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PRAĆENJE HEMATOLOŠKIH PARAMETARA U RANOJ DIJAGNOZI INTRA-ABDOMINALNIH INFEKCIJA

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Sepsa je sistemski odgovor na infekciju. Pojam sepse u abdominalnoj hirurgiji podrazumeva postojanje intra-abdominalnog septičnog fokusa, kao glavnog pokretača niza patofizioloških zbivanja. Sepsa je stanje koje prati poremećaj i hematoloških parametara. Cilj ovog rada bio je da se ispita uticaj intra-abdominalne infekcije praćene septičkim sindromom na hematološke parametre. Određivani su osnovni hematološki parametri: eritrociti, hemoglobin, trombociti, leukociti, neutrofili i limfociti. Ciljnu grupu je činilo 20 pacijenata sa IAI (uglavnom difuzni peritonitis) i kontrolna grupa od 20 pacijenata bez znakova infekcijskog sindroma, a koji su lečeni hirurški (operacija hernije). Broj ćelija određivan je u uzorcima pune krvi, sa antikoagulansom, na hematološkom brojaču Coulter Onyx i manuelno koristeći mikroskop. Srednje vrednosti hemograma i leukocitarne formule u kontrolnoj grupi nisu pokazala odstupanja od referentnih vrednosti. U grupi pacijenata sa IAI, srednje vrednosti (opseg) su iznosile za: leukocite 17,3 (3,8–41,5) × 10^9/L, neutrofile 0,72 (0,55–0,89) i štapičaste leukocite 0,05 (0,02–0,12) i bili su značajno povećani u odnosu na kontrolnu grupu. Trombociti ($\bar{x}$ = 224 × 10^6/L, opseg 76–490), hemoglobin ($\bar{x}$ = 106 g/L, opseg 68–149) i eritrociti ($\bar{x}$ = 3,85 × 10^{12}/L, opseg 2,10–5,20) bili su blago sniženi. U grupi IAI vrednosti limfocita su se kretale u opsegu 0,03–0,26 ($\bar{x}$ = 0,16) i bile su značajno snižene u odnosu na kontrolnu grupu. Na osnovu prikazanih rezultata može se zaključiti da kod pacijenata sa IAI postoji značajno povećanje nivoa leukocita tj. neutrofila što ukazuje na aktivaciju imunog sistema u ovakvim stanjima. Praćenje hematoloških parametara, kao i nekih biohemijskih parametara i parametara hemostaze uz mikrobiološki nalaz, omogućava pravovremenu dijagnozu IAI, a time i njeno lečenje.

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MONITORING OF HAEMATOLOGICAL PARAMETERS IN EARLY DIAGNOSIS OF SEVERE INTRA-ABDOMINAL INFECTIONS

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Systemic response to infection is sepsis. The term sepsis in abdominal surgery designates the presence of septic abdomen as the major trigger of a series of pathophysiological events. Sepsis is frequently associated with disturbances within haematological parameter values. The aim of the study was to analyse haematological disturbances during the severe intra-abdominal infections (IAI) with sepsis syndrome. The following basic haematological parameters were determined: red blood cells (RBC), haemoglobin, platelets, white blood cells (WBC), neutrophils and lymphocytes. The study group consisted of 20 patients with severe IAI (mostly diffuse peritonitis) and control group of 20 surgically treated (hernia repair) patients without signs of infection. The number of cells was determined in blood samples with anticoagulants by haematological counter Coulter Onyx and mannually by microscope. In control group, haematological parameter values were permanently maintained within the normal range. In all patients with IAI, leukocytes (mean 17.3 × 10^9/L, range 3.8–41.5) and neutrophils (mean 0.72, range 0.55–0.89) were significantly increased. Platelets (mean 224 × 10^6/L, range 76–490), haemoglobin (mean 106 g/L, range 68–149) and RBC (mean 3.85 × 10^{12}/L, range 2.10–5.20) were slightly decreased. In group IAI mean of lymphocytes was 0.16 (range 0.03–0.26) and significantly lower than in control group. On the basis of the obtained results the conclusion may be that significant increase in leukocytes and neutrophils levels occurred in patients with IAI, what was the result of activation of immune system. By monitoring haematological parameters, as well as some biochemical and haemostatic parameters and microbiological findings, prompt diagnosis and treatment of IAI will be possible.

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SADRŽAJ HEMOGLOBINA U RETIKULOCITIMA KAO POKAZATELJ KVALITETA TERAPIJE KOD BOLESNIKA NA HRONIČNOJ HEMODIJALIZI

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Sadržaj hemoglobina u retikulocitima (CHr) je direktna mera statusa gvožđa na nivou retikulocita, pouzdan je i vremenski osetljiv test, i predlaže se kao surogat marker therapije gvožđem kod bolesnika na hemodijalizi (HD). Cilj ovog rada je bio da se proceni efikasnost terapije eritropoetinom (rHuEpo) kod HD pacijenata korišćenjem laboratorijskih hematoloskih parametara. Kod 39 bolesnika (starosti 17–78 godina) koji su duže od 6 meseci na HD, najmanje 6 meseci na supstitucionoj terapiji rHuEpo i *i. v.* gvožđem, određivani su uobičajeni hematološki i biohemijski pokazatelji kao i CHr. Rezultati su poređeni sa kontrolnom grupom od 25 zdravih ispitanika. Korišćeni su Bayer Technicon-H3 sistem i Dade Behring komercijalni testovi. Rezultati pokazuju da su broj eritrocita (RBC), hemoglobin (Hb) i hematokrit (Ht) bili značajno niži ($p<0.001$) sa širom RBC i Hb distribucijom od kontrolne grupe. Broj retikulocita, CHr i CHr/CH nisu bili različiti dok je frakcija stepena zrelosti retikulocita (HFR) i širina distribucije hemoglobina retikulocita (HDWr) bili viši ($p<0,01$). Značajni markeri deficita gvožđa, mikrocitoza eritrocita (% mikro) i hipohromija (% hipo), su bili viši ($p<0,01$). Ovakva zapažanja sugeriraju mogućnost identifikovanja pacijenata kojima je potrebna korekcija anemije terapijom gvožđa. Rutinski ferokinetički parametri su bili značajno sniženi, ali su transferin i feritin bili u okviru optimalnih vrednosti. Saturacija transferina, % TSAT (28,0 ± 19,4) je bila u preporučenim okvirima, ali kod 41% pacijenata ispod limita. Srednja vrednost CHr 31,0 pg (raspon 24,5–35,9 pg) kod bolesnika na suplementacionoj terapiji pokazuje dobro zbrinjavanje (»cut-off« > 28 pg), ali 18% njih nije dostiglo taj nivo. Takođe, preporučeni ciljni nivoi za Hb i Ht su bili postignuti (109 ± 13,7 g/L i 0,34 ± 0,05), ali čak oko 45% pacijenata je bilo ispod tih vrednosti. Značajni markeri statusa HD pacijenata, % TSAT, feritin i CHr, pokazuju visoko kvalitetan tretman kod posmatrane grupe, i omogućuju esencijalne informacije za planiranje i vođenje adekvatne rHuEpo terapije.

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RETICULOCYTE HAEMOGLOBIN CONTENT AS INDICES OF THERAPEUTIC QUALITY IN CHRONIC HAEMODIALYSED PATIENTS

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Reticulocyte haemoglobin content (CHr) is a direct measure of iron status at reticulocyte level; it is also an accurate and time sensitive test, and is proposed as a surrogate marker of iron therapy in haemodialysed (HD) patients. The aim of the study was to evaluate the erythropoietin (rHuEpo) therapy efficiency using haematological laboratory parameters. In 39 patients (aged 17–78 years) on HD treatment for >6 months, on rHuEpo therapy for >6 months and *i. v.* Fe substitution, traditional haematological, biochemical parameters and CHr were measured. Results were compared to a control group of 25 healthy volunteers. The Bayer Technicon-H3 system and Dade Behring commercial tests were used. The results showed that RBC, Hb, Ht levels were significantly lower ($P<0.001$) with wider RBC and haemoglobin (Hb) distributions. Reticulocyte count, CHr and CHr/CH were not different, but the high fraction of reticulocytes (HFR) and haemoglobin distribution width reticulocytes (HDWr) were higher ($P<0.01$). The important markers of iron deficiency, red cell microcytosis (% micro) and hypochromia (% hypo), were higher. These observations contibuted identification of patients on dialysis who need correction of their anaemic condition with iron therapy. The routine ferrokinetic parameters were significantly decreased, but transferrin and ferritin concentrations were within the optimal values. The saturation of transferrin,TSAT % (28.0 ± 19.4) was in reference range, but 41% of patients were sublimited. The mean CHr 31.0 pg (range 24.5–35.9 pg) in patients on supplementation therapy showed good results (cut-off > 28 pg), but 18% of them were below that level. The recommended Hb and hematocrit target levels were reached (109 ± 13.7 g/L and 0.34 ± 0.05, respectively), but about 45% of patients were under the limit. The results of important markers of HD patients status, % of TSAT, ferritin and CHr, confirmed a high quality of treatment in the observed group, providing the essential information for planning of an adequate rHuEpo treatment.

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VITAMIN B_{12} I FOLNA KISELINA KOD HEMODIJALIZIRANIH PACIJENATA NA SUPSTITUCIONOJ TERAPIJI

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Megaloblastna anemija je veliki problem kod bolesnika na hemodijalizi (HD) zbog deficita vitamina B_{12} i folne kiseline. Cilj ovog rada je bio da se proceni efikasnost suplementacione vitaminske terapije kod bolesnika na HD, mereći serumske koncentracije vitamina B_{12}, folne kiseline i odgovarajućih laboratorijskih hematoloških parametara. Ispitivanjem je obuhvaćeno 40 bolesnika (starosti 17–78 godina) na HD koji su bili na terapiji eritropoetinom, *i.v.* gvožđem i vitaminima, i 25 zdravih ispitanika kao kontrolnom grupom. Analize su izvođene elektrohemiluminiscentnom metodom na Roche Elecsys 2010 imunoanalizatoru. Hematološki pokazatelji, broj retikulocita (Ret) i frakcije stepena zrelosti Ret (HFR) su određeni Bayer Technicon-H3 protočnom citometrijom. U poređenju sa kontrolnom grupom RBC, hematokrit i hemoglobin su bili statistički značajno niži ($p<0,001$). RDW (15,6 prema 13,5 %) i % makrocita (4,2 ± 2,9 prema 0,9 ± 0,3, $p<0,001$) su bili značajno viši u poređenju sa kontrolnom grupom i ukazivali su na prisustvo anizocitoze. Folna kiselina je bila u očekivanim vrednostima bliskim kontrolnoj grupi (12,8 ± 5,9 nmol/L), a B_{12} značajno viši (648 ± 359 prema 243 ± 61,4 pmol/L, $p<0.001$), čak iznad referentnih vrednosti. MCV (92 ± 7,8 fL) i Ret (1,45 ± 0,5%) nisu bili značajno različiti od kontrole, sa relativnim povećanjem u HFR (6,0 ± 4,5 prema 2,9 ± 1,5%, $p<0,001$). Takvo skretanje retikulocitnih podklasa je karakteristično za neefektivnu eritropoezu, nasuprot dobrom odgovoru na vitaminsku suplementaciju. Dobijeni rezultati ukazuju na naizgled optimalan vitaminski tretman bolesnika na HD ali ipak prisutnu anizocitozu. Može se zaključiti da je za uspešno definisanje statusa bolesnika na HD potrebno, pored optimalne terapije i kontrole koncentracija folne kiseline i vitamina B_{12}, obezbediti dodatne kontrolne testove, gvožđe i hematološke morfološke parametre (HFR, MCV, RDW, % makro).

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VITAMIN B_{12} AND FOLIC ACID IN HAEMODIALYSED PATIENTS ON SUBSTITUTION THERAPY

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Megaloblastic anaemia is a big problem in haemodialysed (HD) patients due to the vitamin B_{12} and folic acid deficiency. The aim of the study was to evaluate the efficiency of supplemented vitamin therapy in HD patients, by measuring the serum concentrations of vitamin B_{12}, folic acid and conventional laboratory haematological parameters. The study included 40 HD patients (aged 17–78 years), subjected to vitamin therapy and 25 healthy volunteers as a control group. Analyses were performed by electrochemiluminescent method on the Roche Elecsys 2010 automatic immunoanalyser. Haematological parameters, reticulocyte count and maturity fractions (HFR) were determined on the Bayer Technicon-H3 flow cytometer. Compared to the control group, the RBC count, hematocrit and haemoglobin values were statistically significantly decreased. The RDW (15.6 vs. 13.5 %) and the percentage of macrocytic RBC (4.2 ± 2.9 vs. 0.9 ± 0.3, $P<0.001$) were higher than in control group suggesting the existance of anisocytosis. Folic acid was within the expected values (12.8 ± 5.9 nmol/L), but B_{12} was significantly increased in comparison to control group (648 ± 359 vs. 243 ± 61.4 pmol/L, $P<0.001$), even over the reference range. The MCV (92 ± 7.8 fL), and reticulocyte count (1.45 ± 0.5%) were not different from those in control group, with a relative increase in the HFR (6.0 ± 4.5 vs. 2.9 ± 1.5%, $P<0.001$). This shift in reticulocyte fractions reflected patients with an ineffective erytropoiesis, despite a good relative response to vitamin supplementation. The obtained results showed both the optimal vitamin treatment of HD patients and the presence of anisocytosis. It can be concluded that patients on HD need an optimal vitamin supplementation and control of their concentration, as well as additional control tests of iron and haematological morphological parameters (HFR, MCV, RDW, % macro) in order to define their precise status.

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REFERENTNE VREDNOSTI HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KOD REGRUTA

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Koncentracije dva hematološka i pet biohemijskih parametara potencijalno vezanih za uzrast određivane su u grupi 74 regruta, osamnaestogodišnjaka. Uključeni su eritrociti (Er), hemoglobin (Hb), alkalna fosfataza (AP), aspartat aminotransferaza (AST) i alanin aminotransferaze (ALT), ukupni i direktni bilirubin. Dobijene vrednosti su upoređivane sa vrednostima kod 20 zdravih, odraslih muškaraca uzrasta 20–30 godina iz iste geografske oblasti. Vrednosti u serumu određivane su na analizatoru »Targa – 3000« komercijalnim testovima »Dialab«; u krvi na hematološkom brojaču »Coulter IG«. Svi parametri osim direktnog bilirubina statistički se značajno razlikuju ($p<0,01$ za AST, ALT, AP i $p<0,001$ za Er, Hb i ukupni bilirubin). Za većinu parametara sem koncentracije Hb i Er nivoi su bili u okviru referentnih vrednosti. Adolescenti imaju niže referentne vrednosti broja Er, Hb, AST i ALT nego odrasli. Vrednosti ukupnog bilirubina i alkalne fosfataze su veće nego u odraslih. Devetnaest regruta imalo je veće vrednosti ukupnog bilirubina od 20,5 μmol/L, ali hiperbilirubinemija nije statistički dokazana. Niže vrednosti hemoglobina i eritrocita kod regruta zahtevaju otkrivanje i prevenciju anemija u ovoj populaciji.

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REFERENCE VALUES OF BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN ADOLESCENT RECRUITS

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Concentration of two haematological and five biochemical parameters, possibly associated with ageing, were determined in 74 adolescent recruits, aged 18 years. Erythrocytes (Er), blood haemoglobin (Hb), alkaline phosphatase (AP), aspartate and alanine aminotransferase (AST and ALT), total bilirubin and direct bilirubin levels were determined, and compared to reference levels in twenty healthy men, aged 20–30 years, from the same geographical area. Parameters were determinated in sera by commercial tests »Dialab« on the analyzer »Targa – 3000«. Erythrocyte and haemoglobin values were determined on the analyzer »CoulterIG«. Analyses of these parameters, except of direct bilirubin, showed a statistically significant difference ($P<0.01$ for AST, ALT, AP and $P<0.001$ for Er, Hb, total bilirubin). Levels were usually in normal reference values, only for Hb and Er were lower. Erythrocytes, haemoglobin, AST and ALT reference values were lower in adolescents than in adult population. Total bilirubin and alkaline phosphatase levels were higher than in adult population. In 19 recruits total bilirubin levels were up to 20.5 μmol /L, but hyperbilirubinaemia was not statistically significant. Lower levels of Hb and Er require screening and prevention of anaemia in adolescent population.

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MEĐUODNOSI TESTA OSMOTSKE REZISTENCIJE ERITROCITA I JAČINE INDIREKTNE HIPERBILIRUBINEMIJE KOD NEKIH HEMATOLOŠKIH BOLESTI DEČIJEG UZRASTA

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Test osmotske rezistencije eritrocita se često koristi u dijagnostici raznih tipova hereditarnih hemolitičkih anemija praćenih hiperbilirubinemijom. Hemolitičke anemije, koje se karakterišu ubrzanom destrukcijom eritrocita, obično su posledica nekih metaboličkih poremećaja kao što su membranski defekti, poremećaji enzima eritrocita ili poremećaji u strukturi hemoglobi-

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RELATIONSHIP BETWEEN OSMOTIC FRAGILITY TEST OF ERYTHROCYTES AND SEVERITY OF INDIRECT HYPERBILIRUBINAEMIA IN SOME HAEMOLYTIC DISORDERS IN CHILDHOOD

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The osmotic fragility test is useful in the diagnosis of different types of hereditary haemolytic anaemias, followed by hyperbilirubinaemia. Haemolytic anaemias, characterized by accelerated destruction of red blood cells, are usually a consequence of many metabolic abnormalities like cellular membrane defect, erythrocytes enzymes defect, or haemoglobin abnormalities –

na–hemoglobinopatije. Predmet istraživanja bio je utvrđivanje odnosa vrednosti osmotske rezistencije eritrocita i stepena indirektne hiperbilirubinemije kod nekih urođenih poremećaja eritrocita. Osmotska rezistencija eritrocita ispitivana je korišćenjem Dacie-ve metode sa normalnim vrednostima za hemolizu eritrocita koja počinje i završava se između 0,48% i 0,34% NaCl (minimalna i maksimalna hemoliza). U slučaju hereditarne sferocitoze fragilnost eritrocita je bila povećana sa početnom hemolizom eritrocita pri koncentraciji NaCL od 0,65% do potpune hemolize eritrocita pri 0,45% NaCl (minimalna 0,65% NaCl i maksimalna 0,45% NaCl). Kod deteta sa α-talasemijom, fragilnost eritrocita je bila smanjena (minimalna na 0,42% NaCl do maksimalna na 0,32% NaCl). Kod novorođenčadi sa visokim vrednostima indirektnog bilirubina u serumu kao posledice fiziološke žutice osmotska rezistencija eritrocita se kretala u normalnim granicama. Dobijeni rezultati ukazuju na dijagnostičku vrednost testa osmotske rezistencije eritrocita u ispitivanju pacijenata sa indirektnom hiperbilirubinemijom. Ovaj jednostavan i značajan dijagnostički test može da se izvodi u malim laboratorijama.

haemoglobinopathies. The aim of our study was to assess the relationship between osmotic fragility test of erythrocytes and severity of indirect hyperbilirubinaemia in some inherited erythrocytes disorders. We performed osmotic fragility test of erythrocytes by using Dacie's method with normal values of erythrocytes haemolysis between 0.48% and 0.34% NaCl (minimal to maximal haemolysis). In hereditary spherocytosis fragility of erythrocytes was increased (min. 0.65% NaCl to max 0.45% NaCl). In a child with α-thalassemia erythrocytes fragility was decreased (min. 0.42% to max 0.32% NaCl). In newborn infants with high levels of indirect bilirubin in serum as a cause of physiological icterus the osmotic fragility test was in normal range. Our findings point to the diagnostic importance of the osmotic fragility test in assessing patients with indirect hyperbilirubinaemia. This simple and important diagnostic test can be performed in small laboratories.

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SOLUBILNI TRANSFERINSKI RECEPTOR I FERITIN U DIJAGNOZI ANEMIJE USLED DEFICITA GVOŽĐA

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Koncentracija feritina manja od 12 μg/L je najbolji biohemijski marker za dijagnozu anemije usled deficita gvožđa (eng. Iron Deficiency Anemia – IDA), ali pošto je reaktant akutne faze, njegova koncentracija može biti normalna ili povećana ukoliko je IDA kombinovana sa anemijom hronične bolesti (eng. Anemia of Chronic Disease – ACD). Solubilni transferinski receptor (sTfR) je biohemijski parametar čija je koncentracija povećana u IDA, a nije reaktant akutne faze. Izračunavanje indeksa sTfR/logFeritin (sTfR/Fer) objedinjuje porast koncentracije sTfR i pad koncentracije feritina u dijagnozi IDA. Cilj rada je bio da se odrede vrednosti sTfR u kontrolnoj i grupi pacijenata sa anemijom i da se uporedi dijagnostička efikasnost sTfR, feritina i sTfR/Fer u dijagnozi IDA. Koncentracije sTfR i feritina je određena u 66 neanemičnih osoba (kontrolna grupa) i 118 anemičnih pacijenata. Anemični pacijenti su podeljeni u dve grupe: ACD grupa – grupa pacijenata (n=31) sa karakteristikama ACD (feritin 241 $\pm$ 208 μg/L) i IDA grupa - pacijenti (n=87) sa karakteristikama IDA (feritin 89 $\pm$ 64 μg/L). Primenom Mann-Whithney testa, u koncentraciji sTfR između kontrolne grupe (1,53 $\pm$

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SOLUBLE TRANSFERRIN RECEPTOR AND FERRITIN IN DIAGNOSIS OF IRON DEFICIENCY ANAEMIA

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Ferritin concentration less than 12 μg/L is the best biochemical parameter in diagnosis of iron deficiency anaemia (IDA), but as an acute phase reactant it could be normal or increased if IDA is accompanied with anaemia of a chronic disease (ACD). Soluble transferrin receptor (sTfR) is a biochemical marker with elevated concentration in IDA, and it is not an acute phase reactant. Calculation of sTfR/logFerritin ratio (sTfR/Fer) unites increase in sTfR and decrease in ferritin concentration in IDA. The aim of the study was to establish the value of sTfR in a control group and anaemic group of patients, and to compare diagnostic accuracy of sTfR, ferritin and sTfR/Fer in diagnosis of IDA. Concentrations of sTfR and ferritin were measured in the sera of 66 non-anaemic persons (control group) and 118 anaemic patients. Anaemic patients were divided in two groups: ADC group – group of 31 patients with characteristic ACD concentrations (mean ferritin 241 $\pm$ 208 μg/L), and IDA group – patients (n = 87) with characteristic IDA concentrations (mean ferritin 89 $\pm$ 64 μg/L). Difference in means was compared with Man-Whitney's test, and concentration of

0,30 mg/L) i IDA grupe (3,66 ± 1,20 mg/L) dobijena je statistički značajna razlika ($p < 0,001$) dok između kontrolne i ACD grupe (1,72 ± 0,51 mg/L) nije bilo razlike ($p = 0,181$). Primenom ROC analize upoređena je ukupna tačnost sTfR, feritina i sTfR/Fer u odnosu na sadržaj hemoglobina u retikulocitima (CHr) koji je najosetljiviji hematološki parametar za detekciju IDA. Površina ispod krive za sTfR bila je 0,962, za feritin 0,789 i za sTfR/Fer 0,944. Koncentracija sTfR u IDA pacijenata je značajno povišena u odnosu na kontrolu i ACD pacijente, i jasno je odražavla status funkcionalnog gvožđa. Međutim nivo feritina je bio značajno viši u odnosu na optimalnu koncentraciju za dijagnozu IDA pa je određivanje sTfR/Fer indeksa povećalo dijagnostičku efikasnost feritina.

sTfR between control group (1.53 ± 0.30 mg/L) and IDA group (3.66 ± 1.20 mg/L) showed a high statistical difference ($p < 0.001$), however, between control and ACD group (1.72 ± 0.51 mg/L) the difference was not found in means ($P = 0.181$). Receiver operator curve (ROC) analysis demonstrated accuracy of sTfR, ferritin and sTfR/Fer contents compared to the reticulocyte haemoglobin content (CHr), the most sensitive haematological parameter in IDA diagnosis. Areas under the curve were 0.962 for sTfR, 0.789 for ferritin and 0.944 for sTfR/Fer. Concentration of sTfR was significantly higher in patients with IDA compared with controls and ACD patients, and clearly demonstrated the functional status of iron. However, ferritin concentration was far above the optimal concentration in IDA diagnosis, and thus the calculation of sTfR/Fer increased the diagnostic efficiency of ferritin.

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LEČENJE PACIJENATA OBOLELIH OD SIDEROPENIJSKE ANEMIJE

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Osnovni znaci ove anemije su: hipohromija, mikrocitoza i hiposideremija. Eritrociti su manji od normalnih zbog smanjenog prečnika, debljine i zapremine (mikrociti). Količina gvožđa u krvnom serumu, tkivnim depoima i koštanoj srži je smanjena. Deficit gvožđa može nastati zbog gubljenja gvožđa, povećane potrebe za gvožđem, smanjene apsorpcije u organima za varenje ili retko, zbog nedovoljne količine u hrani. Organizam nadoknađuje gvožđe apsorpcijom iz hrane kao i mobilisanjem iz tkivnih depoa. Stvara se više transferina, povećane su vrednosti nezasićenog transferina (UIBC) i ukupnog transferina (TIBC) u plazmi. Smanjena je koncentracija hemoglobina, indeksa bojenja, MCH i MCHC. Lečenje sideropenijske anemije podrazumeva lečenje bolesti koja je anemiju izazvala. Daju se i preparati gvožđa najčešće oralno, retko parenteralno. Efikasnost lečenja se prati retikulocitnom krizom. Gvožđe treba davati dovoljno dugo (obično 2–3 meseca) da se popune rezerve u organizmu. U ovom radu praćeno je lečenje 25 pacijenata obolelih od sideropenijske anemije u Zdravstvenom centru Zaječar. Za obradu podataka korišćeni su: neparametarska analiza varijanse – Kruskal-Wallis-ov test i neparametarski Mann-Whitney U test. Dobijene su sledeće vrednosti ($\bar{x}$ ± Sd) na prijemu bolesnika: hemoglobin 93,1 ± 4,9 g/L, gvožđe 5,6 ± 1,0 μmol/L, UIBC 60,4 ± 3,2 μmol/L, TIBC 66,0 ± 2,9 μmol/L, zasićenje siderofilina 0,08 ± 0,04; posle mesec dana lečenja preparatima

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TREATMENT OF PATIENTS WITH SIDEROPENIC ANAEMIA

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The main sings of this type of anaemia are the following: hypochromia, mycrocytosis and hyposideraemia. The erythrocytes are smaller than normal because of the reduced diameter, thickness and volume (microcytes). The amount of iron in blood serum, tissue storages and bone marrow is decreased. Iron deficiency may occur because of lost iron, increased need for iron, reduced absorption in digestive organs, or rarely, because of insufficient amount of iron in food. The body compensates iron by its absorption from food, as well as by mobilising amounts from the tissue storages. A greater amount of transferrine is produced, the values of unsaturated transferrine (UIBC) and total transferrine (TIBC) in the plasma are increased. The concentration of haemoglobin, dying index, MCH and MCHC are reduced. Treatment of sideropenic anaemia includes treatment of disease which has caused anaemia. Iron preparations, most often used orally and rarely parenterally, are prescribed. The treatment efficiency is observed by reticulocytis crisis. Iron should be given for a long period of time (usually for 2–3 months) in order to supplement iron reserves in the body. This study deals with the treatment of 25 patients with sideropenic anaemia treated in the Health Centre in Zaječar. The non-parametric variance analysis Kruskal-Wallis' test and the non-parametric Mann-Whitney's test were used for data processing. The following results were obtained: (mean ± Sd) at attendance of

gvožđa: hemoglobin 103,2 ± 4,9 g/L, gvožđe 7,9 ± 0,9 μmol/L, UIBC 50,1 ± 3,0 μmol/L, TIBC 58,1 ± 2,7 μmol/L, zasićenje siderofilina 0,13 ± 0,05; posle dva meseca lečenja preparatima gvožđa: hemoglobin 113,1 ± 3,7 g/L, gvožđe 12,8 ± 2,4 μmol/L, UIBC 38,0 ± 3,9 μmol/L, TIBC 50,8 ± 3,1 μmol/L, zasićenje siderofilina 0,25 ± 0,06. Rezultati pokazuju da se vrednosti svih praćenih parametara posle mesec dana lečenja statistički značajno ($p<0,01$) razlikuju od vrednosti na početku lečenja. Takođe, vrednosti kod pacijenata posle 2 meseca lečenja se statistički značajno ($p<0,01$) razlikuju od vrednosti posle mesec dana lečenja. Rezultati pokazuju da se sideropenijska anemija može uspešno lečiti ukoliko se leči osnovna bolest i daju preparati gvožđa.

patients: haemoglobin 93.1 ± 4.9 g/L, iron 5.6 ± 1.0 μmol/L, UIBC 60.4 ± 3.2 μmol/L, TIBC 66.0 ± 2.9 μmol/L, siderofiline saturation 0.08 ± 0.04; after a month of treatment by iron preparations: haemoglobin 103.2 ± 4.9 g/L, iron 7.9 ± 0.9 μmol/L, UIBC 50.1 ± 3.0 μmol/L, TIBC 58.1 ± 2.7 μmol/L, siderofiline saturation 0.13 ± 0.05; after a two-month-period of treatment by iron preparations: haemoglobin 113.1 ± 3.7 g/L, iron 12.8 ± 2.4 μmol/L, UIBC 38.0 ± 3.9 μmol/L, TIBC 50.8 ± 3.1 μmol/L, siderofiline saturation 0.25 ± 0.06. The obtained results show that the values of all monitored parameters after a month of treatment statistically significantly ($P<0.01$) differed from the values obtained at the beginning of the treatment. The values obtained after two months of treatment are also statistically significantly ($P<0.01$) different from the results obtained after a month of treatment. Therefore, the obtained results suggest that sideropenic anaemia can be successfully treated if the basic disease is treated with iron preparations.

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D-DIMER I FIBRINOGEN U TRUDNOĆI

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U trudnoći nastaje stanje hiperkoagulabilnosti, hipofibrinolize i povećan je rizik od razvoja tromboembolijskih poremećaja. Pokazatelji smanjene fibrinolize, povećanog stvaranja fibrina i aktivnosti plazmina su povišeni nivoi fibrinogena i D-dimera. Zajedno sa fibrinogenom i antitrombinom III, D-dimer se najčešće kontroliše u trudnoći, naročito u visoko rizičnim trudnoćama. Visoke vrednosti fibrinogena i D-dimera pokazatelji su povećane aktivnosti trombina i aktivacije procesa koagulacije, što može biti uzrok razvoja pretrombotičkog stanja i preeklampsije. U ovom radu su ispitivane trudnice bez komplikacija (grupa I) i trudnice sa više ponavljajućih pobačaja (grupa II). U svakoj grupi je bilo po 50 trudnica, podeljene u 3 različita perioda i to od 1–20 nedelje gestacije (n. g.) (A), 21–30 n.g. (B) i 31– 40 n.g. (C). Određivane su koncentracije fibrinogena i D-dimera u uzorcima krvi uzimane u jutarnjim satima, iz kubitalne vene, sa natrijum citratom kao antikoagulansom. Fibrinogen je određivan Claussovom metodom, a D-dimer kvantitativnim testom zasnovanim na lateks-aglutinaciji, reagensima firme Dade Behring i na analizatoru BCT (Behring Coagulation

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D-DIMER AND FIBRINOGEN IN PREGNANCY

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Pregnancy is a state associated with hypercoagulability, depressed fibrinolysis and risk of development of thromboembolic disorders. However, signs of depressed fibrinolysis and increased fibrin formation followed by plasmin activity can be measured by increasing levels of fibrinogen and D-dimers. In addition to fibrinogen and antithrombin III, D-dimer is frequently checked during pregnancy, in particular during at risk pregnancy. Higher fibrinogen and D-dimer values are signs of higher thrombin activities and activated coagulation and also of acute danger of the development of prethrombotic state and preeclampsia. This study included pregnant women without complications (group I) and high risk pregnant women with repeated miscarriages (group II), 50 subjects in each group, in 3 different periods (A: 1–20 weeks, B: 21–30 weeks, C: 31–40 weeks). We measured plasma levels of fibrinogen and D-dimer. Blood sample was taken during morning hours, from cubital vein, in plastic test-tubes containing 0.11 mmol/L of sodium-citrate as anticoagulant, in 1:10 ratio. Plasma fibrinogen concentrations were measured by clotting method according to Clauss

Timer, Dade Behring). Za statističku analizu koristili smo neparametarski t-test (Mann-Whitney U test) i neparametarsku analizu varijanse. Srednje vrednosti koncentracija fibrinogena u kontrolnoj grupi, u 3 različita perioda, bile su: 3,37, 3,93 i 4,05 g/L. U grupi II srednje vrednosti koncentracija fibrinogena iznosile su 4,92, 5,10 i 5,31 g/L. Srednje vrednosti koncentracija D-dimera u kontrolnoj grupi, u 3 različita perioda, bile su 220, 231 i 315 μg/L, a u grupi II 231, 264 i 385 μg/L. Statistički značajno više vrednosti fibrinogena i D-dimera su dobijene u drugom i trećem periodu u odnosu na prvi period ($p<0,05$), u obe grupe. Može se zaključiti da su koncentracije fibrinogena i D-dimera kao parametara aktivirane koagulacije povećane kod trudnica sa ponavljajućim pobačajima pa su zato one u opasnosti od razvoja tromboembolijskih poremećaja.

using reagents from Behring on BCT (Behring Coagulation Timer). We measured D-dimer levels using a quantitative assay based on agglutination of latex microparticles, Dade Behring, with the analyzer BCT. Statistical significance of difference between the obtained values was checked by non-parametric analysis of variance and non-parametric Mann-Whitney U test. Mean fibrinogen concentrations in control group, in 3 different periods, were 3.37, 3.93 and 4.05 g/L. In group II mean fibrinogen concentrations were 4.92, 5.10 and 5.31 g/L. Mean D-dimer concentrations in control group, in 3 different periods, were 220, 231, and 315 μg/L. In group II mean D-dimer concentrations were 231, 264 and 385 μg/L. Statistically significant differences were found between fibrinogen and D-dimer in the two groups, in periods B and C vs. period A ($P<0.05$). We may conclude that fibrinogen and D-dimer concentrations as parameters of activated coagulation are higher in the group of pregnant women with repeated miscarriages and therefore they carry a greater danger of developing thromboembolic disorders.

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NEOPHODNOST UNAPREĐENJA INR SISTEMA: PREPORUČENA SREDNJA VREDNOST NORMALNOG PROTROMBINSKOG VREMENA U IZRAČUNAVANJU INR

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Mogućnost korigovanja rezultata protrombinskog vremena (PT) je od velikog značaja u praćenju oralne antikoagulantene terapije (OAT). Ozbiljan problem u rutinskim koagulacionim laboratorijama predstavlja definicija i izbor normalne plazme koja se koristi u imeniocu za izračunavanje PT indeksa. Rezultati se jedino mogu izražavati kao indeks PT plazme pacijenta i normalne plazme, izražene u sekundama, da bi se uzele u obzir velike razlike u vremenu koagulacije normalne plazme sa različitim test-reagensima. Srednje normalno protrombinsko vreme (MNPT) predstavlja geometrijsku sredinu PT najmanje 20 plazmi zdravih osoba i koristi se u standardizaciji rezultata protrombinskog vremena. Protrombinsko vreme je određivano iz 178 uzoraka plazmi pacijenata na OAT, sa tri različita tromboplastin test-reagensa koji sadrže različite tromboplastine: rekombinantni zečji tromboplastin (IL), humani placentarni tromboplastin (Thromborel S, Dade-Behring) i kombinovani goveđi tromboplastin

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NECESSARY IMPROVEMENT OF INR SYSTEM: RECOMMENDED MEAN NORMAL PROTHROMBIN TIME FOR INR CALCULATION

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Commutability of results of PT is of major importance especially in the monitoring of anticoagulant therapy (OAT). A serious problem in routine coagulation laboratory is represented by the definition and choice of the normal plasma required as the denominator in the expression of PT ratios. Results should only be expressed as PT ratio of the clotting time of the patient plasma to that of the normal plasma, to account wide differences in the clotting time of normal plasma with the different test-reagents. Mean normal prothrombin time (MNPT) is the geometric mean of the PT from at least 20 fresh samples from healthy individuals and represents a cause of concern in the standardization of the PT. We determined PT in 178 plasma samples from patient on OAT, with three different thromboplastin test-reagents: recombinant thromboplastin (IL), human placental thromboplastin (Thromborel S) and combined bovine thromboplastin (Thrombotest, Nycomed). MNPT was determined with

(Thrombotest, Nycomed). MNPT je određen iz 30 pojedinačnih, svežih normalnih plazmi, sa svakim test-reagensom posebno. Prvo su bazalni rezultati PT indeksa izračunati sa komercijalnom vrednošću 100% normalne plazme (Tromborel S, Trombotest) ili sa kalibracionom plazmom (IL), a zatim su vrednosti PT indeksa korigovane sa MNPT za svaki test-reagens. Srednje bazalne i korigovane PT vrednosti (INR) su bile: 4,10 ± 1,58 i 3,67 ± 1,42 za rekombinantni zečji tromboplastin, 3,60 ± 1,53 i 3,88 ± 1,65 za kombinovani goveđi tromboplastin, 3,14 ± 1,15 i 2,77 ± 1,01 za humani placentarni tromboplastin. Student t-testom nađene su statistički značajne razlike između bazalnih i korigovanih PT vrednosti za sva tri test-tromboplastina: rekombinantni zečji tromboplastin (t=34,54, p= 0,000), kombinovani goveđi tromboplastin (t=31,43, p=0,000) i humani placentarni tromboplastin (t=36,55, p=0,000). Adekvatna interpretacija PT sa različitim test-tromboplastinima zahteva da svaki reagens bude korektno kalibrisan. U preporukama Svetske zdravstvene organizacije preporučuje se da svaka laboratorija odredi MNPT koristeći sopstveni PT sistem.

30 fresh individual normal plasmas using the same test-reagents. First, basal results were expressed as PT ratio of the clotting time of the patient plasma denominated with commercial value of the 100% normal plasma (Thromborel S, Thromboplastin) or calibration plasma (IL), and than, with the correction with MNPT for each test-reagens. Mean basal and corrected PT (INR) was: 4.10 ± 1.58 and 3.67 ± 1.42 with recombinant rabbit thromboplastin, 3.60 ± 1.53 and 3.88 ± 1.65 with combined thromboplastin, and 3.14 ± 1.15 and 2.77 ± 1.01 with human placental thromboplastin. With Student t-test spastically significant differences were observed between basal and corrected results with all three thromboplastin test-reagents: recombinant rabbit thromboplastin (t=34.54, P= 0.000), combined thromboplastin (t=31.43, p=0.000) and human placental thromboplastin (t=36.55, P=0.000). Various types of thromboplastin are prepared commercially and, in order to able to interpret the results of the PT it is essential that each reagent is correctly calibrated. It is recomanded by WHO guidelines that each laboratory should determine MNPT using its own prothrombin-time system.

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UTICAJ HIPERESTROGENIZMA NA MEHANIZAM FIBRINOLIZE TOKOM *IN VITRO* FERTILIZACIJE

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Hemostazni sistem, kao integralni deo biološkog homeostatskog mehanizma čoveka podložan je promenama usled dejstva hormonske terapije korišćene u pripremi za *in vitro* fertilizaciju. Cilj ovog rada je bio ispitivanje uticaja hormonskih promena na fibrinolitičke parametre kod 25 žena koje su bile na pripremi za *in vitro* fertilizaciju. Pacijentkinje su tretirane sa HMG-hCG hormonima u kombinaciji sa GnRH agonistima. Efekti ove terapije na fibrinolitički sistem su ispitivani u četiri vremenska perioda: pre hormonske terapije (I), posle supresije endogene hormonske aktivnosti (II), u periodu maksimalne koncentracije endogenog estradiola (III) i nakon ovulacije izazvane sa humanim horionskim gonadotropinom (IV). Ispitivan je uticaj nivoa estrogena na plazminogen (PLG), α_2-antiplazmin (α_2-APL), plazminogen aktivator inhibitor (PAI-1) i plazmin-2-antiplazmin kompleks (PAP). PLG, α_2-APL i PAI-1 su određivani spektrofotometrijskim metodama, sa Dade Behring testovima, koristeći BCT analizator. Koncen-

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INFLUENCE OF HYPEROESTROGENISM DURING *IN VITRO* FERTILIZATION ON FIBRINOLYTIC MECHANISM

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Haemostatic system, as an integral part of biological homeostatic mechanism in humans, is subject to changes due to the effect of hormonal therapy used in preparation for *in vitro* fertilization. The aim of the study was to analyze the impact of hormonal changes on fibrinolytic parameters in 25 women undergoing *in vitro* fertilization. They were treated with HMG-hCG hormones in combination with a GnRH agonist. Effects on blood fibrinolytic activity were determined four times: before hormonal stimulation (I); after downregulation (II); at maximal E_2 level (III); and on day after ovaluation induced with human chorionic gonadotrophin (IV). We evaluated the effect of estrogen levels on plasminogen (PLG), α_2-antiplasmin (α_2-APL), plasminogen activator inhibitor (PAI-1) and plasmin-α_2-antiplasmin complex (PAP). PLG, α_2-APL and PAI-1 were measured with spectrophotometric method, with Dade Behring tests, using the Behring Coagulation Timer. PAP was determined by sandwich enzyme immunoassay method

tracija PAP kompleksa je dobijena enzim imunoodređivanjem sa Dade Behring testovima na ELISA Behring Procesoru. Analiza varijanse (ANOVA) je pokazala postojanje statistički značajnih promena za sve navedene parametre. Dobijene su sledeće srednje vrednosti za četiri vremenska perioda, za PLG: 100, 99, 97 i 92%; za α_2-APL: 97, 96, 92 i 90%; za PAI-1: 3,1, 3,4, 2,8 i 2,6 U/mL, a za PAP: 267, 304, 369 i 455 μg/L. Kako se serumski estradiol manjao kroz četiri vremenska perioda pripreme, dobijeno je značajno sniženje aktivnosti za PLG, α_2-APL i PAI-1 u periodima III i IV ($p<0,05$). Dobijeni rezultati ukazuju na značajno povećanje PAP koncentracije u III i IV periodu u odnosu na predhodne ($p<0,05$). Značajan porast u koncentraciji PAP kompleksa kao glavnog markera aktivirane fibrinolize uz redukovanu aktivnost inhibitora fibrinolize (α_2-APL i PAI-1) u periodima maksimalne koncentracije endogenog estradiola ukazuje da kontrolisana ovarijalna stimulacija može aktivirati fibrinolitički sistem, što je od velikog značaja za održavanje ravnoteže prokoagulantnim procesima i prevenciji tromboze.

with Dade Behring tests, using the ELISA Behring Processor II. Analysis of variance (ANOVA) revealed statistically significant difference in all parameters. We obtained the following four time average values for PLG: 100, 99, 97 and 92%; for α_2-APL: 97, 96, 92 and 90%; for PAI-1: 3.1, 3.4, 2.8 and 2.6 U/mL and for PAP: 267 μg/L, 304 μg/L, 369 μg/L, 455 μg/L, respectively. As the serum oestradiol level was changed in four periods, a significant decrease was observed in PLG, α_2-APL and PAI-1 concentrations in the III and IV periods ($P<0.05$). The obtained results revealed significantly higher PAP concentrations in periods III and IV in comparison with earlier periods ($P< 0.05$). A significant rise in PAP complex as a major marker of activated fibrinolytic system, with reduced activity of fibrinolysis inhibitors (α_2-APL and PAI-1) in periods with maximal level of endogenous oestradiol demonstrates that ovarian stimulation can produce the activation of fibrinolytic system. It is highly significant for opposing the procoagulant process and prevention the thrombosis.

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ODREĐIVANJE KONCENTRACIJE KOMPLEKSA PLAZMIN/α_2-ANTIPLAZMIN U PLAZMI PACIJENATA SA POVREDOM LOBANJE I MOZGA

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Razgradnja polimera fibrina proteolitičkom degradacijom važan je mehanizam održavanja hemostazne ravnoteže. Ključni enzim fibrinolitičkog sistema je plazmin. On ima osnovnu funkciju u proteolizi, ali ima i centralnu ulogu u aktivaciji degenerativnih i zapaljenskih procesa u tkivima. Deficit ili potpuno odsustvo komponenti fibrinolitičkog sistema vodi tromboembolijskim poremećajima, dok hiperfibrinolitička stanja povećavaju rizik od hemoragija ubrzavanjem procesa degradacije fibrinogena. Plazmin sa svojim inhibitorom 2-antiplazminom formira kompleks plazmin/α_2-antiplazmin (PAP). Koncentracija ovog kompleksa je mera trenutne aktivnosti fibrinolitičkog sistema. Cilj ovog rada bio je da se odrede vrednosti PAP kod pacijenata sa povredom lobanje i mozga po prijemu u Urgentni centar. Koncentracija PAP kompleksa određivana je komercijalnim testom Enzygnost® PAP micro (Behring). Princip određivanja zasniva se na sendvič imuno određivanju. On je namenjen za *in vitro* određivanje PAP u

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DETERMINATION OF PLASMA PLASMIN/α_2-ANTIPLASMIN COMPLEXIN PATIENTS WITH BRAIN INJURY

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The removal of polymerized fibrin from the vascular system by proteolytic degradation is important for maintaining the haemostatic balance. The key enzyme of the fibrinolytic system is plasmin. It has a fibrinolytic function, a central role in the activation of degenerative and inflammatory processes in the tissues. Deficiencies or defects in a component of the fibrinolytic system can lead to thromboembolic disease, until hyperfibrinolytic states increase the risk of haemorrhage through accelerated degradation of fibrinogen. With its inhibitor α_2-antiplasmin plasmin forms a plasmin/α_2-antiplasmin complex (PAP). The plasma PAP concentrations are thus a measure of the current activity of the fibrinolytic system. The aim of this study was to examine PAP values in patients with brain injury after admission to the Emergency Centre. PAP complex was determined by commercial test Enzygnost® PAP micro (Behring), which is based on a sandwich enzyme immunoassay. It is intended for *in vitro* determination

humanoj plazmi i koristi se za dijagnozu hipo- ili hiperfibrinolitičkih stanja. PAP je određivan u citratnoj plazmi 20 pacijenata sa povredama lobanje i mozga, kao i kod 20 zdravih osoba. Na osnovu dobijenih apsorbancija za odgovarajuće koncentracije standarda (50, 200, 2000, 5000 μg/L), dobijena je odgovarajuća kriva na log-log papiru, sa koje su očitane vrednosti koncentracija PAP kompleksa na osnovu izmerenih vrednosti apsorbancija. Pacijenti su imali statistički značajno veće vrednosti PAP u odnosu na zdrave kontrole ($p< 0,01$). Oko 80% ispitivanih pacijenata imalo je PAP vrednosti veće od 700 μg/L, dok su sve kontrole imale vrednosti PAP između 160 i 310 μg/L.

of PAP in human plasma, and for use in the diagnosis of hypo- and hyperfibrinolytic states. PAP was analysed in citrate plasma in twenty patients with brain injury and in twenty healthy persons. After calculating the mean absorbance values of the standards (50, 200, 2000, 5000 μg/L), a reference curve on log-log paper was plotted. Patients had significantly higher values of PAP than healthy controls ($P<0.01$). Almost 80% of examined patients had PAP values higher than 700 μg/L, until all controls had PAP between 160 and 310 μg/L.