. CRP was above the reference range (10 mg/L) in 83.7%, WBC ($>17,8 \times 10^9/L$) in 71.4%, and I/T ($>0,25$) in 2.3% of the newborns. Only two of eight newborns included in blood culture documentation had a "culture-proven" sepsis. In conclusion, the classic markers of neonatal sepsis, CRP and WBC, have a good sensitivity and negative prognostic value. Our results support the opinion that correct interpretation of cut-off values for the respective markers would require such values to be set that follow the physiological dynamics of their variation during the first 48 h of life.

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P19-7

Vrijednost mjerenja koncentracije masnih kiselina vrlo dugih lanaca u ranom otkrivanju bolesnika i heterozigota za X-vezanu adrenoleukodistrofiju

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X-vezana adrenoleukodistrofija (X-ALD) je nasljedna neurometabolična bolest obilježena centralnom ili perifernom demijelinizacijom i adrenalnom insuficijencijom. Gen za X-ALD normalno je odgovoran za sintezu peroksidomskog, transportnog adrenoleukodistrofičnog proteina (ALDP). Defektni ALDP uzrokuje, nepoznatim mehanizmima, poremećaj razgradnje masnih kiselina vrlo dugih lanaca (MKVDL), u najvećoj mjeri C26:0, a posljedica je patološko nakupljanje MKVDL u tkivnim i krvnim lipidima, osobito u korteksu nadbubrežne žlijezde i živčanom sustavu. Metabolična nepravilnost, povećana količina MKVDL i biokemijski poremećaj, te smanjena aktivnost acil-CoA sintetaze vrlo dugih lanaca u peroksidomima osnovne su osobine bolesti. Dok se adrenalna insuficijencija u ovih bolesnika liječi vrlo uspješno hormonskom nadomjesnom terapijom, još uvijek nema mogućnosti zaustavljanja i liječenja progredijentnih neuroloških poremećaja. Provođenje probiranja za otkrivanje nosilaca mutiranog gena na X kromosomu za ALD i prijenatalna dijagnostika od posebne su važnosti za prevenciju ove bolesti. Probiranje na nosioce gena za ALD provodi se kombinacijom nekoliko metoda: određivanjem koncentracije zasićenih MKVDL u serumu i kulturi kožnih fibroblasta, imunocitokemijskim određivanjem ALD proteina u kulturi kožnih fibroblasta te analizom DNA. Prikazujemo analitičku vrijednost i doprinos mjerenja MKVDL u otkrivanju nosilaca za X-ALD. Otkriće prvog bolesnika ponukalo nas je da u 18 dostupnih članova obitelji odredimo razine MKVDL. Koncentracije MKVDL u serumu mjerene su kao metilni esteri metodom plinske kromatografije-spektrometrije masa, uz interne standarde obilježene izotopima (stabilna izotopna dilucijska masena spektrometrija). U svim uzorcima određivano je pet parametara: C22:0, C24:0, C26:0, C24:0/C22:0, C26:0/C22:0. Određivanjem koncentracija MKVDL i njihovog međusobnog omjera te diskriminacijske vrijednosti Y iz seruma članova obitelji nađen je jedan oboljeli hemizigot, a za 3 žene je utvrđeno da su heterozigoti za ALD. Koncentracija C26:0 i omjer C26:0/C22:0 bili su značajno povišeni u serumu te su najvažniji, dijagnostički korisni parametri. Diskriminacijska vrijednost Y upućuje

P19-7

The value of very long chain fatty acid screening in the early detection of patients and heterozygotes for X-linked adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD) is an inherited neurometabolic disease presenting with central or peripheral demyelination and impaired function of adrenals. The responsible X-ALD gene normally encodes the peroxisomal adrenoleukodystrophy protein (ALDP). Defective ALDP causes, by unknown mechanisms, impaired degradation of very long chain fatty acids (VLCFAs), predominantly C26, resulting in pathognomonic accumulation of VLCFAs in tissues and blood lipids, notably in the adrenal cortex and nervous system. The metabolic abnormality, elevated levels of VLCFAs, and the biochemical defect, reduced peroxisomal very long-chain acyl-CoA synthetase (VLCs) activity, are the ubiquitous features of the disease. While adrenal dysfunction in X-ALD can be treated with adrenal hormone replacement therapy, there is no treatment for severe neurologic disabilities. Screening for carriers of mutated relevant gene and prenatal diagnosis are very important for the prevention of the disease. The carrier state can be investigated by determining the concentrations of saturated VLCFAs in serum or cultured skin fibroblasts, by looking for the presence of X-ALD protein in cultured skin fibroblasts, and by carrying out mutation analysis. The aim was to estimate the contribution of VLCFA measurement to the identification of the carrier state of X-ALD. Profiles of saturated VLCFAs were studied in 18 members of an affected family. VLCFA levels were analyzed as methyl esters by gas chromatography-mass spectrometry, using internal standards labeled with isotopes (stable-isotope dilution). Five parameters were determined: C22:0, C24:0, C26:0, C24:0/C22:0, C26:0/C22:0. Laboratory results revealed one patient to be hemizygous and three females heterozygous for ALD. The concentration of C26:0 and C26:00/C22:0 ratios were significantly increased in serum. Among VLCFAs, C26:0 (absolute and in proportion to C22:0) are the most important, diagnostically useful parameters. Discrimination value Y suggested women heterozygous for ALD. In conclusion, serum assay for VLCFAs has made it possible to perform large-scale screening of individuals at risk to identify asymptomatic patients with X-ALD.

na ženu heterozigota za ALD. U zaključku, metoda određivanja koncentracije MKVDL u serumu omogućila je probiranje širokog raspona rizičnih osoba u svrhu otkrivanja asimptomatskih bolesnika s X-ALD. Naglašava se potreba mjerenja MKVDL u serumu svih članova ne samo uže, već i šire obitelji bolesnika s ALD, kako bi se otkrili i drugi bolesnici i/ili heterozigoti za ALD. Otkrivanje heterozigota otvara mogućnost za prevenciju bolesti kroz genetsko savjetovanje. Normalne koncentracije MKVDL u serumu u klinički suspektnih ispitanica ne isključuju heterozigotnost. U takvim slučajevima potrebno je odrediti koncentraciju MKVDL u homogenatu kulture kožnih fibroblasta te eventualno napraviti mutacijske analize gena ABCD1.

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P19-8

Usporedba reagensa dvaju proizvođača za određivanje vrijednosti C-reaktivnog proteina u nedonoščadi i novorođenčadi

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C-reaktivni protein (CRP) je reaktant akutne faze koji je u zdravih osoba prisutan u niskim koncentracijama. Patološka stanja kao što su bakterijske infekcije, upale ili razaranja tkiva praćena su povećanjem vrijednosti CRP uslijed otpuštanja upalnih citokina. Određivanje vrijednosti CRP vrlo je važno u dijagnostici i praćenju infekcije u nedonoščadi i novorođenčadi. U novorođenčadi sepsa može početi nespecifičnim simptomima i dijagnosticira se tek na osnovi patoloških laboratorijskih nalaza. Cilj rada bio je ispitati analitičku pouzdanost i reproducibilnost reagensa Full Range CRP tvrtke Randox za određivanje vrijednosti CRP u serumu/plazmi (mjerno područje 0,1-160,0 mg/L) u usporedbi s CRP reagensima tvrtke Olympus kao referentnim reagensima: Olympus CRP reagensom za koncentracijsko područje 5,0-300,0 mg/L i Olympus CRP Latex reagensom namijenjenom ranoj dijagnozi infekcije u nedonoščadi i novorođenčadi. Za Olympus CRP Latex reagens postoje dvije aplikacije: osjetljiva (SCRP, za koncentracijsko područje 0,5-20,0 mg/L) i visoko osjetljiva (HS-CRP, za koncentracijsko područje 0,05-2,00 mg/L). Za sve reagense primijenjene su izvorne baždarni i kontrolne otopine, kao i aplikacije za instrument Olympus AU 640. Ispitivanja su provedena u skladu s preporukama ECCLS.

Diagnostic tests should be offered to all at risk relatives of X-ALD patients and should include members of the extended family. Identification of heterozygotes provides an opportunity for disease prevention through genetic counseling. Normal concentration VLCFAs in clinically suspected females does not exclude carrier state. In these cases, the combined use of fibroblast VLCFA level analysis and possibly ABCD1 gene mutation analysis could detect X-ALD carriers correctly.

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P19-8

Comparison of reagents from two manufacturers for C-reactive protein determination in preterm and term neonates

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C-reactive protein (CRP) is an acute phase reactant, which is found in low concentrations in healthy individuals. Pathologic states such as bacterial infection, inflammation or tissue lesions are associated with an increase in CRP level due to the release of inflammatory cytokines. Determination of CRP is of utmost importance in the diagnosis and monitoring of infection in preterm and term neonates. In neonates, sepsis may begin with unspecific symptoms and is only diagnosed on the basis of pathologic laboratory findings. The aim of the study was to assess analytical reliability and reproducibility of the Full Range CRP reagent (Randox) for CRP determination in serum/plasma (measuring range 0.1-160.0 mg/L) in comparison with Olympus CRP reagents as reference reagents: Olympus CRP reagent for the concentration range of 5.0-300.0 mg/L and Olympus CRP Latex reagent intended for early diagnosis of infection in preterm and term newborns. The Olympus CRP Latex reagent has two applications: sensitive (SCRP, for concentration range of 0.5-20.0 mg/L) and highly sensitive (HSCR, for concentration range of 0.05-2.00 mg/L). The original calibrated and control solutions and applications for the Olympus AU 640 instrument were used for all reagents. Tests were performed in line with

Analitička procjena obuhvatila je nepreciznost u seriji, nepreciznost iz dana u dan, netočnost i usporedna određivanja vrijednosti CRP u uzorcima bolesnika. Analizirano je 139 uzoraka seruma nedonoščadi i novorođenčadi. Uzorci s vrijednostima CRP manjim od 2,00 mg/L (skupina 1, n=35) analizirani su pomoću Olympus Latex reagensa (visoko osjetljiva aplikacija, mjerno područje 0,05-2,00 mg/L) i Randox Full Range CRP reagensa. Uzorci seruma s vrijednostima CRP manjim od 20,0 mg/L (skupina 2, n=63) analizirani su pomoću Olympus Latex reagensa (osjetljiva aplikacija, mjerno područje 0,5-20,0 mg/L) i Randox Full Range CRP reagensa, a uzorci seruma s vrijednostima CRP većim od 5,0 mg/L (skupina 3, n=41) analizirani su pomoću Olympus CRP reagensa (mjerno područje 5,0-300,0 mg/L) i Randox Full Range CRP reagensa. Dobiveni su zadovoljavajući rezultati za analitičku nepreciznost iz dana u dan (KV=1,64%), nepreciznost u seriji (KV <2,18%) i netočnost (R <3,67%). Rezultati usporednih određivanja vrijednosti CRP s reagensima dvaju proizvođača u tri skupine ispitanika (tri koncentracijska područja) statistički obrađeni linearnom regresijskom analizom pokazali su visok stupanj korelacije ($r > 0,99$). Na temelju dobivenih rezultata može se zaključiti da je Full Range Randox reagens pouzdan i reproducibilan reagens za određivanje koncentracije CRP u nedonoščadi i novorođenčadi.

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P20 – Laboratorijski informacijski sustavi, P20-1

Analizator IL GEM Premier 3000 za pretrage uz bolesnika: umreženje i prednosti

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Razvojem biomedicinske i informatičke tehnologije rad na analizatorima koji su vezani uz bolesnika (engl. *point-of-care*, POC) znatno se pojednostavljuje. Uređaji se međusobno povezuju u lokalnu mrežu (engl. *Local Area Network*, LAN) koja omogućava nadzor nad umreženim analizatorima u jedinicama za pretrage uz bolesnika. Reorganizacijom laboratorijskog rada 2005. godine u Kliničkom zavodu za laboratorijsku dijagnostiku KBC Zagreb uvode se analizatori acidobaznog statusa tvrtke Instrumentation Laboratory (zastupnik tvrtka MDLab d.o.o.) GEM Premier 3000 na 11 lokacijskih jedinica (Klinika za anesteziologiju, reanimaciju i intenzivno liječenje, jedinice intenzivne skrbi i operacijska dvorana Klinike za neurokirurgiju, Klinike za kirurgiju, Klinike za kardijalnu kirurgiju, Klinike za

ECCLS recommendations. Analytical evaluation included serial imprecision, day to day imprecision, inaccuracy, and parallel CRP determination in patient samples. A total of 139 serum samples from preterm and term babies were analyzed. Samples with CRP levels lower than 2.00 mg/L (group 1, n=35) were analyzed by use of Olympus Latex reagent (highly sensitive application, measuring range 0.05-2.00 mg/L) and Randox Full Range CRP reagent. Serum samples with CRP levels lower than 20.0 mg/L (group 2, n=63) were analyzed by use of Olympus Latex reagent (sensitive application, measuring range 0.5-20.0 mg/L) and Randox Full Range CRP reagent, whereas those with CRP level greater than 5.0 mg/L (group 3, n=41) were analyzed by use of Olympus CRP reagent (measuring range 5.0-300.0 mg/L) and Randox Full Range CRP reagent. Satisfactory results were obtained for analytical day to day imprecision (CV=1.64%), serial imprecision (CV <2.18%) and inaccuracy (R <3.67%). The results of parallel CRP determinations with the use of reagents from two different manufacturers in three study groups (three concentration ranges), statistically processed by linear regression analysis, showed a high degree of correlation ($r > 0,99$). Based on the results obtained, it is concluded that the Randox Full Range reagent is a reliable and reproducible reagent for CRP determination in preterm and term newborns.

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P20 – Laboratory information system, P20-1

IL GEM Premier 3000 POCT analyzer: networking and advantages

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Development of biomedical and computer technology has substantially contributed to increasing simplification of the operation of point-of-care (POC) analyzers. These instruments are interconnected in a local area network (LAN), which allows their surveillance in POC units. Following the restructuring of laboratory practice conducted during 2005 at Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, GEM Premier 3000 analyzers manufactured by Instrumentation Laboratory (represented in Croatia by MDLab d.o.o.) were introduced to determine acid-base status at 11 hospital locations: Department of Anesthesiology, Resuscitation and Intensive Care, intensive care units and operating rooms of University Departments of Neurosurgery, Surgery, Cardiac Sur-

pedijatriju; Zavod za dijalizu; Zavod za hitnu i intenzivnu medicinu; Klinika za ženske bolesti i porode, Zavod za perinatalnu medicinu, te hitni laboratoriji Kliničkoga zavoda za laboratorijsku dijagnostiku). Uporabom programa GEM Web koji je instaliran na dva računala smještena u hitnom i redovnom laboratoriju medicinskim su biokemičarima omogućeni 24-satni nadzor i praćenje udaljenih analizatora. Program omogućava dobivanje svih potrebnih izvješća za pretrage uz bolesnika koji uključuju: pregled svih rezultata bolesnika, aktivnu kontrolu kvalitete rada, uvid u pogreške s identifikacijom analitičara, te uvid o broju izvršenih analiza i potrošnji reagensa. Program ima mogućnost povezivanja podataka u LIS i BIS. Tijekom 6 mjeseci na analizatorima za pretrage uz bolesnika KBC Zagreb obrađeno je 53 711 uzoraka krvi bolesnika i zabilježeno 511 prijeanalitičkih pogrešaka (ugrušak i/ili interferencija, 0,9%). Pojavnost prijeanalitičkih pogrešaka manja od 1% bila je moguća zahvaljujući trajnoj izobrazbi osoblja na odjelima koje provodi analize od strane laboratorijskih stručnjaka kojima umreženje uređaja omogućava stalan pregled nad analitičkim radom.

Vrijeme laboratorijskih djelatnika provedeno uz instrument svedeno je na minimum, a kontrole i rezultati retrogradno se mogu provjeriti za svakog bolesnika.

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P20-2

Primjena informacijskih tehnologija u dostavi laboratorijskih nalaza

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Cilj rada bio je ispitati dostavu nalaza vanjskim korisnicima u Kliničkom zavodu za laboratorijsku dijagnostiku KBC Zagreb, te uvesti one metode koje će omogućiti brzu i pouzdanu dostavu nalaza te spriječiti nepotrebne troškove. U radu smo od metoda primijenili laboratorijski informacijski sustav (LIS), anketu, te statističku obradu nepodignutih nalaza. Glavne značajke primjene LIS su potpuna automatizacija u izradi laboratorijskih pretraga te brza razmjena informacija. Sam proces stvaranja informacija definira strukturne module LIS. Jedan od modula je izrada i dostava nalaza korisnicima, a uključuje mrežnu razmjenu podataka s bolničkim odjelima i ambulantom, te pretva-

gery, Pediatrics; Dialysis Unit, Department of Emergency and Intensive Care; University Department of Gynecology and Obstetrics, Department of Perinatal Medicine, and emergency laboratories of the Clinical Institute of Laboratory Diagnosis. GEM Web application has been installed on two computers located in emergency and central laboratory to allow medical biochemists 24-h surveillance and monitoring of remote analyzers. The application provides all the necessary POC test reports, comprising the list of all patient results, active quality management, survey of errors including analyst identification, and data on the number of analyses performed and reagent consumption. The application can also be integrated into LIS or HIS. A total of 53,711 patient blood samples were processed by POCT analyzers at Zagreb University Hospital Center during 6 months, and 511 preanalytical errors (clot and/or interference, 0.9%) were recorded. A less than 1% incidence of preanalytical errors was possible owing to continuous education of nurses that perform assays by laboratory professionals who can permanently monitor analytical performance due to analyzer integration. The time that laboratory staff spend by the analyzers has been reduced to minimum, and control and test results may be retrogradely checked for any patient.

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P20-2

Use of information technologies in laboratory finding delivery

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The aim of the study was to investigate the delivery of laboratory findings to outpatients at Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, and to introduce methods that will allow for rapid and reliable laboratory result delivery and eliminate unnecessary costs. Regarding study methods, we used laboratory information system (LIS), survey, and statistical processing of uncollected results. Major LIS characteristics are complete automation of laboratory test performance and rapid information exchange. Structural models of LIS are defined by the actual information-producing process itself. One of the modules comprises preparation and

ranje validiranih nalaza u PDF-format (engl. *Portable Document Format*) i njihovu dostavu pomoću elektroničke pošte za vanjske korisnike. Kod takvog slanja nalaza osobito je važna zaštita podataka kod određenih genetičkih i drugih pretraga. Za anketno ispitivanje pripremljen je poseban upitnik na temelju kojega je proveden terenski dio istraživanja među posjetiteljima kliničkog laboratorija o njihovoj navici dobivanja nalaza, o poznavanju interneta, te o mogućnosti dobivanja nalaza elektroničkom poštom. Analiza zatvorenih pitanja iz upitnika pokazala je da 86% ispitanika sami dolaze u laboratorij po nalaze, 59% ispitanika se služi internetom, a 55% ispitanika je čulo za mogućnost slanja nalaza internetom. Jedna četvrtina ispitanika (25%) izjasnila se za dobivanje nalaza elektroničkom poštom i to pretežito oni iz dobne skupine od 25-50 godina. Otvorena su pitanja dala uvid o stavovima bolesnika kod odabira specijalističkih laboratorija. Statistička obrada nepodignutih nalaza kroz razdoblje od 6 mjeseci pokazala je kako svakog mjeseca ostaje oko 20% nepodignutih nalaza, od kojih su najbrojniji oni iz biokemije proteina i tumorskih biljega (33%), endokrinologije (23%), te imunologije (20%). Rezultati ispitivanja pokazuju da primjena informacijskih tehnologija (elektronička pošta ili elektronička razmjena podataka s liječnicima primarne zaštite) u dostavi laboratorijskih nalaza vanjskim korisnicima omogućuje smanjenje nagomilavanja nepodignutih nalaza, posjeta bolesnika i gužve u čekaonicama, ali i nepotrebnog rasipanja zdravstvenih sredstava. Također doprinosi racionalizaciji i uštedi u reorganizaciji zdravstvene zaštite.

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P21 – Automatizacija i robotika, P21-1

Automatizirani sustav za sveobuhvatnu analizu mokraće Iris IQ 200 – iskustva nakon godinu dana svakodnevnog rada

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Iris IQ 200 jedna je od dviju danas dostupnih automatiziranih alternativa tradicionalno "ručnoj" i subjektivnoj analizi mokraćnog sedimenta. Inicijalna procjena instrumenta ukazala je na dobru korelaciju nalaza dobivenih svjetlosnom mikroskopijom i upotrebom sustava Iris IQ

delivery of laboratory reports to users, involving network data exchange with hospital wards and outpatient units, conversion of validated results into PDF (portable document format) files, and their delivery by electronic mail to outpatients. However, data protection during such delivery of laboratory reports is of particular importance for some genetic and other tests. A specific questionnaire was prepared for polling which was the basis for the field investigation among clinical laboratory users on the usual manner of receiving reports, their knowledge of internet, and the possibility of receiving laboratory results by e-mail. The analysis of closed-ended poll questions revealed that 86% of respondents collected their laboratory reports themselves, 59% confirmed the use of internet, and 55% of respondents had heard of the possibility of laboratory report delivery by the internet. One fourth of respondents (25%) declared in favor of receiving reports by e-mail, mainly those from the 25-50 age group. Open-ended questions provided information on the patient attitudes regarding selection of specialist laboratories. Statistical processing of reports uncollected over 6 months demonstrated that 20% of reports were left uncollected *per* month, mostly those on protein biochemistry and tumor markers (33%), endocrinology (23%), and immunology (20%). Study results showed that the use of information technologies, i.e. electronic mail or electronic data exchange with primary health care physicians, in delivery of laboratory findings to outpatients led to reduction in the number of uncollected reports, patient visits, waiting room queues, and unnecessary wasting of healthcare funds. The application of these technologies also contributes to rationalization and savings in healthcare restructuring.

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P21 – Automatization and robotics, P21-1

Iris IQ 200 fully automated urinalysis system – one-year experience in routine use

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Iris IQ 200 is one of the two currently available alternatives to traditionally manual urine microscopy. In our hands, initial instrument evaluation has indicated good correlation between light microscopy and Iris IQ 200 findings, provided all the elements were sorted manually by an

200, uz uvjet neizostavne preklasifikacije elemenata od strane iskusnog operatera za sve uzorke. Koeficijenti korelacije za tipične elemente sedimenta poput eritrocita, leukocita, različitih epitelnih stanica i cilindara kretali su se između 0,763 i 0,989. Nepreciznost brojanja bila je, prema očekivanjima, bitno manja nego za ručnu metodu i iznosila je, izražena kao CV%, od 5,4 do 9,7 za pojedine elemente unutar dana. Ukupno vrijeme potrebno za izdavanje nalaza pojedinačnog uzorka mokraće smanjeno je s prosječnih 10-15 na 2-5 minuta. Mogućnost izdavanja nalaza broja nađenih elemenata po vidnom polju učinila je prijelaz s tradicionalne na automatiziranu analizu mokraće jednostavnom za kliničare i bolesnike. Unutar inicijalne procjene instrumenta provjerili smo i prenošenje uzorak-uzorak (*carry-over*) za osnovne elemente sedimenta. Proizvođač se u tom smislu ograđuje od analize uzoraka s "velikom količinom krvi", što se, pretpostavljamo, odnosi na makrohaturiju. Ispitujući *carry-over* na uzorcima s mikrohematurijom (masa eritrocita, mokraća makroskopski uredna) ustanovili smo da zaista ne postoji značajno prenošenje uzorak-uzorak. Isto je ustanovljeno za leukocite i stanice pločastog i prijelaznog epitela. Međutim, tijekom rutinskog rada uočili smo slučajeve prenošenja uzorak-uzorak, i to za elemente koji nisu bili uključeni u inicijalnu validaciju – spermatozoide i gljivice. Kako ovakvo prenošenje nigdje nije opisano u literaturi vezanoj za Iris IQ 200, smatramo potrebnim izvijestiti o ovom problemu, kako bi se izbjeglo izdavanje pogrešnih nalaza, odnosno potencijalno neugodne situacije.

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P21-2

Analitička procjena plinskog analizatora Gem® PremierTM 3000

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U novije vrijeme na tržištu se pojavljuju novi biokemijski analizatori glavna namjena kojih je određivanje analiza uz bolesnički krevet (POCT, *point of care testing*). Jedan od takvih analizatora je Gem® PremierTM 3000 (Inc. Lexington, MA, SAD), analitičku procjenu kojega smo proveli prema preporukama Europskog odbora za kliničko-laboratorijske standarde (ECCLS). Mjerali smo parametre: pH, pCO₂, pO₂, iCa²⁺, Na⁺, K⁺, laktat, glukozu i hematokrit u uzorcima

experienced technician. Correlation coefficients obtained for typical elements such as erythrocytes, leukocytes, epithelial cells and casts were between 0.763 and 0.989. As expected, counting imprecision was considerably lower than the manual method with within day variation coefficients (CV%) between 5.4 and 9.7. Total average time needed for single urinalysis decreased from 10-15 to 2-5 minutes. The possibility of reporting the findings number/high power field has eased the transition from manual to automated urinalysis for both the clinicians and patients. Our initial evaluation protocol included sample to sample carry-over studies as well. However, we included only common sediment elements such as erythrocytes, leukocytes and squamous epithelial cells in these protocols. The manufacturer's instructions state "not to analyze samples with great quantities of blood", which presumably concerns macrohematuric samples. Indeed, we found no carry-over with microhematuria, i.e. in samples that were macroscopically normal but contained great quantities of erythrocytes. Similar results were obtained for leukocytes and squamous epithelial cells, with no carry-over detected. However, during routine use we spotted and documented isolated cases of sample to sample carry-over, which involved urine elements not included in initial evaluation studies – spermatozoa and fungi. Although occurring only with fairly high amounts of the mentioned elements, we think it is important to be aware of this possibility, in order to avoid false results and/or potentially embarrassing situations. Up to now, this kind of carry-over with Iris IQ System has not been reported in the literature.

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P21-2

Analytical evaluation of the Gem® PremierTM 3000 analyzer

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New biochemical analyzers for point-of-care testing (POCT) have recently become available. One of these is Gem® PremierTM 3000 (Inc. Lexington, MA, USA). We conducted analytical evaluation of its performance based on the guidelines issued by the European Committee for Clinical Laboratory Standards (ECCLS). We measured pH, pCO₂, pO₂, iCa²⁺, Na⁺, K⁺, lactate, glucose and hematocrit in whole blood samples. Analytical evaluation of analyz-

pune krvi. Analitička procjena analizatora obuhvaća ispitivanje nepreciznosti iz dana u dan, nepreciznost u seriji i usporedbu s konvencionalnim laboratorijskim metodama provedenim na biokemijskim analizatorima (Dimension RxL, Dade Behring Inc., Newark, DE, USA; Olympus AU 400, Olympus Corporation, Mishima, Japan), hematološkom (Cell Dyn 1700, Abbott Laboratories, SAD) i acidobaznom (Rapid 348, Bayer Corporation, SAD) analizatoru. Usporedna mjerenja pojedinih analiza pokazuju zadovoljavajuće koeficijente korelacije ($r=0,8233-0,9843$). Nepreciznost u seriji kod svih analiza ukazuje na vrlo nizak koeficijent varijacije ($KV=0,00-3,73\%$). Nepreciznost iz dana u dan je bila dobra ($KV=0,23-3,76\%$), osim za glukozu za koju je bila nešto veća ($KV=5,57\%$) i za laktat ($KV=9,29\%$). Analitičkom procjenom analizatora Gem® Premier™ 3000 ustanovili smo da je dovoljno pouzdan za svakodnevnu primjenu u sustavu POCT. Analizator je lagan za upotrebu, jednostavan za rukovanje, lako prenosiv, pouzdan, odgovarajući je za operacijske sale, za jedinicu intenzivne njege, za analize tijekom hitnih slučajeva, na udaljenim mjestima od centralnog laboratorija. Primjena ovakvih analizatora zahtijeva i dobru komunikaciju kliničkog i laboratorijskog osoblja, što je vrlo važno u organizaciji provođenja laboratorijskih pretraga uz bolesnika. Treba istaknuti da analizator ima mogućnost povezivanja s bolničkim, laboratorijskim informacijskim sustavom, što je budućnost svih naših laboratorija.

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P21-3

Analitička procjena analizatora ADVIA 1200

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ADVIA 1200 je automatizirani biokemijski analizator koji izvodi pretrage u serumu, plazmi i mokraći. Pretrage se obavljaju nasumice, u seriji i u STAT modu brzinom od 800 fotometrijskih testova na sat i 600 testova za elektrolite na sat. Cilj je prikazati analitičku procjenu biokemijskog analizatora ADVIA 1200, koja je provedena prema preporukama Europskog odbora za kliničko-laboratorijske standarde (ECCLS). Ispitivani su: glukozu, ureja, kreatinin, mokraćna kiselina, ukupni bilirubin, AST, ALT, ALP, GGT, CK, LDH, amilaza, kolesterol, trigliceridi i željezo. Procjena je obuhvatila nepreciznost unutar serije, nepreciznost iz dana u dan, netočnost i usporedno određivanje s analiza-

ers includes assessment of within-run and between-run imprecision, and comparison with traditional laboratory methods of biochemical analyzers (Dimension RxL, Dade Behring Inc., Newark, DE, USA; Olympus AU 400, Olympus Corporation, Mishima, Japan), hematology (Cell Dyn 1700, Abbott Laboratories, USA) and blood gas analyzers (Rapid 348, Bayer Corporation, USA). Comparison measurements showed satisfactory correlation coefficients ($r=0.8233-0.9843$). Within-run imprecision for all parameters revealed a very low coefficient of variation ($CV=0.00-3.73\%$). Between-run imprecision was good ($CV=0.23-3.76\%$), except for glucose where it was slightly elevated ($CV=5.57\%$), and for lactate ($CV=9.29\%$). On the basis of data obtained by analytical evaluation of the Gem® Premier™ 3000 analyzer, we conclude that it is sufficiently reliable for daily use in POCT. The analyzer is easy to use, simple to operate, portable and reliable, therefore, it is suitable for operating rooms, intensive care units, emergency rooms, and decentralized locations. The application of such analyzers requires adequate communication between clinical and laboratory staff, resulting in an important link in the organization of point-of-care testing. It should also be stressed that the analyzer has the possibility of connection to hospital laboratory information system, what constitutes the future of all our laboratories.

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P21-3

Analytical evaluation of ADVIA 1200 analyzer

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The ADVIA 1200 Chemistry System is an automated clinical chemistry analyzer that can run tests on human serum, plasma or urine in random access, batch and STAT modes at a throughput rate of 800 photometric tests per hour and 600 electrolyte tests per hour. The objective is to present analytical evaluation of the ADVIA 1200 clinical chemistry analyzer, performed according to the guidelines of the European Committee for Clinical Laboratory Standards (ECCLS). The following analytes were tested: glucose, urea, creatinine, uric acid, total bilirubin, AST, ALT, GGT, ALP, CK, LDH, cholesterol, triglycerides, amylase and iron. The evaluation consisted of determination of

torom Hitachi 911. Nepreciznost unutar serije određivana je u 30 uzastopnih mjerenja u kontrolnim serumima Bayer QC Control 1, Control 2, Precinorm U, Precipath U (Roche). Nepreciznost iz dana u dan određivana je u triplikatu u navedenim kontrolnim serumima tijekom 10 dana. Netočnost mjerenja prikazana je kao postotak odstupanja (R%) srednje izmjerenih vrijednosti od srednje deklariranih vrijednosti kontrolnih seruma. Usporedba analizatora provedena je pomoću 67 uzoraka u različitim koncentracijskim područjima. Rezultati su pokazali prihvatljiv koeficijent varijacije za nepreciznost unutar serije i nepreciznost iz dana u dan te zadovoljavajući stupanj točnosti u odnosu na kriterije temeljene na veličini biološke varijacije, osim za željezo u kontrolnom serumu Bayer QC Control 2 i visok stupanj korelacije s Hitachi 911 (koeficijent korelacije veći od 0,985). Provedena analitička procjena pokazala je da je ADVIA 1200 pouzdan biokemijski analizator koji osigurava brz i siguran rad uz uporabu minimalnih količina uzoraka i reagensa.

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P22 – Procjena analitičkih sustava, P22-1 (UP4-1)

Epruvete različitih proizvođača i vrijednosti biokemijskih parametara

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Najčešći čimbenici koji utječu na promjenjivost laboratorijskih nalaza pripadaju skupini predanalitičkih metodoloških čimbenika. U toj skupini važno mjesto pripada pravilnom uzorkovanju krvi, odnosno uporabi odgovarajuće epruvete s podtlakom. Globalizacija tržišta donosi sa sobom i različite proizvođače ovoga potrošnog materijala koji u potpunosti ne uklanjaju interferenciju dodatka u epruvetama (antikoagulansi, konzervansi) s metodama različitih instrumenata koji se upotrebljavaju u laboratorijima. Poučeni vlastitim iskustvom o mogućnosti interferencije sastojka epruvete s rezultatima laboratorijskih nalaza (Crnokrak S. 16th IFCC-FESCC Congress, Glasgow, 2005.), ispitali smo utjecaj biokemijskih epruveta pet različitih proizvođača na vrijednosti biokemijskih parametara. Ispitivanje je izvršeno na 20 uzoraka seruma uzorkovanih u slijedeće biokemijske epruvete: Venosafe Vacuette, BD Vacutainer, Improve (s gelom i bez njega), Greiner-vacuette. U svim uzorcima izmjerene su vrijednosti 21 biokemijskog parametra (opća biokemija i hormoni štitnjače). Vrijednosti su izmjerene na biokemijskom analizatoru

between-day imprecision, within-run imprecision, inaccuracy and correlation with Hitachi 911. Within-run imprecision was determined on 30 samples of four control sera, Bayer QC Control 1, Control 2, Precinorm U, Precinorm P (Roche). Day-to-day imprecision was determined in triplicate in four control sera during 10 days. Inaccuracy was calculated as percentage of deviation of the mean determined value from the mean declared value of the control sera. Comparison of the analyzers was performed using 67 samples in various concentration ranges. The results of analytical performance were as follows: low within-run and day-to-day imprecision, a satisfactory level of accuracy according to the criteria based on biological variation except for iron in control sera, Bayer QC Control 2, and a high rate of correlation with Hitachi 911 (coefficient of correlation above 0.985). Analytical evaluation showed ADVIA 1200 to be a reliable biochemistry analyzer, which ensures rapid and safe work, using minimal quantities of samples and reagents.

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P22 – Evaluation of analytical systems, P22-1 (UP4-1)

Use of different test tubes and values of biochemical parameters

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The most common factors affecting laboratory test results belong to the group of preanalytical methodology factors. Within this group, correct blood sampling, i.e. by use of appropriate vacuum test-tubes, has a very important role. Market globalization implies different manufacturers of this kind of expendable supplies. Some of them do not eliminate the interference between test-tube additives (e.g., anticoagulants, preservatives) and methods of various instruments that are used in laboratories. Based on our own experience considering the possibility of test-tube interference with laboratory test results (Crnokrak S. 16th IFCC-FESCC Congress, Glasgow, 2005), we assessed the influence of biochemistry test-tubes by five different manufacturers on the values of biochemical parameters. Testing was conducted on 20 samples in the following biochemistry test-tubes: Venosafe Vacuette, BD Vacutainer, Improve (with and without gel), and Greiner-vacuette. In all samples the values of 21 biochemical parameters were measured (general biochemistry and thyroid hormones). Values were measured on an Archi-

Architect c8000 i imunokemijskom analizatoru Architect i2000 (Abbott, SAD) uz primjenu preporučene analitičke metode. Dobiveni rezultati statistički su obrađeni i iskazani srednjom vrijednosti, standardnom devijacijom (SD) i koeficijentom varijacije (KV), te koeficijentom korelacije (r). Vrijednosti općih biokemijskih parametara (glukoza, urea, kreatinin, ALT, AST, GGT, AP, ukupni bilirubin, CK, LDH, amilaza, željezo, kolesterol, trigliceridi, natrij, kalij, kloridi, kalcij) nisu pokazala značajna odstupanja u ovisnosti o vrsti uporabljene epruvete. Najniži koeficijent varijacije pokazale su vrijednosti natrija (KV=0,05%), a najviši vrijednosti GGT (KV=3,7%). Koeficijenti varijacije za izmjerene vrijednosti hormona štitnjače iznosili su od 0,84% za fT4 do 1,38% za T3. Najniži koeficijent korelacije pokazale su epruvete s gelom Improve (r =od 0,86 do 0,93) pri određivanju T3. S obzirom na statističke pokazatelje epruvete svih navedenih proizvođača smatraju se prihvatljivima za uzorkovanje krvi i ne pokazuju značajne interferencije s uporabljenom metodologijom.

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P22-2 (UP4-2)

Određivanje hipoksantina, ksantina i mokraćne kiseline u likvoru tekućinskom kromatografijom visoke djelotvornosti (HPLC)

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Priključeni podaci ukazuju na to da oksidacijski stres igra glavnu ulogu u patogenezi mnogobrojnih neuroloških bolesti (moždani udar, traumatska ozljeda mozga, epilepsija, meningitis, neurodegenerativne bolesti, multipla skleroza, hipoksijsko-ishemijska encefalopatija novorođenčeta, preeklampsija itd.). Hipoksantin je proučavan kao biljeg hipoksije/ishemije budući da se nakuplja u tkivu kod pojačane razgradnje adeninskih nukleotida i kod inhibicije ksantin-oksidade zbog nedostatka kisika. Smatra se da mokraćna kiselina ima važnu ulogu u uklanjanju endogenih radikala. Također se smatra da za vrijeme recirkulacije, nakon privremene ishemije ili nakon nepotpune ishemije, sustav hipoksantin-ksantin stvara slobodne radikale koji mogu izazvati novu ozljedu tkiva u razdoblju reoksigenacije (tzv. paradoks kisika). Cilj ovoga rada bio je uvesti u rutinsku likvorsku dijagnostiku određivanje

tect c8000 biochemistry analyzer and Architect i2000 (Abbott, USA) immunochemistry analyzer. We used the recommended analytical methods. The results obtained were statistically processed and expressed as mean, standard deviation (SD), coefficient of variation (CV) and correlation coefficient (r). The values of general biochemistry parameters (glucose, urea, creatinine, ALT, AST, GGT, AP, total bilirubin, CK, LDH, amylases, iron, cholesterol, triglycerides, sodium, potassium, chlorides, calcium) did not show any significant deviation related to the type of the test-tube used. Sodium showed the lowest and GGT highest coefficient of variation (CV=0.05% and 3.7%, respectively). The coefficients of variation for thyroid hormones were from 0.84% for fT4 to up to 1.38% for T3. The lowest correlation coefficient was recorded for the Improve test-tubes with gel (r =0.86-0.93) on T3 measurement. Considering statistical indicators, the test-tubes of all the above mentioned manufacturers were found acceptable for blood sampling and showed no significant interference with the tested methodology.

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P22-2 (UP4-2)

Determination of hypoxanthine, xanthine and uric acid in human cerebrospinal fluid by high performance liquid chromatography (HPLC)

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Accumulating data indicate that oxidative stress plays a major role in the pathogenesis of numerous neurological diseases (stroke, traumatic brain injury, epilepsy, meningitis, neurodegenerative diseases, multiple sclerosis, perinatal hypoxic-ischemic injury, preeclampsia, etc.). Hypoxanthine has been studied as a marker of hypoxia/ischemia since it accumulates in the tissue due to the increased breakdown of adenine nucleotides and inhibition of xanthine oxidase activity because of the lack of oxygen. Uric acid has been implicated as an important endogenous radical scavenger. It has also been suggested that reactive oxygen species (ROS) produced by the hypoxanthine-xanthine oxidase system might trigger secondary injury in the reoxygenation period ("oxygen paradox") during recirculation after temporary ischemia or during incomplete ischemia. The aim of the study was

hipoksantina, ksantina i mokraćne kiseline metodom tekućinske kromatografije visoke djelotvornosti (HPLC). Hidrofilni analiti razdvojeni su izokratski metodom HPLC na koloni C18 pri temperaturi od 30 °C. Proteini iz likvora uklonjeni su ultrafiltracijom. Mobilna faza sastojala se je od 0,06 M fosfatnog pufera (pH 5,1) i metanola (97:3). Rabio se je protok 0,7 mL/min i UV detektor pri 254 nm. Kalibracijska krivulja bila je linearna u rasponu od 1-50 µmol/L ($R^2=0,996$, $R^2=0,999$) za hipoksantin i ksantin te 1-100 µmol/L ($R^2=0,997$) za mokraćnu kiselinu. Ponovljivost izražena koeficijentom varijacije bila je 6%, 5% i 4% za mokraćnu kiselinu, hipoksantin i ksantin ($n=10$). Reproducibilnost izražena koeficijentom varijacije bila je 6%, 9% and 5% za mokraćnu kiselinu, hipoksantin i ksantin ($n=10$). Rezultat testa iskorištenja za mokraćnu kiselinu, hipoksantin i ksantin bio je od 80% do 101% ($n=9$). Dobiveni rezultati pokazuju da opisana metoda HPLC ima zadovoljavajuću nepreciznost i točnost te se može rabiti za određivanje metabolita purina u likvoru.

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P22-3

Dugoročna procjena vanjske procjene kvalitete rada za srčane biljege

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Za određivanje srčanih biljega još uvijek ne postoje referentne metode, a referentni materijali još uvijek nisu uspostavljeni. Revizija preporuka Komiteta za standardizaciju srčanih biljega pri IFCC (engl. *IFCC Committee on Standardization of Markers of Cardiac Damage*, IFCC C-SMCD) je u tijeku. Sudjelovanje u vanjskoj procjeni kvalitete rada laboratorijima daje uvid u stupanj međulaboratorijskih razlika, što je preduvjet usklađivanja dobivenih rezultata. Cilj studije bio je evaluirati rezultate određivanja mioglobina i troponina I (cTnI) u vanjskoj procjeni kvalitete *Randox International Quality Assessment Scheme* (RIQAS EQA) potvrđenoj prema međunarodnom standardu ISO:9002 proizvođača Randox Laboratories. Zavod za kliničku kemiju KB Merkur potvrđen prema međunarodnom standardu ISO:9001:2000 sudjeluje u vanjskoj procjeni kvalitete rada za srčane biljege *RIQAS-Cardiac Programme* od 2001. godine. Liofilizirani kontrolni serum humanog podrijetla se distribuiraju u 150 laboratorija, a rezultati se grupiraju prema metodama i procjenjuju u odnosu na srednju vri-

to introduce HPLC method for simultaneous determination of hypoxanthine (Hx), xanthine (Xa) and uric acid (UA) in routine cerebrospinal fluid (CSF) analysis. The hydrophilic analytes were separated isocratically by HPLC method using C18 column at a temperature of 30 °C. In this method, CSF proteins were removed by ultrafiltration. Mobile phase was composed of 0.06 M phosphate buffer (pH 5.1) and methanol (97:3). The flow rate was at 0.7 mL/min and UV detector at 254 nm. Calibration curves were linear at 1-50 µmol/L for Hx and Xa ($R^2=0.997$, $R^2=0.999$, respectively) and for UA at 1-100 µmol/L ($R^2=0.997$). Within-day precision of the method defined by CV% for UA, Hx and Xa was 6%, 5%, and 4% ($n=10$), respectively. Between-day precision of the method defined by CV% for UA, Hx, and Xa was 6%, 9% and 5% ($n=10$), respectively. Recovery of the method for UA, Hx, and Xa was from 80% to 101% ($n=9$). These results indicate that the described HPLC method for purine metabolites in CSF has satisfactory precision and accuracy, so it can be introduced in laboratory practice.

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P22-3

Long-term evaluation of external quality assessment for cardiac markers

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No reference methods or reference materials have yet been established for cardiac markers. The IFCC Committee on Standardization of Markers of Cardiac Damage (IFCC C-SMCD) recommendations are still in the process of revision. Through their participation in the external quality assessment (EQA), laboratories are informed on the extent of inter-laboratory variation, which is a prerequisite for result harmonization. The aim of the study was to evaluate the results on myoglobin and cardiac troponin I (cTnI) in the Randox International Quality Assessment Scheme (RIQAS EQA) external quality assessment by Randox Laboratories. Department of Clinical Chemistry, Merkur University Hospital, certified according to ISO:9001:2000, participates in the RIQAS EQA-Cardiac Programme since 2001. Lyophilized control samples of human origin were distributed to 150 laboratories, results were grouped according to the method used and estimated by comparison with the group average. Results within ± 2 standard deviation index (SDI) are considered

jednost grupe, pri čemu se prihvatljivima smatraju oni koji se nalaze unutar dozvoljenog odstupanja od ± 2 relativne standardne devijacije (SDI). U Zavodu za kliničku kemiju koncentracija mioglobina se određuje imunoturbidimetrijskom metodom na analizatoru Olympus AU 400, a rezultati se procjenjuju prema grupi ostalih metoda (4 laboratorija). Koncentracija cTnI se određuje imunokemijskom (RPIA) metodom na analizatoru Dade Stratus CS, a rezultat se procjenjuje prema metodi RPIA (Dade Behring Stratus CS; 9 laboratorija). Procjena rezultata bez obzira na metodu je pokazala veliku varijabilnost (KV %): za mioglobin 26,6% i za cTnI 77,7%. Grupiranje rezultata prema metodama je pokazalo manju varijabilnost: za mioglobin 0,5–31,4% i za cTnI 3,6–20,7%. Varijabilnost rezultata grupe u kojoj se procjenjuju naši rezultati za mioglobin je bila 25,9%, što je očekivana vrijednost s obzirom na broj sudionika i heterogenost grupe. Varijabilnost rezultata za cTnI u grupi metoda RPIA je iznosila 6,1%, što je očekivana vrijednost s obzirom na homogenost grupe. S obzirom na visoku varijabilnost rezultata bez obzira na metodu neophodna je standardizacija metoda za cTnI primjenom metoda definiranih vezanjem protutijela isključivo na epitope stabilnoga dijela molekule cTnI i KV manjim od 10%. Iako su rezultati za mioglobin usklađeniji, varijabilnost unutar pojedinih metoda nameće standardizaciju metoda za mioglobin prema certificiranom referentnom materijalu.

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P22-4

Prijeanalitičke pogreške u laboratorijskoj medicini: vrsta i učestalost

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Kvaliteta analitičkog materijala kritični je čimbenik za kvalitetu rezultata laboratorijskih pretraga. Kako bi se liječnicima osigurali kvalitetni nalazi treba poštivati pravila dobre laboratorijske prakse i uvesti strategiju za prevenciju pogreške odgovornim usvajanjem standardiziranog prijeanalitičkog postupka. Nepoštivanje prijeanalitičkih standarda uzrok je za oko 93% pogrešaka u dijagnostičkom laboratorijskom testiranju. Cilj rada bila je obrada ukupnih podataka o prijeanalitičkim pogreškama na uzorcima bolesnika u svrhu dobivanja uvida u njihovu učestalost i broj te uspostava strategije za njihovo smanjenje. Istraživanje je obuhvatilo 237 600 uzoraka bolesnika od kojih je 3307 obilježeno prijeanalitičkim pogreškama,

acceptable and satisfactory. Myoglobin concentrations were determined by immunoturbidimetric method (Olympus AU 400 analyzer) and results estimated within the group of other methods (4 participants). cTnI concentrations were determined by RPIA method (Dade Behring Stratus CS analyzer) and the results estimated within the group of RPIA method (9 participants). The results estimated irrespective of the method used showed high variability (CV %) for myoglobin and cTnI (26.6% and 77.7%, respectively). Variability of the results estimated within the group was lower: 0.5%–31.4% for myoglobin and 3.6%–20.7% for cTnI. The variability of myoglobin results within the group of other methods (25.9%) was quite expected because of the small number of participants and group heterogeneity. The variability of cTnI results within the group of RPIA methods (6.1%) was lower as the result of group homogeneity. Accordingly, harmonization of cTnI results is necessary due to the high variability of results estimated regardless of the method. That is why cTnI methods with antibodies on epitopes on the stable part of the molecule are recommended. Although the results for myoglobin showed a higher degree of harmonization, the variability within the methods imposes the need of harmonization according to the certified reference material.

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P22-4

Preanalytical errors in laboratory medicine: type and frequency

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The quality of analytical material is a factor critical for the quality of test results. To provide physicians with quality laboratory results, it is necessary to comply with the rules of good laboratory practice and to introduce strategy for error prevention by responsible adoption of standardized preanalytical procedure. Failure to comply with this standard is the cause of approximately 93% of errors in diagnostic laboratory testing. The aim of the study was to process all data on preanalytical errors in patient samples in order to get an insight in their frequency and number, and to establish a strategy for their reduction. The study included 237 600 patient samples; 3307 of them involved preanalytical errors, with 0.06%–2.4% relative frequency

što u relativnim frekvencijama za bolničke i izvanbolničke bolesnike iznosi od 0,06% do 2,4%. Na temelju klasifikacije prema specifičnim vrstama prijeanalitičkih pogrešaka najveća pojavnost pogrešaka zabilježena je kod hemoliziranih uzoraka (48%) te zgrušanih uzoraka (15%), dok ostale vrste pogrešaka bilježe pojavnost od 1% do 7%. Dokazano je kako je najčešći uzrok nepouzdanih laboratorijskih rezultata u neposrednoj svezi s nepoštovanjem prijeanalitičkog standardiziranog postupka od strane kliničkog osoblja. Zbog toga je potrebno provoditi sustavni program koji je usmjeren na uvažavanje prijeanalitičkog standarda za kvalitetu uzorka kroz izobrazbu, nadzor nad uzorcima i izvještavanje, te afirmaciju medicinsko-laboratorijske struke.

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P22-5

Određivanje koncentracije NT-pro BNP na analizatoru Dimension RXL MAX

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Određivanje N-terminalnog pronatriuretskog peptida mozga (NT-pro BNP) primjenjuje se u dijagnostici sumnjivih stanja koja mogu biti kongestivno zatajivanje srca, te u određivanju rizika u bolesnika s akutnim koronarnim sindromom i kongestivnim zatajenjem srca. Cilj rada bio je usporediti metode određivanja NT-pro BNP za Dimension RXL Max NT-pro BNP i Elecsys 1010 NT-pro BNP prije primjene metoda u laboratoriju. U ovom radu procijenili smo dvije metode za mjerenje NT-pro BNP i izvršili korelaciju rezultata u plazmi 29 bolesnika s Odjela za kardiologiju naše Kliničke bolnice. Kontrolnu skupinu činilo je 62 zdravih ispitanika, 38 muškaraca i 24 žene, raspon dobi 20-71 godina. Mjerenje koncentracije NT-pro BNP izvršeno je pomoću enzimskog imuno testa na automatskom biokemijskom analizatoru Dimension RXL (Dade Behring) i elektrokemiluminiscentnog imuno testa na Diagnostics Elecsys 1010 (Roche). Statističkom obradom između vrijednosti za Dimension RXL Max NT-pro BNP (x) i Elecsys 1010 NT-pro BNP (y) dobivena je korelacija ($y=0,94x-0,26$, $r=0,96$, $p<0,01$). Kod zdravih ispitanika ženska skupina ispitanika ($72,3\pm 31,4$ pg/mL) pokazala je značajno više vrijednosti nego muška skupina ispitanika ($63,9\pm 24,5$ pg/mL). Kao što se i očekivalo, vrijednosti koncentracija NT-

for inpatients and outpatients. Classification of preanalytical errors according to specific error type revealed the highest error frequency in hemolyzed (48%) and clotted samples (15%), while the frequency of other error types was 1%-7%. The most common cause of unreliable laboratory results was demonstrated to be directly related to noncompliance with preanalytical standardized procedure by non-laboratory staff. Therefore, it is necessary to implement a systematic program focused on compliance with preanalytical standard of sample quality, which involves education, sample surveillance and reporting, and assertion of medical laboratory profession.

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P22-5

Determination of NT-pro BNP on a Dimension RXL MAX analyzer

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Determination of N-terminal pro-brain natriuretic peptide (NT-pro BNP) is used as an aid in the diagnosis of individuals suspected of having congestive heart failure as well as for risk stratification of patients with acute coronary syndrome and congestive heart failure. The aim was to compare Dimension RXL Max NT-pro BNP assay and Elecsys 1010 NT-pro BNP assay before use in our laboratory. We evaluated two methods of NT-pro BNP measurement and correlated the results obtained in human plasma samples of 29 patients from Department of Cardiology of our Hospital. Control group included 62 healthy subjects, 38 men and 24 women, aged 20-71. NT-pro BNP was measured using one-step enzyme immunoassay on a Dade Behring Dimension RXL Max automated biochemistry analyzer and electrochemiluminescence immunoassay on a Roche Diagnostics Elecsys 1010 instrument. The correlation using the least square method between Dade Behring Dimension RXL Max NT-pro BNP (x) and Elecsys NT-pro BNP (y) was very good ($y=0.94x-0.26$, $r=0.96$). Healthy women showed significantly higher values (72.3 ± 31.4 pg/mL) than men (63.9 ± 24.5 pg/mL). As expected, the NT-pro BNP values were significantly higher in patients with heart failure than in normal subjects. In conclusion, the

pro BNP u bolesnika sa zatajivanjem srca bile su značajno više od vrijednosti koncentracija izmjerenih u zdravih ispitanika. Zaključuje se kako je metoda NT-pro BNP za Dade Behring pokazala odlične analitičke performanse koje su u suglasnosti s rezultatima metode NT-pro BNP dobivenim na instrumentu Elecsys.

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P22-6

Iskustvo Zavoda za kliničku kemiju Kliničke bolnice Merkur u međunarodnoj procjeni kvalitete rada iz laboratorijske koagulacije

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Zavod za kliničku kemiju, atestiran prema standardu ISO 9001:2000 od 2003. godine, aktivni je sudionik međunarodne kontrole kvalitete u koagulaciji (IEQAS-Coagulation) pod pokroviteljstvom Svjetske zdravstvene organizacije (SZO) od 1992. godine. Cilj programa je provjera kvalitete vlastitih analitičkih sustava putem neovisnog sustava i usklađivanje prema međunarodnim standardima i preporukama uz obvezu organiziranja Nacionalne kontrole kvalitete. Provođenje programa kontrole se odvija kroz 3 ciklusa na godinu i sadrži određivanje protrombinskog vremena (PV, PV- INR) aktiviranog parcijalog tromboplastinskog vremena (APTV), APTV za praćenje heparinske terapije, fibrinogena, trombinskog vremena (TV) i anti-trombina III (AT III). Rezultati 77 laboratorija iz 49 zemalja svijeta procjenjuju se kao postotak odstupanja od medijana referentne skupine koju sačinjavaju svi sudionici Nacionalne kontrole Velike Britanije koji rabe isti analitički sustav (*peer group*). Prihvatljivi su rezultati s odstupanjem <15% za sve parametre. Za određivanje PV-INR u oralnoj antikoagulantnoj terapiji prednost se daje reagensima visoke osjetljivosti. Kod testova probiranja (PV, APTV, TV) rezultati se izražavaju u omjeru, te se kao takvi uspoređuju i procjenjuju. Najčešće rabljene metode za određivanje fibrinogena su metoda po Clausu i određivanje deriviranog fibrinogena. Rezultati se procjenjuju prema medijanu pripadajuće metode. Rezultati Zavoda za kliničku kemiju u razdoblju 1992.-2006. godine zadovoljili su kriterije procjene uz udio pozitivnih rezultata od 80–100%. Rezultati potvrđuju važnost međulaboratorijske suradnje koja omogućuje razmjenu stručnih i praktičnih znanja u smislu

NT-pro BNP Dade Behring assay demonstrated excellent analytical performance characteristics and agreement with NT-pro BNP results on Elecsys instrument.

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P22-6

Our experience in IEQAS-Coagulation: Department of Clinical Chemistry, Merkur University Hospital, Zagreb

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Department of Clinical Chemistry has been accredited on the basis of ISO 9001:2000 since 2003, and has participated in the WHO IEQAS-Coagulation for 16 years now, since 1992. The aim of the program is to provide external quality assessment for blood coagulation tests and to promote high standards of laboratory performance and practice. Additionally, the aim is to encourage participants to set up the National External Quality Assessment Schemes (NEQAS) in their countries. According to the scheme design, every participant receives control samples four times a year for INR-determination, PT for diagnosis, APTT for heparin dosage assessment, fibrinogen, APTT, TT and ATIII. There are 77 participants from 49 countries all over the world. Results for the scheme are compared to a reference group of results from UK NEQAS. Acceptable results are designated as "results within consensus" (<15%). For PT-INR most participants prefer thromboplastin reagents with low ISI. For other screening tests, PT for diagnosis, APTT and TT results must be expressed as the ratio of measurement clotting time/median of the reference interval. For fibrinogen determination, the Claus and PT- derived fibrinogen method is most widely used. Results are evaluated according to the method-specific median. During the 1992-2006 period, our results were designated as "satisfactory performance" in 80%-100% of cases. Inter-laboratory collaboration on promoting high standards of Good Laboratory Practice is important for allowing useful exchange of professional and practical knowledge. The obligation of using high sensitivity reagents for PT and the unique form of reporting PTT

unapređivanja Dobre laboratorijske prakse, a to podrazumijeva primjenu visokoosjetljivih reagensa za određivanje PV i uniformnost izražavanja rezultata (PV-INR u oralnoj antikoagulantnoj terapiji i omjera za PV i APTV kao testova probiranja). Provođenje vanjske procjene kvalitete na nacionalnoj razini organizirano je od 1994. i provodi se neprekidno.

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P22-7

Imunofiksacija – automatizirani sustav Sebia Hydrasys

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Imunofiksacija je dvostupanjski postupak koji se sastoji od elektroforeze na agarozu i imunoprecipitacije. Proteini su razdvojeni elektroforetski na oštire vrpce u gelu. Na svaku vrpcu doda se specifični antiserum za molekulu pojedinačnih razreda imunoglobulina. Ako su specifični razredi laganog lanca imunoglobulina prisutni, nastati će netopivi kompleks s antiserumom koji se tada može obojiti i dokazati. Imunofiksaciju obilježava pojačana osjetljivost, laka interpretacija, brzi rezultati testiranja, vrhunsko razdvajanje, kao i mogućnost dokazivanja monoklonskog imunoglobulina koji nije vidljiv rutinskom elektroforezom. Pojačana osjetljivost testa jednim se dijelom postiže uporabom boje *acid violet* te jasnoćom gela, čime je olakšano tumačenje. Primarna upotreba ovoga testa je identifikacija i praćenje pojedinog monoklonskog imunoglobulina (IgG, IgM, IgA, IgD, IgE, laganih lanaca lambda i laganih lanaca kapa) koji su prisutni u multiplom mijelomu i Waldenstromovoj makroglobulinemiji. Imunofiksacijski test Sebia dizajniran je za identifikaciju gamopatija u humanom serumu ili ostalim humanim biološkim tekućinama, uključujući ukoncentriranu mokraću. Test rabi inovacijsku laboratorijsku metodu koja uvelike pojednostavljuje nanošenje antiseruma. Patentirana imunofiksacijska maska (IF Dynamic Mask) omogućuje znatno smanjenje ručnog pipetiranja antiseruma. Zamjenom višekratno upotrebljene IF maske Sebia je promijenila nanošenje antiseruma uvođenjem raspoloživog segmenta za svaki gel. Manji volumen antiseruma nanosi se na segment i tada premaže preko gela, čime je smanjena količina pipetiranog antiseruma, a istodobno je omogućeno nanošenje antiseruma na sve vrpce razdvojenih proteina. Upotrebom pojedinačnih

results ensure high reliability and accuracy of laboratory test results.

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P22-7

Immunofixation – Sebia Hydrasys automated system

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Immunofixation is a two-stage process combining agarose gel electrophoresis with immunoprecipitation. Proteins are separated electrophoretically in distinct tracks on the gel. Antisera specific to individual classes of molecules are added to each track. If specific classes of light chain immunoglobulins are present, insoluble complexes form with the antisera, which can then be stained and detected. Immunofixation is characterized by its enhanced sensitivity, ease of interpretation, quick test results, excellent resolution, and it may detect monoclonal immunoglobulins that are not visible on routine electrophoresis. The enhanced sensitivity of the assay, in part achieved by using an acid violet stain and gel clarity, facilitates interpretation. The primary use of this test is to identify and monitor certain monoclonal immunoglobulins (IgG, IgM, IgA, IgD, IgE, lambda light chain and kappa light chain) such as those present in multiple myeloma and Waldenstrom's macroglobulinemia. Sebia's immunofixation assay is designed for identification of gammopathies in human sera or other human biological fluids, including concentrated urine. An innovative labor saving method is used to greatly simplify antiserum application. The patented Dynamic Mask provides a significant reduction in manual antiserum pipetting. Replacing the reusable IF masks, Sebia has changed the method of antiserum application by introducing disposable segments for each gel. A smaller volume of antiserum is applied onto the segment and then spread across the gel, reducing the amount of antiserum pipetting and providing simultaneous application of antisera to all tracks. There is no template cleaning with the single use antiserum segments and so cross con-

nih segmenata za antiserume nije potrebno čistiti predložke te je uklonjena i mogućnost kontaminacije vrpca proteina. Obradeni su serumi 30 bolesnika i verificirani imunofiksacijom kao: 18 monoklonskih imunoglobulina, 5 hipogamaglobulinemija, 3 poliklonske hipergamaglobulinemije i 4 normalna nalaza. Imunofiksacija je metoda izbora za dokazivanje monoklonskih proteina zbog njene brzine, specifičnosti, fleksibilnosti i lakoće u interpretaciji, naročito kada su monoklonski imunoglobulini prisutni u niskim koncentracijama u serumu i/ili u mokraći.

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P22-8

Procjena rezultata vanjske procjene kvalitete pH, plinova u krvi i ioniziranih elektrolita

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Cilj rada bila je procjena rezultata pH, plinova u krvi i ioniziranih elektrolita Zavoda za kliničku kemiju, KB Merkur u vanjskoj procjeni kvalitete koju provodi neovisni organizator Labquality – WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finska, tijekom 3 godine u svrhu utvrđivanja analitičke kvalitete. Dva puta na godinu se 3 različita uzorka pripremljena tonometrijski uvođenjem smjese CO₂, O₂ i N₂ u fiziološki puferirani matriks distribuiraju u 290 laboratorija iz 15 zemalja i analiziraju na 426 analizatora. Rezultati se procjenjuju u odnosu na dozvoljeni analitički bias koji iznosi ±0,5% za pH, ±2,0% za Na⁺, ±3,0% za Ca⁺⁺ i Cl⁻, ±4,0% za pCO₂ i K⁺, ±5,0% za Mg⁺⁺, ±6,0% za nisku i 8,0% za normalnu i visoku razinu pO₂. U Zavodu za kliničku kemiju KB Merkur analizirano je 12 uzoraka na analizatoru Stat Profile pHox Plus (pHox) i 9 uzoraka na analizatoru Stat Profile Critical Care Xpress tvrtke Nova Biomedical (SP CCX). Rezultati su pokazali da postoje značajne razlike u mjerenju pH i plinova u krvi između različitih tipova analizatora. SP CCX pokazuje negativan bias u mjerenju pH u odnosu na pHox, te pozitivan bias u odnosu na Radiometrove (ABL 700-735, 800-835), Bayerove (248,348, 840-865) i Ilove analizatore (1610-1640, Gem Premier 3000). Vrijednosti pCO₂ na analizatoru SP CCX sukladne su vrijednostima na Radiometrovim (ABL 700-735, 800-835) i Bayerovim analizatorima (840-865, Rapid Point 400-405), imaju pozitivan bias u odnosu na pHox, te negativan bias

tamination of tracks is eliminated. The sera of 30 patients were analyzed and verified by immunofixation as follows: 18 with monoclonal immunoglobulins, 5 hypogammaglobulinemias, 3 polyclonal hypergammaglobulinemias, and 4 normal results. Immunofixation is the method of choice for detecting monoclonal protein because it is fast, specific, flexible and easy to interpret, especially when monoclonal proteins are present in low concentration in serum and/or urine.

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P22-8

Evaluation of external quality assessment results in acid base and ionized electrolyte analysis

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The aim of the study was to evaluate the results obtained at Department of Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia, in acid base and ionized electrolyte analysis in the external quality assessment schemes performed by an external, independent body, Labquality – WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finland, during 3 years, to determine analytical quality of the results. Two times a year, 3 different specimens prepared tonometrically by applying mixtures of CO₂, O₂ and N₂ into physiologically buffered matrix were distributed to 290 laboratories from 15 countries and analyzed on 426 instruments. Results were evaluated according to total analytical error limits of ±0.5% for pH, ±2.0% for Na⁺, ±3.0% for Ca⁺⁺ and Cl⁻, ±4.0% for pCO₂ and K⁺, ±5.0% for Mg⁺⁺, ±6.0% for low and 8.0% for normal and high level pO₂. We analyzed 12 samples on Stat Profile pHox Plus (pHox) and 9 samples on Stat Profile Critical Care Xpress from Nova Biomedical (SP CCX). The results showed marked bias in the measurement of pH, pCO₂ and pO₂ between the analyzers. SP CCX showed negative bias for pH vs pHox, and positive bias vs Radiometer (ABL 700-735, 800-835), Bayer (248, 348, 840-865) and IL analyzers (1610-1640, Gem Premier 3000). The results for pCO₂ on SP CCX were comparable with Radiometer (ABL 700-735, 800-835) and Bayer analyzers (840-865, Rapid Point 400-405), showed positive bias vs pHox and negative bias vs IL Gem Premier 3000 analyzer.

u odnosu na Gem Premier 3000. Vrijednosti pO_2 na analizatoru SP CCX sukladne su vrijednostima pO_2 na Radiometrovom ABL 500-555, imaju pozitivan bias u odnosu na Bayerove analizatore (248, 348, 840-865), ILOV 1610-1640, te negativan bias u odnosu na Novin pHox i ILOV Gem Premier 3000. Naši rezultati na oba analitička sustava bili su 100% prihvatljivi za pH i Na^+ , 80-90% za pO_2 , pCO_2 i K^+ , a manje od 80% za Ca^{++} , Mg^{++} i Cl^- , zbog male zastupljenosti analizatora SP CCX, malog broja sudionika (3 za Mg^{++}), te strogih kriterija prihvatljivosti rezultata. Rezultati ukazuju na problem kontrolnih materijala koji se različito ponašaju na različitim analitičkim sustavima. Ion selektivne elektrode vrlo su osjetljive na utjecaj matriksa, što znatno otežava usporedbu rezultata, poglavito kad se radi o malom broju sudionika.

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P22-9

Procjena mjerne nesigurnosti pri određivanju standarda bakra spektrofotometrijskom metodom

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Kao dugogodišnji proizvođač dijagnostičkih reagensa Herbos Dijagnostika je s ciljem osiguranja standardne kvalitete proizvoda od siječnja 2004. godine uvela sustav upravljanja kvalitetom ISO 9001:2000. Naši zahtjevi za stalnim poboljšanjem kvalitete proizvoda provode se u proces proizvodnje vodenih standarda sljedljivih prema međunarodno potvrđenom referentnom materijalu (National Institute of Standards and Technology, NIST). Prvi uvedeni standard je standard bakra u sastavu našega dijagnostičkog testa TR-0171 Bakar. Cilj rada bio je kalibrirati vrijednost standarda bakra Herbos Dijagnostike prema ISO uputi za iskazivanje mjerne nesigurnosti (Guide to the Expression of Uncertainty in Measurement, GUM, 1993.) preko NIST SRM (referentnog standardnog materijala). Bakar se određuje direktnom metodom sa specifičnim kromogenom 5-Br-PSAA u kiselom mediju. 5-Br-PSAA s bakrom tvori obojeni kompleks intenzitet boje kojega je proporcionalan koncentraciji bakra i mjeri se fotometrijski na 580 nm u kivetu 10 mm pri sobnoj temperaturi. Kvantifikacija standarda bakra provodi se pomoću kalibracij-

The results for pO_2 on SP CCX were comparable with Radiometer ABL 500-555, showed positive bias vs Bayer (248, 348, 840-865) and ILOV 1610-1640 analyzers, and negative bias vs pHox and ILOV Gem Premier 3000 analyzers. Our results from both analyzers were within the quality specifications, 100% for pH and Na^+ , 80%-90% for pO_2 , pCO_2 i K^+ , and less than 80% for Ca^{++} , Mg^{++} and Cl^- due to the low proportion of SP CCX analyzers, small number of participants (3 for Mg^{++}) and strict criteria for the acceptability of results. The results obtained emphasize the problem of control materials, which behave differently on various blood gas analyzers of the same or different manufacturers. The ion selective electrodes are very sensitive to matrix effects, which makes the comparability of the results difficult, especially when a small number of laboratories participate in the assessment.

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P22-9

Estimation of measurement uncertainty in the determination of copper standard by spectrophotometric method

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As a long-standing manufacturer of diagnostic reagents, Herbos Dijagnostika has introduced the ISO 9001:2001 quality management system as of January 2004 in order to ensure product quality standards. Our requirements for continuing improvements of product quality are implemented within the manufacturing process of water standards following the internationally certified reference material (National Institute of Standards and Technology, NIST). The first standard introduced was copper standard making part of our diagnostic test TR-0171 Copper. The aim of the study was to calibrate the value of the Herbos Dijagnostika copper standard on the basis of the Guide to the Expression of Uncertainty in Measurement (GUM) 1993, through the NIST SRM (standard reference material). Copper is determined using direct method with a specific chromogen 5-Br-PSAA in acid medium. Combined with copper, the 5-Br-PSAA forms a colored complex whose color intensity is proportional to copper concentration and is measured photometrically at 580 nm in a 10-mm cuvette at room temperature. The

skog pravca, a rezultati se izražavaju u $\mu\text{mol/L}$. Validacija metode provedena je kroz dvadeset dana po četiri mjerenja na dvjema razinama, te je validirana točnost testom iskorištenja. Za procjenu mjerne nesigurnosti i računanje sastavljene i proširene mjerne nesigurnosti rabljeni su podatci dobiveni validacijom metode (obnovljivost, točnost), kalibracijski pravac, priprema radnog standarda iz koncentrata. Usporedbom relativne standardne nesigurnosti je vidljivo da najveći doprinos mjerne nesigurnosti dolazi od kalibracijskog pravca i dugotrajne obnovljivosti, a najmanji od primarnog SRM. Mjerna nesigurnost za standard bakra za vrijednost $15,75 \mu\text{mol/L}$ iznosi $0,25 \mu\text{mol/L}$ s obuhvatnim faktorom $k=2$.

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P22-10

Iskustva Zavoda za kliničku kemiju Kliničke bolnice Merkur u programu međunarodne procjene kvalitete rada u kvalitativnoj analizi mokraće

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Od 1997. godine Zavod za kliničku kemiju KB Merkur sudjeluje u međunarodnom programu EQA Labquality, utemeljenom 1971. godine. U programu sudjeluje 3743 laboratorija iz 41 zemlje. Cilj je procjena kvalitete putem neovisnog sustava kvalitete potvrđenog standardom ISO 9001:2000 kao sustav provjere kvalitete vlastitih rezultata kako u kemijskoj analizi mokraće test trakom, tako i u prepoznavanju elemenata sedimenta mokraće te njihovo usklađivanje s Europskim preporukama kvalitete. Program se izvodi 3 puta na godinu za: 1) kemijsku analizu mokraće test trakom za glukozu, ketone, eritrocite, pH, proteine, nitrite, leukocite, relativnu volumnu masu i brojanje eritrocita (Erc) i leukocita (Lkc) i 2) prepoznavanje elemenata sedimenta mokraće pomoću 4 slike u boji priređene prema preporukama Europske grupe za analizu mokraće uz prikaz slučaja. Rezultati za test traku se obrađuju prema vrsti trake i načinu očitavanja, a pokazuju da se očitavanje izvodi isključivo instrumentalno uz uporabu 5-7 vrsta traka. Iako je osjetljivost traka različita, ukupni rezultati

copper standard quantification is carried out by use of calibration curve and results are expressed in $\mu\text{mol/L}$. The validation of the method is executed over 20 days, with four 2-level measurements *per* day, and the accuracy is validated based on the recovery test. For the purpose of assessing uncertainty in measurement and calculation of the combined and extended uncertainty in measurement the data obtained from the method validation (reproducibility, accuracy), calibration curve, preparation of working standards from the concentrate are used. The comparison of relative standard uncertainty makes it evident that the most significant input to measurement uncertainty derives from the calibration curve and long-term reproducibility while the least one comes from the primary SRM. Uncertainty in the measurement of copper standard for the value of $15.75 \mu\text{mol/L}$ is $0.25 \mu\text{mol/L}$ with the coverage factor $k=2$.

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P22-10

Results of international EQA program for qualitative urinalysis at Department of Clinical Chemistry, Merkur University Hospital

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Since 1997, Department of Clinical Chemistry, Merkur University Hospital has participated in the International Labquality EQA Programme established in 1971 and encompassing 3743 participating laboratories from 41 countries. The aim is quality assessment by an independent quality system certified according to ISO 9001:2000 standard as a system of quality assessment of each laboratory's own results, both in chemical urinalysis using a test strip and in the identification of cell elements, and their harmonization with the European quality recommendations. The program is performed 3 times a year including the following: 1) chemical urinalysis using a test strip for glucose, ketones, erythrocytes (Erc), pH, proteins, nitrites, leukocytes (Lkc) relative volume mass, and Erc and Lkc counting; and 2) identification of urine sediment elements using 4 color pictures prepared according to the European Urinalysis Group with a case report. Results are analyzed for the type of test strip and way of reading. Five to seven types of strips are in use, and measuring is

pokazuju manji rasap vrijednosti za ista koncentracijska područja zbog primjene standardiziranog postupka. Brojanje Erc i Lkc iskazuje se prema metodi brojanja stanica kao srednja vrijednost i razlika od ciljne vrijednosti izražene kao srednja vrijednost $\pm 50\%$. Dobiveni rasap vrijednosti unutar metoda, iako su metode standardizirane, pokazuje još uvijek prisutnu mogućnost utjecaja prije- i poslijeanalitičkih grješaka. Prepoznavanje elemenata sedimenta mokraće se izvodi isključivo tzv. shemom "e-mail" putem interneta. Mogućnost manipulacije slikom olakšava prepoznavanje elemenata. Rezultati su dostupni na web stranicama Labquality. Sudjelovanjem u međunarodnom programu EQA uspostavili smo stalnu provjeru vlastitih analitičkih sustava u kemijskoj analizi mokraće test trakom i u kvantificiranju i identifikaciji staničnih elemenata; time dobiveni rezultati postaju pouzdani nalaz koji zadovoljava europske ciljeve analitičke kvalitete.

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P22-11

Iskustva vanjske procjene kvalitete rada UKNEQAS u području imunofenotipizacije stanica

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Točnost određivanja apsolutnog broja i udjela subpopulacija limfocita i CD34 hematopoetskih matičnih stanica je važna za kliničku obradu imunokompromitiranih osoba i bolesnika sa zloćudnom bolešću koji se podvrgavaju transplantaciji perifernim matičnim stanicama. Sudjelovanje u vanjskoj procjeni kvalitete rada je stoga neophodno da bi se laboratorijski rezultati mogli usporediti i vrednovati radi povećanja vjerodostojnosti i njihove usporedivosti širom svijeta. Cilj je prikazati rezultate sudjelovanja u međunarodnoj kontroli UKNEQAS iz područja imunofenotipizacije dobivene upotrebom standardiziranih i danas preferiranih postupaka za kvantifikaciju stanica protočnom citometrijom.

Od kraja 2005. godine Zavod za kliničku kemiju KB Merkur atestiran prema međunarodnom standardu ISO: 9001:2000 sudjeluje u vanjskoj procjeni kvalitete rada UKNEQAS for Leukocyte Immunophenotyping u dva programa: Immune Monitoring (imunološki status) i CD34 Stem Cell (CD34+ stanice). Kao uzorak se rabi stabilizirana

mainly instrumental. In spite of varying sensitivity of test strips, overall results show lower dispersion of values due to the use of standardized methods. Erc and Lkc count is expressed according to the method of cell counting as mean and difference from the target value expressed as mean $\pm 50\%$. Despite standardization of the methods, dispersion of values still implies the possibility of pre- and postanalytical errors. Identification of urinary sediment elements is performed exclusively by use of the "e-mail" scheme *via* internet. The possibility of picture manipulation facilitates element analysis. Results are available on the Labquality web pages. By participating in the International EQA Program, we have established permanent control of our own analytical systems in chemical urinalysis with test strips and in quantification and identification of cell elements. In this manner, we have ensured the results obtained to be a relevant finding that meets the European standards in analytical quality.

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P22-11

The experience in UKNEQAS External Quality Assessment Scheme for Immunophenotyping

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Accurate determination of absolute and percentage values for lymphocyte subsets and CD34 hematopoietic stem cells is important for clinical management of immunocompromised individuals and cancer patients undergoing peripheral blood stem transplantation. It is essential, therefore, that laboratory data can be compared and evaluated through participation in an external quality assurance program to improve the credibility of results and their comparability worldwide. The objective of this presentation is to point to the results achieved in the UKNEQAS external quality control for immunophenotyping using the state-of-the-art flow cytometry methodologies for cell quantification.

Since the end of 2005, the Institute of Clinical Chemistry of the Merkur University Hospital certified according to ISO: 9001:2000 has participated in the UKNEQAS external quality assessment for leukocyte immunophenotyping in two programs: immune monitoring and CD34 stem cell. Stabilized whole blood was used as a specimen in

puna krv. Uzorci za čitanje na protočnom citometru EPICS XL obrađeni su amonij klorid (NH₄Cl) lizatorom, postupkom "liziraj-ne ispiri". Za određivanje parametara imunološkog statusa rabili smo preporučenu analizu CD45/postranično raspršenje (SSC), a za mjerenje broja CD34+ stanica protokol ISHAGE. Apsolutni broj pozitivnih stanica je određen na protočnom citometru metodom s česticama definiranog broja (*Flow Count beads*). Svi rezultati relativnog i apsolutnog broja pozitivnih stanica imunološkog statusa bili su unutar dozvoljenih granica s tendencijom grupiranja u području ciljne vrijednosti ($\chi \pm 1SD$ kod imunološkog statusa i 25.-75. centila za broj CD34+ stanica). Predobrada uzoraka za čitanje na protočnom citometru s lizatorom NH₄Cl postupkom "liziraj-ne ispiri" je dominantan pripremni postupak i rabi ga više od trećine sudionika. Nadalje, upotrijebljena CD45/SSC analiza na protočnom citometru je najzastupljenija vrst analize (>75%) kod imunološkog statusa, dok je protokol ISHAGE najzastupljeniji (>90%) u programu CD34+ stanica. Metoda *Flow Count beads* za određivanje apsolutnog broja pozitivnih stanica na protočnom citometru druga je po zastupljenosti (>30%). Upotrebom preporučenih postupaka i protokola u imunofenotipizaciji stanica, koji uključuju pripremu uzoraka za mjerenje i analizu na protočnom citometru, moguće je osigurati visok stupanj međulaboratorijske usklađenosti uz potpunu pouzdanost dobivenih rezultata.

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P22-12

Vanjska procjena kvalitete u laboratorijskoj hematologiji (IEQAS-H) – dvadeset godina iskustva Zavoda za kliničku kemiju Kliničke bolnice Merkur

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Zavod za kliničku kemiju Kliničke bolnice Merkur od 1985. godine sudjeluje u međunarodnoj sponzoriranoj kontroli iz područja laboratorijske hematologije Shema za internacionalnu vanjsku procjenu kvalitete iz hematologije (International External Quality Assessment Scheme for Haematology, IEQAS-H) pod pokroviteljstvom Svjetske zdravstvene organizacije. Godine 1987. Zavod je dobio

all send-outs. For sample processing on an EPICS XL flow cytometer, the lyse/no wash preparation technique with ammonium chloride (NH₄Cl) lysing reagent was employed. In the immune monitoring program, the CD45/sideward light scatter (SSC) proposed gating strategy was adopted for lymphocyte subsets, while ISHAGE protocol was used for CD34+ cell enumeration. Absolute count determination was performed on a flow cytometer using the Flow Count beads solution with the known number of particles. The relative and absolute enumeration results from both programs were within the tolerable limits, and tended to group within the region around the target value (value within $\chi \pm 1SD$, and 25th-75th centile in CD34+ in immune monitoring and stem cell program, respectively). Sample treatment in the lyse/no wash technique using NH₄Cl lysing solution for acquisition on flow cytometer was a dominant procedure used by more than one-third of participants. Moreover, in immune monitoring and CD34+ cell determination the most frequent analyses were CD45/SSC gating strategy (>75%) and ISHAGE protocol (>90%), respectively, whereas the Flow Count beads method for absolute count enumeration on flow cytometer was the second of the bead-based techniques (>30%). The use of the recommended procedures and protocols for cell quantification, which include sample preparation and flow cytometric analysis, can ensure a high degree of interlaboratory harmonization accompanied by absolute reliability of the results obtained.

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P22-12

International External Quality Assessment Scheme for Hematology – 20-year experience of Department of Clinical Chemistry, Merkur University Hospital

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Department of Clinical Chemistry, Merkur University Hospital, has been involved in the International External Quality Assessment Scheme for Haematology (IEQAS - H) organized by the World Health Organization (WHO) since 1985. In 1987, the Department received a certificate with the obligation of active participation in external quality control at regional level, which was fulfilled. The aim

certifikat s obvezom aktivnog sudjelovanja u provedenju vanjske procjene kvalitete na regionalnoj, razini što je učinjeno. Cilj vanjske procjene kvalitete rada je usklađivanje rezultata u laboratorijskoj hematologiji neprekidnom izobrazbom zasnovanom na povratnim informacijama o rezultatima svakog pojedinog ciklusa kontrole. Kroz 6 kontrola na godinu prođe ukupno 12 uzoraka za hemoglobin, leukocite i trombocite na hematološkom brojaču (specijalni pripravci); 8 uzoraka za leukogram, 8 uzoraka za parazite (pripravci obojeni po Pappenheimu); i 8 uzoraka za retikulocite (pripravci obojeni briljant krezil modrilom). Rezultati za hemoglobin, leukocite, trombocite i retikulocite vrednuju se prema indeksu odstupanja DI (DI >3,0 neprihvatljiv). Rezultati za diferencijalnu krvnu sliku – leukogram uspoređuju se s deklariranim intervalima za pojedinu vrstu leukocita, a nađene morfološke promjene na krvnim stanicama opisno, do 5 najbitnijih morfoloških obilježja u razmazu.

Za retikulocite dozvoljeno odstupanje je $\pm 50\%$. Za točan nalaz krvnih parazita potrebno je definirati vrstu i podvrstu parazita. Prihvatljivost rezultata tijekom 20-godišnje kontrole: za hemoglobin 95,2% , za leukocite 80,5%, za trombocite 95,2%, za retikulocite 80,5%, za leukogram 97,0% i za vrstu parazita 99,0%. Različiti analitički sustavi u laboratorijskoj hematologiji postavljaju apsolutni zahtjev za usklađivanje rezultata. To se može postići kroz nekoliko stupnjeva: svakodnevnim provođenjem unutarnje kontrole kvalitete rada i statističkom obradom rezultata nepreciznosti i netočnosti; sudjelovanjem u nacionalnoj vanjskoj procjeni kvalitete rada i usklađivanjem rezultata među laboratorijima. U takovom ustroju treba predvidjeti i sudjelovanje u međunarodnim procjenama. Kontrola IEQAS pod pokroviteljstvom Svjetske zdravstvene organizacije je svakako jedna od najvažnijih.

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P23 – Ostalo, P23-1

Oksidacijski stres u miševa: učinak kukuruznog ulja i željeza

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Antioksidacijski status predstavlja ravnotežu između nusproizvoda oksidacijskog stresa i zaštitnog antioksidacijskog sustava. Oksidacijski stres najčešće se mjeri indirektno preko enzimskih i neenzimskih antioksidacijskih pokazatelja. Unos velikih količina željeza (Fe) rezultira ok-

of external quality assessment is harmonization of the results in laboratory hematology by continuous education based on feedback on the results from each control step. Through 6 controls *per year*, 12 samples undergo EQA for hemoglobin, leukocytes and platelets on a blood counter (special preparations); 8 samples for leukogram, 8 samples for parasites (preparations stained according to Pappenheim); and 8 samples for reticulocytes (preparations stained with brilliant cresyl blue). The results on hemoglobin, leukocytes, platelets and reticulocytes are evaluated according to the deviation index DI (DI >3.0 is unacceptable). The results on differential blood count – leukogram are compared with declared intervals for each type of leukocytes, and morphological changes observed in blood cells are descriptively presented, including up to 5 most important morphological features *per smear*. For reticulocytes the allowed deviation is $\pm 50\%$. For accurate identification of blood parasites, it is required to define the parasite type and subtype. Acceptable results during 20 years of control: for hemoglobin 95.2%, for leukocytes 80.5%, for platelets 95.2%, for reticulocytes 80.5%, for leukogram 97.0%, and for parasites 99.0%. Different hematologic analytical systems require a system for harmonization of the results. This can be accomplished through several steps: daily conduction of internal quality control and statistical analysis of the results on imprecision and inaccuracy; and participation in national external quality control and assessment of the results between laboratories. The structure should also include participation in international controls. IEQAS control under WHO supervision is certainly one of the most important.

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P23 – Other, P23-1

Oxidative stress in mice: effect of dietary corn oil and iron

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Antioxidant status represents the balance between by-products of oxidative stress and antioxidant defense system. Oxidative stress is most commonly measured indirectly *via* enzymatic and nonenzymatic antioxidant markers. High Fe ingestion may result in oxidative stress

sidacijskim stresom i štetnim reakcijama kao što su peroksidacija višestruko nezasićenih masnih kiselina i smanjena aktivnost antioksidacijskih enzima. Mehanizam kojim je Fe uključen u započinjanje ili poticanje oksidacijskog oštećenja nije sasvim jasan. Cilj ovoga istraživanja bio je ispitivanje učinka velikih količina Fe na antioksidacijski status miševa čija je hrana obogaćena kukuruznim uljem. Mužjaci miševa Balb/c hranjeni su 3 tjedna standardnom prehranom obogaćenom s 5% kukuruznog ulja i s optimalnom količinom Fe (skupina FCO), odnosno obogaćenom s 1% karbonilnog Fe (skupina FCOFe). Kontrolna skupina je hranjena standardnom prehranom. Količina Fe, bakra (Cu) i cinka (Zn) u tkivu jetre određeni su induktivno spregnutom plazma spektrometrijom. Aktivnosti Cu/Zn superoksid dismutaze (CuZnSOD) i glutation peroksidaze (GPx) određene su spektrofotometrijski u nadsloju homogenata jetre. Lipidna peroksidacija određena je mjerenjem količine reaktivnih spojeva s tiobarbiturnom kiselinom (TBARS). Prehrana s velikim udjelom Fe povećala je količinu Fe u jetri 2 puta. Međutim, porast količine Fe u timusu izazvan je isključivo masnoćama u hrani. Sadržaj Cu u jetri blago se smanjio u skupini FCO. U slezeni je porast količine Fe izazvan prehranom obogaćenom Fe negativno korelirao s količinom Cu. Na antioksidacijski status utjecali su oboje, i prehranska masnoća i Fe. Miševi čija je hrana bila obogaćena kukuruznim uljem imali su povećanu količinu TBARS, s većim porastom u skupini FCOFe. Aktivnost CuZnSOD u jetri smanjena je u skupini FCO, dok je dodatak Fe u prehrani uzrokovao daljnje smanjenje aktivnosti enzima. Rezultati ukazuju na to da prehrana obogaćena kukuruznim uljem uzrokuje oksidacijsko oštećenje i smanjenje antioksidacijske enzimske zaštite. Dodatak velikih količina Fe dodatno je utjecao na antioksidacijski sustav povećavajući osjetljivost jetrenog tkiva na lipidnu peroksidaciju. Uz to, prehrana obogaćena kukuruznim uljem i Fe povećala je u miševa potrebe za Cu.

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P23-2

Povezanost koncentracije serumskog kolesterola i suicidalnog ponašanja

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Ranije studije pokazale su povezanost niske koncentracije ukupnog kolesterola i suicidalnog ponašanja. Cilj ispitivanja bio je ustanoviti postoje li razlike u koncentraciji serumskog kolesterola u bolesnika s određenim psihijatrij-

and deleterious reactions such as peroxidation of polyunsaturated fatty acids and decreased antioxidant enzyme activities. The mechanism by which Fe is involved in initiating or promoting oxidative damage is not entirely clear. The aim of the study was to analyze the effect of high dietary iron (Fe) on liver antioxidant status in mice fed corn oil-enriched diet. Male Balb/c mice were fed for 3 weeks standard diets enriched with 5% by weight of corn oil, with adequate Fe (FCO group), or supplemented with 1% carbonyl Fe (FCOFe group). Control group was fed standard diet. Fe, copper (Cu), and zinc (Zn) levels in liver tissue were determined by inductively coupled plasma spectrometry. Cu/Zn superoxide dismutase (CuZnSOD) and glutathione peroxidase (GPx) activities were determined spectrophotometrically in liver homogenate supernatant. Lipid peroxidation was evaluated by measuring thiobarbituric acid-reactive species (TBARS). High-Fe diet induced 2-fold increase of hepatic Fe level. However, the increase of thymic Fe level was induced solely by dietary fat. Hepatic Cu level slightly decreased on FCO diet. In spleen, the high-Fe diet induced increase of Fe level correlated negatively with Cu level. The antioxidant status was influenced by both dietary fat and Fe. Mice fed corn oil-enriched diets had a higher concentration of TBARS, with a greater increase on FCOFe diet. Hepatic CuZnSOD activity was decreased in FCO diet, and Fe supplementation caused further decrease in the enzyme activity. These results suggest that feeding with corn oil-enriched diets increases oxidative damage by decreasing antioxidant enzyme defense. The high-Fe diet additionally affects the antioxidant defense system, further increasing the tissue susceptibility to lipid peroxidation. Additionally, both corn oil- and Fe-enriched diets increased Cu requirements in mice.

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P23-2

Relationship between serum cholesterol and suicidal behavior

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Previous studies have pointed to a relationship between low level of total serum cholesterol and suicidal behavior. The aim of our study was to investigate the possible differences in cholesterol level between psychiatric patients

skim dijagnozama u odnosu na bolesnike koji su pokušali suicid. Uzorak se sastojao od 677 bolesnika raspoređenih u skupine s dijagnozama prema MKB-10 (F10 sindrom ovisnosti-alkoholizam, F20 shizofrenija, F23 psihotični poremećaji, F25 shizoafektivni potemećaji, F32 depresivni poremećaj, F43 reakcija na stres, F60 poremećaji ličnosti, X61 nenasilni pokušaj suicida, X70 nasilni pokušaj suicida), kojima je određena koncentracija ukupnog kolesterola standardnom metodom kod prijma na liječenje u Psihijatrijsku bolnicu Sveti Ivan u razdoblju od 1. siječnja do 1. travnja 2005. Razlike u koncentraciji kolesterola prema dijagnozama testirane su analizom varijance, budući da je test homogenosti varijance pokazao da se varijance statistički značajno ne razlikuju (Bartlett $\chi^2=11,12$, st.sl=8, $p=0,19$). Analiza varijance pokazala je kako postoji statistički značajna razlika između skupina bolesnika s različitim dijagnozama ($F=2,47$, st.sl.=8, $p=0,012$). Višestrukim post hoc testom (LSD) pokazalo se da se skupina s dijagnozom X61 koja ima najmanju vrijednost kolesterola statistički značajno ne razlikuje od skupine s dijagnozama F10, F23 i X70, dok se statistički značajno razlikuje od skupina F20, F25, F32, F43 i F60. Dobiveni rezultati potvrdili su hipotezu o povezanosti niske koncentracije ukupnog kolesterola i suicidalnog ponašanja. Određivanje koncentracije ukupnog kolesterola u serumu bolesnika s dijagnozama F10 i F23 može poslužiti kao koristan biološki biljeg pri procjeni rizika suicidalnog ponašanja psihijatrijskih bolesnika.

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with particular diagnoses and patients with a history of suicide attempts. Total cholesterol level was determined in serum samples of 677 patients admitted to Sveti Ivan Psychiatric Hospital from January 1 till April 1, 2005, distributed into groups according to ICD10 diagnoses. The ICD-10 diagnoses were: F10, syndrome of alcohol dependence; F20, schizophrenia; F23, psychotic disorder; F25, schizoaffective disorder; F32, depressive disorder; F43, stress reaction; F60, personality disorder; X61, nonviolent suicide attempt; and X70, violent suicide attempt. Analysis of variance was used to analyze differences in cholesterol levels between patient groups because the test of homogeneous variance yielded no statistically significant difference. A significant difference was found between the groups with different diagnoses ($F=2.47$, $p=0.012$). Multiple post hoc test (LSD) showed the group of patients with X61 diagnosis, which had the lowest cholesterol level, not to differ from the groups of patients with the F10, F23 and X70 diagnoses, but to differ statistically significantly from the patient group with the F20, F25, F32, F43 and F60 diagnoses. In conclusion, our results approve the hypothesis on the relationship between low levels of total cholesterol in serum and suicidal behavior. It is also suggested that determination of total serum cholesterol in patients with the F10 and F23 diagnoses could provide a valuable biological risk marker on assessing suicidal behavior in these patients.

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