PLENARNE SEKCIJE

PLENARY SESSIONS

Sekcija 1 Session 1

BIOLOŠKI I KLINIČKI ZNAČAJ AZOT-MONOKSIDA I INTERMEDIJATA METABOLIZMA L-ARGININA

NITRIC OXIDE AND L-ARGININE METABOLISM INTERMEDIATES- BIOLOGICAL AND CLINICAL SIGNIFICANCE

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Plenary sessions
Plenarne sekcije

PATOFIZIOLO[KI ZNA^AJ AZOT-MONOKSIDA U KORONARNOJ BOLESTI SRCA

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Azot-monoksid (NO) se sintetiše u mnogim ćelijama organizma, ali je njegova produkcija u vaskularnom endotelu naročito važna u regulaciji protoka krvi. Vaskularni efekti uključuju: dir ektnu vazodilataciju, indirektnu vazodilataciju inhibicijom vazokonstriktora, antitrombotični efekat, antiinflamator ni i antipr oliferativni efekat. Zbog njegove važnosti u vaskular noj funkciji, abnormalna produkcija NO, koja se javlja u različitim bolestima, može imati nepovoljan efekat na protok krvi i druge vaskularne funkcije. Smatra se da je poremećaj sinteze NO kritičan uzr ok oštećenja u ishemičnom srcu. Biološka veza između endotelnog oštećenja i aterosklerotske bolesti koronarnih arterija pre svega se odnosila na smanjenje raspoloživog NO kroz predisponiranje adhezije leukocita i tr ombocita, vazokonstrikcije i pr oliferacije glatkomišićnih ćelija. Međutim, precizni mehanizam por emećaja sinteze NO još nije jasan i postoje kontr overzne tvrdnje o tome da li miokardna ishemija dovodi do povećanog ili smanjenog stvaranja NO . Asimetrični dimetil - arginin (ADMA) prir odni je kompetitivni inhibitor i jedan od primarnih faktora koji kontrolišu produkciju NO. Koncentracija asimetričnog dimetilar ginina je povećana i usko kor elira sa por emećenom vazodilatacijom u uslovima kada postoji endotelna disfunkcija kao što su hiperholesterolemija, hipertenzija, rezistencija na insulin i dijabet Tip 2 i r enalna insuficijencija. Takođe, ADMA je uključen u miokar dnu ishemiju i na osnovu njegove vr ednosti u plazmi mogu se pr edvideti budući kor onarni događaji kod pacijenata sa povećanim kar diovaskularnim rizikom. Nedavno je saopšteno da je povećana koncentracija ADMA u akutnim koronarnim događajima nezavisan kardiovaskularni faktor rizika.

Ključne reči: azot monoksid, asimetrični dimetil-arginin, koronarna bolest srca

THE PATHOPHYSIOLOGICAL IMPORTANCE OF NITRIC OXIDE IN CORONARY HEART DISEASE

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Nitric oxide (NO) is pr oduced by many cells in the body; however , its pr oduction by the vascular endothelium is particularly important in the r egulation of blood flow. Vascular actions of NO include the following: direct vasodilation, indirect vasodilation by inhibiting vasoconstrictor influences, anti-thr ombotic effects, anti-inflammatory effects, anti-pr oliferative effects. Because of its importance in vascular function, abnormal production of NO, as occurs in different disease states, can adversely affect blood flow and other vascular functions. It has been suggested that alterations in NO generation ar e a critical cause of injury in the ischemic heart. A biologic link between endothelial damage and ather osclerotic coronary arterial disease has been pr esumably related to decreased arterial bioavailability of NO through predisposing to leucocyte and platelet adhesion, vasoconstriction and smooth muscle cell pr oliferation. However, the precise alterations in NO generation which occur are not known, and there is considerable controversy regarding whether myocar dial ischemia results in incr eased or decr eased NO formation. Asymmetric dimethylar ginine (ADMA) is a natural, competitive inhibitor, and one of the primary factors controlling nitric oxide pr oduction. ADMA was found to be elevated and closely cor related with the impaired vasodilator function in conditions associated with endothelial dysfunction, such as hyper - cholesterolemia, hypertension, insulin resistance and type 2 diabetes, and r enal insufficiency. But ADMA also seems to be involved in myocar dial ischemia, since its plasma levels predict future coronary events in patients with elevated cardiovascular risk. Recently, elevations of plasma ADMA concentrations in acute coronary events wer e observed independent of cardiovascular risk factors.

Keywords: nitric oxide, asymmetric dimethyl - arginine, coronary heart disease

SIGNALIZACIJA POSREDOVANA AZOT-MONOKSIDOM I NITROZATIVNIM STRESOM U NEUROPATOLOGIJI

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Azot monoksid je važan signalni molekul u brojnim fiziološkim procesima. Ovaj gas stvara se iz L-arginina dejstvom tri izoforme azot monoksid sintaze i medijator je važnih fizioloških funkcija, kao što su posredovanje u regulaciji tonusa krvnih sudova i komunikaciji ćelija nervnog sistema. Nasuprot ovim ulogama, kao slobodni radikal, NO može izazvati ćelijsko oštećenje indukcijom nitrozativnog stresa, što ima značajne implikacije u bolestima nervnog sistema. Mada je mehanizam neurodegeneracije posredovane azot-monoksidom još nerazjašnjen, brojne studije ukazuju na njegovu ključnu ulogu u modifikaciji funkcije proteina kroz procese S-nitrozilacije i nitrovanja tirozina. On doprinosi glutamatnoj ekscitotoksičnosti, učestvuje u fragmentaciji organela, inhibiše mitohondrijalne respiratorne komplekse i mobiliše cink iz unutrašnjih depoa. Nedavno je ukazano da može biti medijator epigenetske genske ekspresije i promena hromatina. Pored toga, NO je ključni posrednik u regulaciji inflamatornog i imunog odgovora CNS. On učestvuje u nishodnoj regulaciji nekoliko aspekata inflamacije CNS, ali takođe ispoljava dualističke efekte u uslovima inflamacije.

Ključne reči: azot monoksid, nitrozativni stres, neurodegeneracija, neuroinflamacija

NITRIC OXIDE-MEDIATED SIGNALIZATION AND NITROSATIVE STRESS IN NEUROPATHOLOGY

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Nitric oxide (NO) is an important signaling molecule in a variety of physiological processes. NO, a gas, is produced from L-arginine by different isoforms of nitric oxide synthase and serves as a mediator in important physiological functions, such as promoting vasodilation of blood vessels and mediating communication between nervous system cells. Contradictory to its physiologic actions, free radical activity of NO can cause cellular damage by the induction of nitrosative stress with significant implications on nervous system diseases. Although the mechanism of NO-mediated neurodegeneration still remains unclear, numerous studies suggest its crucial role in the modification of protein functions by nitrosylation and nitrotyrosination. NO contributes to glutamate excitotoxicity, participates in organelle fragmentation, inhibits mitochondrial respiratory complexes and mobilizes zinc from internal stores. Recently, NO has emerged as a mediator of epigenetic gene expression and chromatin changes. Besides, NO is a key mediator in the regulation of inflammatory and immune response of the central nervous system. It is involved in the downregulation of several aspects of CNS inflammation, but also has a dual role in that it is required for inflammation in some situations.

Keywords: nitric oxide, nitrosative stress, neurodegeneration, neuroinflammation

DIMETILARGININI – BIOMARKERI U PRAĆENJU BUBRE@NIHBOLESTI

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Hronična inflamacija je jedan od najvažnijih uzroka morbiditeta i mortaliteta kod pacijenata sa hroničnom bubrežnom insuficijencijom (HBI). Sma - njenje koncentracije NO i/ili nedovoljna raspoloživost ovog molekula kod pacijenata sa HBI može biti razlog povećanja krvnog pritiska, kar diovaskularnih bolesti (KVS) i progresije bubrežnog oštećenja. Metilarginini koji uključuju NG -monometil-L-arginin (LNMMA), NG,NG-dimetil-L-arginin (asimetrični dimetilar ginin; ADMA) i NG,N'G-dimetil-L-arginin (simetrični dimetilarginin; SDMA) nastaju u pr ocesu proteolize post-translaciono metilisanih ar gininskih rezidua u pr oteinima. Asimetrični dimetilar ginin (ADMA) jeste kompetitivni inhibitor azot oksid sintaze (NOS). Naj - važnija uloga bubr ega u eliminaciji ADMA podrazu - meva procese urinarne ekskrecije (samo 10%) i raz-gradnju ovog molekula pod uticajem dimetilar ginin dimetilaminohidrolaze (DDAH). Na aktivnost DD AH utiču oksidativni str es i inflamacija. Studije poka - zuju da visoke koncentracije ADMA (između 2 i 10 μmol/L) značajno inhibiraju vaskular nu produkciju NO, retenciju natrijuma i povećavaju kr vni pritisak. Neuhranjenost i ubr zani metabolizam pr oteina kod pacijenata na dijalizi udr uženi su sa povećanim oksidativnim stresom koji dir ektno inhibira DD AH i smanjuje razlaganje ADMA. ADMA nije samo ur e-mijski toksin već i značajan marker endotelne dis-funkcije i ater oskleroze kao i nezavisan pr ediktor mortaliteta i kardiovaskularnih bolesti kod pacijenata sa HBI. Osnovni uzr oci koji dovode do akumulacije ADMA su povećana metilacija pr oteina, njihov povećan metabolizam, smanjena aktivnost DD AH i smanjena urinarna ekskrecija. Klirens SDMA u plaz-mi zavisi samo od r enalne funkcije (pozitivna ko re-lacija sa kreatininom) i njena akumulacija predstavlja nespecifični indikator ur emijskih toksina. R edukova-na količina NO praćena akumulacijom ADMA, udru-žena sa inflamacijom može biti važan patogeni faktor

DIMETHYLARGININES – BIOMARKERS FOR MONITORING THE PROGRESION OF KIDNEY DISEASE

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Chronic inflammation is one of most the impor-tant reasons for morbidity and mortality in patients with chronic kidney disease (CKD). Decr eased nitric oxide (NO) production and/or impaired NO bioavail-ability may occur in patients with CKD, and could contribute to the elevation of blood pr essure, cardio-vascular disease (CVD) and the pr ogression of renal injury in these patients. F ree guanidino-methylated (NG) arginine residues occur endogenously as a result of proteolysis of post-translationally methylated tissue proteins. The ar ginine analogues identified to date include NG -monomethyl-L-arginine (LNMMA), NG,NG-dimethyl-L-arginine (asymmetric dimethyl - arginine; ADMA) and NG,N'G -dimethyl-L-arginine (symmetric dimethylarginine; SDMA). The asymmet-rically methylated ar ginine residues (L-NMMA and ADMA), but not the symmetrically methylated ar gi-nine (SDMA), ar e competitive inhibitors of the nitric oxide synthase (NOS) enzymes. The kidney has a predominant role in ADMA elimination by combining 2 mechanisms: urinary excretion and metabolization of ADMA. The degradation of ADMA is accompli - shed intracellularly by the enzyme dimethylar ginine dimethylaminohydrolase (DDAH). The activity of DDAH may be dysr egulated in several pathophysio-logical situations including oxidative str ess and inflammation. Data from several experimental studies suggest that ADMA concentrations in a pathophysio-logically high range (i.e., between 2 and 10 μmol/L) significantly inhibit vascular NO pr oduction. ADMA decreases effective r enal plasma flow and incr eases renovascular resistance in a dose-r elated manner. Moreover, administration of ADMA causes significant sodium retention and blood pr essure increase. Malnutrition and enhanced pr otein turnover are common in CKD and these alterations are associated with oxidative str ess, a factor which inhibits DD AH, and hence ADMA degradation inkpatients with kid-

endotelne disfunkcije kod bubrežnih pacijenata. Dobijeni rezultati pokazuju da porast koncentracije ADMA može biti veza između KVS i HBI.

Ključne reči: asimetrični dimetilarginin, inflamacija, bubrežna insuficijencija

ney disease. ADMA is not only a uremic toxin, but also a strong marker of endothelial dysfunction and atherosclerosis and a stronger independent predictor of all-cause mortality and cardiovascular outcome in patients with chronic renal failure than some traditional risk factors. There are at least four possible mechanisms that may explain the accumulation of ADMA in CKD: increased methylation of proteins; increased protein turnover; decreased metabolism by DDAH; and impaired renal excretion. A strong positive correlation between SDMA and creatinine observed in our study suggests that SDMA might be of value as a marker of renal function. Reduced NO elaboration secondary to accumulation of ADMA and elevated inflammation may be important pathogenic factors for endothelial dysfunction in patients with renal disease. These findings suggest that elevation of ADMA may be a missing link between CVD and CKD.

Keywords: asymmetric dimethylarginine, inflammation, chronic kidney disease

Sekcija 2 Session 2

SIMPOZIJUM IZ KLINIČKE IMUNOLOGIJE | SYMPOSIUM IN CLINICAL IMMUNOLOGY

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VASKULITISI INDUKOVANI ANTITIROIDNIM LEKOVIMA: DIFERENCIJALNO DIJAGNOSTI^KIZNA^AJ IMUNOSEROLO[KIH PARAMETARA U ODNOSU NA IDIOPATSKE VASKULITISE

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Klinička slika idiopatskih sistemskih vaskulitisa (ISV) i lekom indukovanih vaskulitisa (LIV) slična je, tako da se LIV kasno dijagnostikuju. Supr otno ISV (Wegenerova granulomatoza, mikroskopski poliangiitis, Churg-Strauss sindrom), LIV se najčešće ne leče citostaticima, ali zahtevaju hitno obustavljanje suspektnog leka. Antitiroidni lekovi (ATL) – propiltiouracil i metimazol – mogu indukovati vaskulitise udr užene sa antineutr ofilnim citoplazmatskim antitelima (ANCA). U retrospektivnoj studiji (srednje vreme praćenja 6,5 god) poredili smo kliničke i serološke markere ISV udr uženih sa ANC A (n=56) sa A TL-indukovanim oboljenjima (n=20). ANC A, antinukleusna (ANA) i anti-dsDNK antitela odr eđena su indirektnom imunofluorescencijom. Koncentracije antikardiolipinskih (aCL), anti-beta-2 glikopr otein I, antihistonskih antitela i ANCA specifičnih za proteinazu 3, mijeloperoksidazu (MPO), elastazu (EL), catepsin G, lizo zim, laktoferin i protein koji povećava permeabilnost odr eđene su ELISA testovima (A eskulisa, Nemačka). C3, C4 komponente komplementa i krioglobulini me reni su standardnim metodama. Četiri od 20 pacijenata lečenih ATL imalo je sistemski vaskulitis, 4/20 izolovani vaskulitis kože, dok je 12/20 imalo bolest sličnu lupusu. Zahvatanje kože bilo je češće kod A TL-indukovanih oboljenja, dok su lezije bubr ega bile češće kod ISV (p<0,01). Pacijenti lečeni ATL imali su češće polispecifična ANCA (najčešće MPO-ANCA, EL-ANCA), ANA, antihistonska, aCL, anti-beta-2 gli kopro-

VASCULITIS INDUCED BY ANTITHYROID DRUGS: THE IMPORTANCE OF IMMUNOSEROLOGICAL PARAMETERS IN DIFFERENTIAL DIAGNOSIS INBRELATION TO IDIOPATHIC SYSTEMIC VASCULITIS

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The clinical pictur e of idiopathic systemic vasculitis (ISV) and drug-induced vasculitis (DIV) may be similar, and the diagnosis of DIV is of ten delayed. Contrary to ISV (W egener's granulomatosis, micr oscopic polyangiitis, Chur g-Strauss syndrome), DIV are often not treated with immunosuppressants, but demand immediate discontinuation of the suspected drug. Antithyroid drugs (ATL), propylthiouracil and methimazole, can induce vasculitis associated with antineutrophil cytoplasmic antibodies (ANC A). In a retrospective study (mean follow -up time 6.5 years) we compared the clinical and ser ological markers of ANCA-associated ISV (n=56) and A TL-induced diseases (n=20). ANC A, antinuclear (ANA) and anti-dsDNA antibodies wer e determined by indir ect immunofluorescence. Concentrations of anticar diolipin (aCL), anti-beta 2 glycopr otein I, antihistoneantibodies, and ANC A specific for pr oteinase 3, myeloperoxidase (MPO), elastase (EL), cathepsin G, lysozyme, lactoferrin and bactericidal per meability protein were determined by ELISA (A eskulisa, Germany). C3, C4 complement components and cryoglobulins were measured by standar d methods. 4/20 patients treated with ATL had systemic vasculitis, 4/20 had isolated cutaneous vasculitis, while 12/20 had lupus-like disease. Skin involvement was more frequent in ATL-induced diseases, while r enal lesions were more common in ISV (p<0.01). P atients treated with ATL more frequently had polyspecific ANCA (MPO-ANCA with EL-ANCA), ANA, anti-

tein I antitela, krioglobuline i nizak C4 (p<0,01). Trinaest od 56 pacijenata sa ISV je umrlo, 8/56 je razvilo terminalnu bubrežnu insuficijenciju (TBI). U gr upi ATL-indukovanih oboljenja nije bilo smrtnog ishoda, a samo jedna pacijentkinja je razvila TBI. Supr otno ISV, kod A TL-indukovanih oboljenja, ANC A su udružena sa dr ugim autoantitelima i potr ošnjom komplementa. Različit serološki profil pomaže u diferencijalnoj dijagnozi ANCA-pozitivnih pacijenata i ukazuje na različite patofiziološke mehanizme ISV i ATL-indukovanih oboljenja.

Ključne reči: antineutrofilna citoplazmatska antitela, lekom indukovani vaskulitis, antitir oidni lekovi

histone, aCL, anti-beta-2 glycopr otein I antibodies, cryoglobulins and low C4 (p<0.01). Three out of 56 patients with ISV died, 8/56 developed ter minal renal failure (TRF). In the gr oup of ATL-induced disease there was no lethality , and only one patient developed TRF. Contrary to the ISV , in the A TL-induced diseases, ANC A are associated with other autoantibodies and complement consumption. Different serological profile helps in the differ ential diagnosis of ANC A-positive patients and indicates different pathophysiological mechanisms of ISV and ATL-induced diseases.

Keywords: antineutrophil cytoplasmic antibo - dies, drug-induced vasculitis, antithyroid drugs

KRIOGLOBULINEMIJA – KLINIČKE I LABORATORIJSKE KARAKTERISTIKE

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Krioglobulini su abnormalni imunoglobulini koji reverzibilno precipitiraju kada se ser um inkubira na temperaturi nižoj od 37 °C i ponovo rastvaraju pri zagrevanju seruma. Krioglobulinemija podrazumeva prisustvo jednog (monoklonska krioglobulinemija) ili više imunoglobulina (mešovita krioglobulinemija). Mešovita krioglobulinemija (MC), II i III tipa, odnosi se na prisustvo cirkulišućih krioprecitabilnih imunskih kompleksa u ser umu i klinički se manifestuje klasič - nim trijasom: purpura, malaksalost, artralgije. Može biti praćena različitim patološkim stanjima, kao što su hronični hepatitis, membranoproliferativni glomerulonefritis, periferna neuropatija, kožne ulceracije, difuzni vaskulitis. Zbog svojih kliničkih i histoloških karakteristika, MC se klasifikuje u podgrupu vaskulitisa malih krvnih sudova. Upadljiva je povezanost me - šovite krioglobulinemije i HCV infekcije. Dijagnoza MC postavlja se na osnovu kliničkog i laboratorijskog nalaza. Cirkulišući mešoviti krioglobulini, nizak C4 i purpura su ključni kliničko -laboratorijski pokazatelji i glavni dijagnostički kriterijumi. Leukocitoklastični vaskulitis je histološko obeležje i može obuhvatiti male kao i kr vne sudove sr ednjeg kalibra. Autoimunske i limfoproliferativne bolesti i MC mogu ispoljiti mnogo preklapajućih kliničkih i ser oloških karakteristika, te ova mimikrija može stvarati poteškoće u difer encijalnoj dijagnostici. Neke laboratorijske analize inter feriraju sa prisustvom krioglobulina, pa odr eđivanje RF, komplementa, imunoglobulina, EF proteina i imuno-

CRYOGLOBULINEMIA – CLINICAL AND LABORATORY CHARACTERISTICS

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Cryoglobulin are abnormal immunoglobulins that reversibly precipitate when serum is incubated at temperatures below 37 °C, and then dissolve when serum is heated. Cr yoglobulinemia implies the pr esence of one (monoclonal cr yoglobulinemia) or more immunoglobulins (mixed cr yoglobulinemia). Mixed cryoglobulinemia (MC), type II and type III, r efers to the presence of cir culating cryoprecitable immune complexes in the ser um and manifests clinically by a classical triad of purpura, weakness and arthralgias. It may be accompanied by a variety of pathological conditions such as chr onic hepatitis, membranopr oliferative glomerulonephritis, peripheral neur opathy, skin ulcers, diffuse vasculitis. Because of its clinical and histological features, MC is classified in the subgroup of small vessel vasculitis. MC may be associ - ated with the pr esence of certain diseases such as lymphoproliferative, autoimmune and infectious diseases or may occur in idiopathic for m designated as essential cryoglobulinemia. The striking correlation is noticed between mixed cr yoglobulinemia and HCV infection. The diagnosis of MC is set on the basis of clinical and laborator y findings. Cir culating mixed cryoglobulins, low C4 and skin purpura ar e the hallmarks of the disease and major diagnostic criteria. Leucocytoclastic vasculitis is a histopathological hallmark and involves small and medium-sized blood vessels. Autoimmune and lymphopr oliferative disease and MC may show many overlapping clinical

fiksacije u serumu, kao i krvne slike, može biti neadekvatno ukoliko se ne uzme u obzir prisustvo krioglobulina. Prikazujemo naše rezultate u pogledu nekih značajnih kliničkih i laboratorijskih obeležja krioglobulinemije.

Ključne reči: krioglobulinemija, mešovita krioglobulinemija, krioglobulinemijski vaskulitis

and serological characteristics, and this mimicry can create differential diagnostic difficulties. Some laboratory tests interfere with the presence of cryoglobulins, and determination of RF, complement, immunoglobulins, EF protein and immunofixation in serum as well as blood cell count, may be inadequate if it fails to take into account the presence of cryoglobulins. We present our results in terms of some important clinical and laboratory characteristics of cryoglobulinemia.

Keywords: cryoglobulinemia, mixed cryoglobulinemia, cryoglobulinemic vasculitis

KRIOGLOBULINEMIJSKI VASKULITIS – PRIKAZ SLU^AJA

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Krioglobulinemijski vaskulitis je sistemski vaskulitis koji se karakteriše serološkim markerima (krioglobulini, nizak C4) i kliničkim pr omenama (kožne promene i multiple organske lezije). U osnovi se radi o imunskim kompleksima posr edovanom vaskulitisu malih i srednjih krvnih sudova. Kod više od 90% pacijenata, mešovita krioglobulinemija je povezana sa hepatitis C virusnom (HCV) infekcijom, pa se smatra da ovaj vir us ima ključnu ulogu u etiopatogenezi. Hepatitis C vir us pored hepatotropnih ima i limfotropne karakteristike, čime se može objasniti mono i oligoklonska ekspanzija B limfocita. Karakteristika kliničke slike je prisustvo purpur e, artralgija i malaksalosti (Meltzerov trijas), ali i dr ugih patoloških stanja, uključujući hronični hepatitis, membranoproliferativni glomerulonefritis, perifernu neuropatiju, ulkuse kože, difuzni vaskulitis i, ređe, hematoloških i hepatoloških maligniteta. Terapija se sastoji u primeni anti virusnih i imunosupresivnih lekova i terapijskih izmena plazme. Prikazaćemo slučaj pacijentkinje sa hr oničnom HCV infekcijom i teškom formom krioglobulinemijskog vaskulitisa.

Ključne reči: krioglobulinemijski vaskulitis, he patitis C virusna infekcija

CRYOGLOBULINEMIC VASCULITIS – A CASE REPORT

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Cryoglobulinemic vasculitis is a systemic vasculitis characterized by serological (cryoglobulins, low C4) and clinical featur es (skin changes and multiple organ involvement). It is an immune complex-mediated vasculitis of the small to medium-sized vessels. In more than 90% of patients mixed cr yoglobulinemia is associated with chronic hepatitis C virus infection, which is consider ed the triggering factor in the etiopathogenesis. Hepatitis C vir us besides hepatotropic also has lymphotr opic characteristics, which may explain the mono and oligoclonal B-lymphocyte expansion. Cryoglobulinaemic vasculitis is characterized clinically by purpura, weakness, arthralgias (Meltzer's triad) and by a series of pathological conditions, including chronic hepatitis, membranoproliferative glomerulonephritis, peripheral neur opathy, skin ulcers, diffuse vasculitis, and, less fr equently, by lymphatic and he patic malignancies. The tr eatment is based on antiviral and immunosuppr essive drugs, and therapeutic plasma exchange. We will present a case of a patient with chr onic HCV infection and severe form of cryoglobulinemic vasculitis.

Keywords: cryoglobulinemic vasculitis, hepatitis C virus infection

HEREDITARNI ANGIOEDEM: KLINI^KA I LABORATORIJSKA DIJAGNOSTIKA

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Hereditarni angioedem (HAE) je r etko autozomno dominantno oboljenje koje se odlikuje sni - ženom funkcijom C1 inhibitora (C1 INH) nastalom usled poremećene transkripcije ili stvaranja nefunk - cionalnog proteina. Ustanovljena pr evalenca ovog oboljenja je 1/50 000. P acijenti sa HAE- om imaju ponavljane epizode subkutanog i submukoznog oto - ka bilo kog dela kože, r espiratornog ili gastr ointestinalnog trakta. Napadi koji zahvataju or ofarinks ili abdomen mogu dovesti do značajnog rizika od traj - nog oštećenja i smrti. Koncentracija C4 komponente komplementa u serumu je dobar skrining test za deficijenciju C1 INH, pošto je uvek snižena kod neleče - nih pacijenata sa HAE- om. Ukoliko je koncentracija C4 niska, trebalo bi odrediti koncentraciju C1 INH i njegovu funkcionalnu aktivnost. Dijagnoza I tipa HAE-a (85% slučajeva) postavlja se dokazivanjem niske koncentracije C1 INH pr oteina imunohemijskim metodama. Ukoliko je koncentracija C1 INH normalna (uz nizak C4) ispitivanje bi tr ebalo usmeriti na utvrđivanje funkcije C1 INH. Odsustvo ili značajno sniženje funkcije C1 INH upućuje na postojanje II tipa HAE. Svi testovi bi tr ebalo da budu obavljeni sa svežim (ili sveže zamr znutim) uzorcima seruma i plazme. Sva testiranja se obavljaju kad pacijenti nisu na specifičnoj terapiji. Interpr etacija rezultata kod male dece je otežana zbog malog br oja podataka o referentnim vrednostima kod dece. Ispitivanja bi va - ljalo ponoviti kad dete navrši prvu godinu. Ukoliko se utvrdi niska koncentracija ili funkcija C1 INH , a normalna koncentracija C4, dijagnoza HAE se dovodi u pitanje. Za sada se r utinski ne rade genetska ispitivanja. Do danas je u Srbiji r egistrovano 50 pacijenata sa HAE-om. Naši pokušaji usmereni su ka: 1) definisanju laboratorijskih i kliničkih karakteristika pacijenata sa dijagnostikovanim HAE- om prema važećim kriterijumima; 2) skriningu članova por odice koji nose rizik od oboljevanja i utvrđivanju njihove polne i starosne distribucije, kao i definisanju laboratorijskih i kliničkih obeležja novootkrivenih pacijenata i 3) formiranju nacionalnog registra pacijenata obolelih od HAE-a.

Ključne reči: komplement, C1 inhibitor, hereditarni angioedem

HEREDITARY ANGIOEDEMA: CLINICAL AND LABORATORY DIAGNOSIS

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Hereditary angioedema (HAE) is a rar e autosomal dominant condition characterized by r educed function of C1 inhibitor (C1 INH) due to impair ed transcription or pr oduction of non-functional pr otein. The prevalence of the disease has been estimated at 1/50 000. P atients with HAE experience r ecurrent episodes of subcutaneous and submucosal swellings in any part of the skin and the r espiratory and gastrointestinal tracts. Attacks affecting the or opharynx or abdomen can be associated with significant risk of illness and death. Ser um C4 level is a good scr eening test for C1 INH deficiency, as serum C4 is invariably low in untreated HAE. If the C4 level is low, then C1 INH level and function should be assessed. The diagnosis of type I HAE (85% of cases) is made by demonstrating low amounts of C1 INH pr otein, as assessed by immunochemistr y. If C1 inhibitor value appears normal (and C4 is low), a test of C1 INH function should be car ried out. An absence or signif - icant reduction of C1 INH function suggests a type II defect. All such tests should be car ried out on fr esh (or freshly frozen) serum and plasma samples. All testing should be undertaken off tr eatment. Interpretation in very young children is difficult, owing to a paucity of data regarding reference ranges in children. Investigations should be r epeated when the child is over 1 year. In the pr esence of a low C1 INH level or function, but a nor mal C4 the diagnosis of HAE must be questioned. Cur rently, genetic tests ar e not indi - cated routinely. By now, fifty patients have been diagnosed with HAE in Serbia. Our attempts ar e: 1) to define laboratory and clinical characteristics of pa - tients with diagnosed HAE accor ding to the cur rent criteria; 2) screening of family members and r elatives who are under risk, deter mine gender and age distribution, define the laborator y and clinical featur es of newly discovered patients and 3) to for m the national patient register of HAE patients.

Keywords: complement, C1 inhibitor, hereditary angioedema

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BIOCHEMISTRY AND METABOLISM OF VITAMIN D – INTRODUCTION

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Vitamin D is not technically a vitamin, since it is not an essential dietary factor. It is rather a prohormone produced photochemically in the skin from 7-dehydrocholesterol. Vitamin D and its metabolites may be categorized as either cholecalciferols or ergocalciferols. Cholecalciferol (vitamin D3) is the parent compound of the naturally occuring family and is produced in the skin from 7-dehydrocholesterol on exposure to the ultraviolet B portion of sunlight. Vitamin D2 (ergocalciferol), the parent compound of the other family, is manufactured by irradiation of ergosterol produced by yeasts and its potency is less than one-third of vitamin D3's potency. The steps in the vitamin D endocrine system include the following: 1) the photoconversion of 7-dehydrocholesterol to vitamin D3 in the skin or dietary intake of vitamin D3; 2) metabolism of vitamin D3 by the liver to 25-hydroxyvitamin-D3 [25(OH)D3], the major form of vitamin D circulating in the blood compartment; 3) conversion of 25(OH)D3 by the kidney (functioning as an endocrine gland) to the hormone 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and the candidate hormone 24R,25(OH)2D3; 4) systemic transport of the dihydroxylated metabolites 24R,25(OH)2D3 and 1,25(OH)2D3 to distal target organs; and 5) binding of 1,25(OH)2D3 to a nuclear receptor (VDR) at target organs, followed by generation of appropriate biological responses. The activation of vitamin D to its hormonal form is mediated by cytochrome P450 enzymes. Six cytochrome P450 (CYP) isoforms have been shown to hydroxylate vitamin D. Four of these, CYP27A1, CYP2R1, CYP3A4 and CYP2J3, are candidates for the enzyme vitamin D 25-hydroxylase that is involved in the first step of activation. The highly regulated, renal enzyme 25-hydroxyvitamin D-1 – hydroxylase contains the component CYP27B1, which completes the activation pathway to the hormonal form 1,25(OH)2D3. A five-step inactivation pathway from 1,25(OH)2D3 to calcitroic acid is attributed to a single multifunctional CYP, CYP24A1, which is transcriptionally induced in vitamin D target cells by the action of 1,25(OH)2D3. An additional key component in the operation of the vitamin D endocrine system is the plasma vitamin D binding protein (VDBP), which carries vitamin D3 and its metabolites to their metabolism and target organs. VDBP is a specific, high-affinity transport protein. It is synthesized by the liver and circulates in great excess, with fewer than 5% of the binding sites normally occupied. 1,25(OH)2D3 acts as a ligand for a nuclear transcription factor, VDR, which like all other nuclear receptors regulates gene transcription and cell function. The widespread presence of VDR and the key activating (1-hydroxylase, CYP27B1) and inactivating (24-hydroxylase, CYP24A1) enzymes in most mammalian cells means that the cells in these tissues have the potential to produce biological responses, depending on the availability of appropriate amounts of vitamin D3. Thanks to this widespread presence of elements of the vitamin D endocrine system, its biological features are being recognized outside bone tissue, i.e. calcium and phosphate metabolism.

VITAMIN D – CHALLENGES IN DIAGNOSING AND MONITORING HYPOVITAMINOSIS D

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Vitamin D3 (cholecalciferol) arises from an endogenous precursor by a photochemical reaction in the skin. It is the precursor of 1,25-(OH)2-vitamin D (D-hormone), a key hormone in the homeostasis of calcium, which has calcitropic action and probably also exerts multiple paracrine regulatory functions in a variety of tissues. Since food – except for fatty fish or fortified dairy products – contains only small amounts of vitamin D, sufficient sun exposure or vitamin supplementation is required to maintain an adequate substrate for the D-hormone system and is necessary for optimal mineralisation of the bone.

Monitoring of the individual vitamin D status is probably best achieved by the quantification of serum 25-hydroxyvitamin D (25-OHD), an intermediate metabolite of vitamin D. Decreased serum calcium together with increased alkaline phosphatase is typically observed only in severe D-hypovitaminosis with 25-OHD serum concentrations <10 ng/mL. The active metabolite 1,25-(OH)2-vitamin D, on the other hand, may counter-intuitively remain in the normal range also in patients with severe D-hypovitaminosis due to secondary hyperparathyroidism and is therefore not useful for monitoring the vitamin D status. Vitamin D deficiency can be defined as a condition where administration of vitamin D results in a decrease of PTH blood concentrations. According to epidemiological studies, 25-OHD concentrations above 20 to 30 ng/mL are considered sufficient for bone health, whereas lower concentrations are recognized as a risk factor for falls and fractures. A substantial problem in the interpretation of such results, however, is the fact that 25-OHD serum levels show a high degree of seasonal variability in many regions and consequently one single measurement is probably not appropriate to characterize a person's individual long-term vitamin D status.

During the last decade, a variety of potential extra-skeletal health outcomes associated with a poor vitamin D status have been discussed extensively (including diabetes, hypertension, cardiovascular diseases, malignancies, psychiatric disorders, and infections). However, the causality of such an association is highly controversial at present: a good vitamin D status is typically a result of an active lifestyle with extensive out-door physical activities which are per se highly protective against many non-commutable diseases. Thus, to date it is uncertain to what extent the worldwide burden of osteoporosis and chronic diseases is actually influenced by chronic and long-standing hypovitaminosis D. Indeed, given the abovementioned target ranges, a large proportion of the world's population must be considered to have a poor vitamin D status, at least seasonally. This definitely also includes sunny countries. As a consequence, vitamin D supplementation may be considered for billions of people. According to present data, such supplementation probably has a favourable safety profile and is rather inexpensive. However, the extent of long-term health effects of a widespread vitamin D supplementation on a population level is still speculative; such global recommendations warrant further very robust and comprehensive epidemiological data. A prerequisite for such large scale studies are reliable analytical methods which can be traced back to mass spectrometric reference methods and reference material preparations. Guidelines for the monitoring of vitamin D supplementation are not available to date but may be relevant e.g. for populations with potentially impaired intestinal absorption, as is the case in the elderly. Furthermore, it seems reasonable to investigate innovative laboratory tools to characterize the individual vitamin D status beyond single 25-OHD measurement, potentially including sequential measuring schedules throughout the year as well as measurement of PTH, urinary calcium, and vitamin D-binding protein.

VITAMIN D: NEEDS DURING PREGNANCY AND THE PERINATAL PERIOD

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Pregnancy and the perinatal period are two key stages in bone metabolism, as they both impose major stress on this calcium resource. Vitamin D and parathyroid hormones are major players in the calcium absorption and bone resorption processes during these critical periods. Maternal serum 25-hydroxyvitamin D (25-OHD) concentration is the marker of the vitamin D nutritional status. The foetus is entirely dependent on mother for its vitamin D, which is believed to cross the placenta. At birth, the neonate has to adapt quickly to an environment with lower mineral supply. This leads to a transient hypocalcemia and increased parathyroid hormone secretion that induce synthesis of 1,25-dihydroxyvitamin D, the hormonal form of vitamin D. Under these circumstances, serum 25-OHD concentrations are the rate-limiting step in the hormone synthesis. Thus, at equivalence and equivalent time post-partum, circulating 1,25-dihydroxyvitamin D concentrations are higher in vitamin D-repleted infants than in infants from vitamin D-depleted mothers. In countries in which dairy products are not routinely supplemented with vitamin D, or in populations in which milk products are not consumed, vitamin D supplementation during pregnancy and in the perinatal period is necessary. However, in the normal population, the daily intakes should always follow the guidelines given by the groups of experts, and there are no indications for the use of pharmacologic doses of vitamin D or its metabolites.

ROLE OF VITAMIN D IN OSTEOPOROSIS

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For many years, the vitamin D status of individuals was defined simply by the presence or absence of rachitic bone disease (osteomalacia in adults), a relatively rare clinical problem in the 21st century. Serum levels of 25OHD <20 nmol/L (8 ng/mL) were considered a marker of vitamin D-deficiency. However, a variety of studies carried out over the last 10 years have suggested that suboptimal vitamin D status can occur in the absence of rickets/osteomalacia. This new perspective on vitamin D status arose from reports indicating that serum levels of 25OHD continue to correlate inversely with serum parathyroid hormone (PTH) levels up to concentrations of approximately 75 nmol/L (30 ng/mL). Similar observations were also made for intestinal calcium uptake, leading to the conclusion that optimal vitamin D status occurred at serum concentrations >75 nmol/L. The term vitamin D 'insufficiency' was proposed in the 1980s to define subjects with suboptimal vitamin D status (<75 nmol/L serum 25OHD) who did not have rachitic bone disease (<20 nmol/L 25OHD). A panel of scientists and clinicians at the Institute of Medicine (IOM), USA defined in 2010 the reference values that best represent the levels of vitamin D and calcium that are optimal for bone health. The term 'vitamin D' specifically refers to vitamin D derived from sunlight or diet which undergoes metabolism, firstly to circulating 25-hydroxyvitamin D (25OHD) which is nowadays used to define the vitamin D status. Whilst recognizing that 75 nmol/L of 25OHD is optimal for calciotropic function, the IOM report highlighted other publications that contradicted this. In some studies, a lower optimal serum concentration of 25OHD was defined, and in some cases it was not possible to define an optimal plateau point. The IOM report concludes that classical effects on skeletal homeostasis remain the most clinically robust health outcome associated with vitamin D (rickets, PTH, falls, BMD, fractures). Based on this, the IOM suggested that a serum level of 50 nmol/L (20 ng/mL) 25OHD is sufficient to optimize bone mineral density (BMD) as a marker of skeletal health for most populations in the United States and Canada. The Task Force of the Endocrine Society however suggested optimal levels of vitamin D of 75 nmol/L (30 ng/mL). Primary osteoporosis is an age-related disease defined as »low bone mineral density« and mostly affects the elderly (>65 years). Vitamin D is deficient in most of the patients with osteoporosis. The IOM proposes only 600 IU/day, however, this might not be sufficient for lowering the fracture risk in osteoporosis. Thus most specialists in the field of osteoporosis comply to the recent guidelines of the Task Force of the Endocrine Society recommending at least 800 IU/day for the anti fracture efficacy of vitamin D in patients with osteoporosis.

VITAMIN D IN THE PREVENTION OF RENAL AND CARDIOVASCULAR DISEASE

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Vitamin D belongs to a group of secosteroids and is produced in the skin by ultraviolet, i.e. UVB radiation. The role of vitamin D in mineral metabolism has been well known for more than one century. During the last 20 years it has been recognized that the biological effects far of vitamin D reach beyond mineral metabolism. There are considerable experimental and observation data that demonstrate links between vitamin D and cardiovascular and renal disease. First, vitamin D receptors have been identified on vascular endothelial and smooth muscle cells and cardiomyocytes. Second, there are *in vitro* data that vitamin D regulates the growth and proliferation of these cells. Moreover, vitamin D is involved in the regulation of the renin-angiotensin-aldosterone system, has anti-inflammatory effects and by the reduction of PTH it could have vasculoprotective effects. Based on these experimental data it could be concluded without any doubt that vitamin D is involved in the regulation of the cardiovascular system and renal function. Several observation studies have been conducted in an attempt to correlate vitamin D levels with hypertension, cardiovascular disease, diabetes, metabolic syndrome as well with renal disease. In the majority of studies inverse correlations between the vitamin D level and hypertension, heart failure, myocardial infarction, stroke and peripheral vascular disease were observed. Also, vitamin D insufficiency was associated with diabetes mellitus, insulin resistance and metabolic syndrome, well-known risk factors for cardiovascular and renal disease. Unfortunately it is still unclear if these associations are causal. Until now there have been few interventional studies of the vitamin D effects on blood pressure, cardiovascular and renal protection remains undefined. The results are promising, but still inconclusive. The optimal dose of vitamin D or if there is any advantage of in using vitamin D analogs in cardiovascular and renal protection. Very recent results obtained with the vitamin D analog paricalcitol in renal protection are very promising. Undoubtedly we need more prospective randomized studies to explain the causal relationship between the vitamin D level and cardiovascular and renal disease and to investigate the appropriate level and dose of vitamin D in renal and cardiovascular protection.

THE ROLE OF VITAMIN D IN MULTISYSTEM SARCOIDOSIS

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Recently published data suggest that patients vitamin D abnormalities are common in sarcoidosis. The purpose of this study was to compare serum vitamin 25(OH)D levels among our sarcoidosis patients with different clinical courses of the disease. The study also included the first observations on cognitive functions (i.e. the feeling of depression and fatigue syndrome) in relation to vitamin D deficiency in sarcoidosis patients.

In the Biochemical Laboratory of the Clinical Center of Serbia, Belgrade, vitamin D -25(OH)D was measured using the Elecsys ® vitamin D total test. Two hundred twenty-six patients with biopsy -positive sarcoidosis were analyzed.

The average median value of serum vitamin D was 9.47 μg/L, indicating severe deficiency. A statistically significant correlation was found in patients with chronic disease and low levels of serum vitamin 25(OH)D (Chi-Square=6.044; df=2; p=0.014). The patient group with vitamin D serum levels higher than 20 μg/L showed higher levels of the mean forced vital capacity (FVC) by 380 mL, and forced expiratory volume in one second (FEV1) by 220 mL when compared to the patient group with lower serum vitamin D. A statistically significant role was established serum vitamin 25(OH)D levels as a predictor of fatigue (R^2=0.878; p=0.038 (β=0.216)) and depression in patients with sarcoidosis (R^2=0.80; p=0.000 (β=0.391)).

The insufficiency of 25(OH)D seems to be in important factor in predicting the course of chronic disease, significant lung function impairments and cognitive failures such as fatigue and depression. The fact that the majority of the analyzed sarcoidosis patients had totally deficient levels serum 25(OH)D made this importance even more notable.

BIOLOGICAL AND CLINICAL EFFECTS OF VITAMIN D IN THE ELDERLY POPULATION

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The high prevalence of vitamin D depletion in the French elderly population with consequences on the global health and mortality is the reason of an important public health campaign providing information on the inherent risk and on the strategies of prevention and treatment.

We shall discuss the role of vitamin D in parathyroid activity, muscle function and falls prevention in the elderly, and the correlation of vitamin D depletion with cognitive decline, risk of cancer and global mortality.

Concerning the modality of vitamin D repletion in order to preserve optimal vitamin D blood levels, we shall present the point of view of the French GRIO.

CONTRIBUTION OF DIET TO VITAMIN D STATUS

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Vitamin D is a fat-soluble vitamin and prohormone that is naturally found in very few foods, is added to others, and is available as a dietary supplement. Humans acquire vitamin D through skin photosynthesis and digestive intake. This nutrient plays an important role in numerous biological functions, including regular bone growth, blood glucose levels control, the process of inflammation and in adequate functioning of the innate immune system. The relationship between vitamin D and health outcomes is not linear, and there are probably various optimal vitamin D levels influencing different endpoints. Although some results are controversial, low serum 25(OH)D levels have been linked to all-cause, cardiovascular, cancer and infection related morbidity and mortality. The clinical symptoms of severe vitamin D deficiency (rickets) are well known and can be easily detected. The signs and symptoms beyond deficiency, however, remain to be elucidated. The present paper evaluated the contribution of diet to vitamin D status and analyzed the prevalence of inadequacy of vitamin D intake in different countries. Dietary sources of vitamin D include fatty fish species, whole eggs, beef liver, fish liver oils, and UV-irradiated mushrooms and yeast are the only known significant vegan sources of vitamin D. Since 2000, many relevant international societies have proposed new reference values that are based on much more information and higher-quality studies than were available when the values for this nutrient were set before. The recommended daily amount for vitamin D in the European Union is 5 μg and supplementing is possible for the elderly and adolescent girls as the risk groups for vitamin D deficiency. Some experts believe in food fortification, as a way of increasing vitamin D intake. Also, too much vitamin D may be harmful. The data on vitamin D intake in most European countries are sparse, but it was estimated that 90% of Europeans have intakes below recommendations. Dietary habits, intake of supplements and high calcium intake influence the vitamin D status. Relevant food databases on vitamin D contents in food could be helpful. Appropriate lifestyle changes, such as regular short exposures to sunlight (15 min a day), and an adequate diet that includes vitamin D rich components, are not always easily accomplished, so dosages used for vitamin D supplementation should be higher than those traditionally suggested. To conclude, vitamin D holds great promise as an agent for primary and secondary prevention of disease, and during times of reduced exposure to sunlight vitamin D status is related to dietary vitamin D intake. Health professionals must be aware of the contribution that dietary intake makes to the vitamin D status of the population.

VITAMIN D MEASUREMENT – CHALLENGES IN EXACT DIAGNOSIS, PITFALLS, STANDARDIZATION, RESULTS OF THE ELECSYS VITAMIN D TOTAL ASSAY

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During recent years vitamin D has gained significant interest not only in the scientific literature and medical community but also in the public domain. Called a vitamin but being a hormone due to its paracrine, autocrine and endocrine function, together with parathyroid hormone (PTH) it is acknowledged as an essential player in the regulation of bone remodeling and mineral homeostasis. The discovery that most cells in the body have a vitamin D receptor and are able to convert the »substrate« 25-hydroxyvitamin D [25(OH)D] to its active form 1,25-dihydroxyvitamin D has provided new insights into the non-musculoskeletal function of this vitamin. According to numerous observational studies vitamin D deficiency has been linked to chronic illnesses including various cancers, autoimmune and infectious diseases, inflammatory response, and cardiovascular diseases. The lack of vitamin D is widespread and looked at as a major health and economic burden worldwide. Measurement of 25(OH)D, providing the best insight into the vitamin D supply, is not an easy task. For 25(OH)D quantification a number of different analytical techniques have been employed with immunoassays still being the primary method and the most widely used technique. Potential limitations of 25(OH)D quantification by means of immunoassays have led to the development of more sophisticated technologies like liquid chromatographic and tandem mass spectrometric methods (LC-MSMS), respectively. Due to continuous further development of the tandem mass spectrometry technology in regard to equipment and test design, this method is increasingly used in clinical laboratories and acknowledged as the reference method for the quantification of 25(OH)D levels with high selectivity and specificity. To date all major diagnostic companies have launched 25(OH)D assays claiming standardization against LC-MSMS, traceability to reference measurement procedures (RMP) and therefore postulating trueness of the measured value, which is not always given. Immunoassays are prone to various factors having a significant impact on the quality and trueness of measured value. Setting free 25(OH)D from its specific binding protein, type of sample employed, equimolar detection of 25(OH)D3 and 25(OH)D2 and detection of additional 25(OH)D relevant metabolites are only a few of the existing pitfalls in 25(OH)D immunoassay testing. Even though the quality of measurement of vitamin D by means of immunoassay has improved significantly, it is still not an easy task.

CHALLENGES IN VITAMIN D ANALYSIS

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Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and plays an important role in calcium homeostasis. Vitamin D is essential for the formation and maintenance of healthy bones. The research in the past decade has revealed a diverse range of biological actions that include: immunomodulatory functions, anti-infective action, a stimulatory effect on insulin secretion from pancreatic beta cells and an inhibitory effect on angiogenesis. Vitamin D deficiency is an important concern and results from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism or vitamin D resistance. The 25-OH Vitamin D is an accepted marker for vitamin D nutritional status that has not been standardized. Recognition of prevalent vitamin D deficiency in most patient populations resulted in an unexpected and marked increase in the volume of testing of 25- OH Vitamin D in clinical laboratories. Various methods are now available for measuring circulating concentrations of 25-OH Vitamin D. Until recently, RIA was the only method used for this purpose. Current methods include: automated chemiluminescence immunassays, HPLC and LC -MSMS. These new methods have aroused controversy. Recent correlation studies between immunoassay and LC -MSMS methods for

25-OH Vitamin D r evealed reasonable correlations but with significant differences. LC-MSMS is becoming the technique of choice in many clinical laboratories. Many in-house methods have also been developed. Laboratories per forming 25-OH Vitamin D testing by the LC -MSMS method exhibit differ ences in their standard operating procedures. For that reason, interlaboratory variations are around 20%.

In the pr esentation, the advantages and disadvantages of differ ent methods will be evaluated and a comparison of the differ ent methodologies will be given by examples.

ANALYTICAL METHODS AND PERFORMANCE OF IMMUNOASSAY METHODS FOR DETERMINATION OF VITAMIN D IN COMPARISON TO MASS SPECTROMETRY

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Demand for vitamin D testing has been on a constant rise worldwide, partially due to mounting evidence linking vitamin D status to overall health and well-being. Currently available assays measur e 25-hydroxy vitamin D (25OH-D), a major cir culating form of vitamin D. Methodologies available include immunoassays, HPLC and mass spectr ometry based methods (LC-MS/MS). Until recently the only immunoassays available for diagnostic use in the US wer e DiaSorin radioimmunoassay (RIA) and an automated immunoassay on a LIAISON® platfor m. Within the last year Siemens and Abbott have successfully launched immunoassays for the deter mination of total vitamin D on their r espective automated platfor ms, Centaur® and ARCHITECT®. Development of r obust and precise Vitamin D immunoassays has historically been plagued with difficulty. One of the major challenges is development of specific antibodies against such a small antigen. Vitamin D is also highly hydrophobic molecule pr edominantly bound to vitamin D binding pr otein (VDBP). It is likely , therefore, that immunoassays might be affected to varying extent by the VDBP concentration. Adoption of mass spectrometry (LC-MS/MS) into clinical laboratories has enabled development of accurate and almost fully automated methods that could handle incr easing volume demands, especially in lar ge-volume reference laboratories. Smaller to mid-size hospital laboratories as well as physician offices have neither the funds nor technical expertise to implement LC -MS/MS based testing.

Our laboratory at the University of Chicago Medical Center has also seen an incr ease in the vitamin D testing volume and cur rently performs close to 20,000 25OH-D assays per year . We have r ecently developed an LC -MS/MS method for the quanti -tation of 25OH-D2 (obtained fr om plant sour ces) and 25OH-D3 (endogenous and animal sour ces). Prior to the acquisition of an LC -MS/MS instrument we performed 25OH-D analysis by RIA. During the transition period, we encountered several challenges, including the necessity to str eamline sample pr e-paration as well as the bias intr oduced by calibration differences. We chose to match our LC -MS/MS method to the RIA method in order to make this transition transparent to the clinician. W e also evaluated the ADVIA Centaur® vitamin D competitive immunoassay. Although this assay is clearly positively biased in comparison to RIA and LC -MS/MS and most likely susceptible to the variability in VDBP , it is acceptable for clinical use and might be a method of choice for smaller laboratories. Lar ger clinical laboratories and academic institutions that possess technical expertise, particularly the ones with a lar ge pediatric population where assay sensitivity and specificity may be important, might find the LC -MS/MS methodology a more suitable choice.

COMPARISON OF THREE DIFFERENT METHODS FOR 25(OH)-VITAMIN D DETERMINATION AND VITAMIN D STATUS IN THE GENERAL POPULATION – A SERBIAN EXPERIENCE

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Determination of 25-hydroxyvitamin D [25(OH)D] represents a unique challenge, considering its lipophilic nature. Because of the widespread prevalence of vitamin D deficiency, which has lead to an increasing number of requests for 25(OH)D determination, immunoassay measurements adjusted to automated analyzers are being developed. Due to the variability among assays, it is often difficult to monitor the vitamin D status and supplementation. The aim of this study was to compare the results of two immunoassays against high performance liquid chromatography with ultraviolet detection (HPLC-UV). Also, the aim was to estimate the vitamin D status, since up to date the prevalence of vitamin D deficiency in Serbia has not been examined.

We evaluated the analytical characteristics of the electrochemiluminescent immunoassay Elecsys® Vitamin D total (Roche Diagnostics GmbH, Mannheim, Germany) performed on a Cobas® e601 analyzer, and of the chemiluminescent microparticle immunoassay ARCHITECT 25-(OH) vitamin D (Abbott Diagnostics, Wiesbaden, Germany) performed on an Architect® ci8200 analyzer. For comparison studies we used HPLC analysis of 25-(OH)- Vitamin D3/D2 (Chromsystems Instruments & Chemicals GmbH, Munich, Germany) using the Waters isocratic HPLC-UV system, because it involves pretreatment of samples which minimizes the influence of interferences. In order to estimate the vitamin D status in the general population, we searched the database of the laboratory information system and analyzed the data from 533 patients whose 25(OH)D was determined together with intact parathyroid hormone (iPTH).

For imprecision assessment, four serum pools were prepared with the following 25(OH)D concentrations: ~10 μg/L, ~20 μg/L, ~30 μg/L and ~50 μg/L. Obtained CVs for the Roche method were 1.5–2.8% for within-run and 4.0–6.7% for between-run imprecision. For the Abbott method, CVs were 0.7–4.4% for within-run and 3.8–7.2% for between-run imprecision. Inaccuracy was analyzed using commercial control sera. Obtained deviations from the target value were 2.1% for the Roche assay and 1.3–1.5% for the Abbott method, and were not statistically significant ($P>0.05$). Comparison of Roche and HPLC-UV methods using Passing-Bablok regression analysis gave the following equation for the regression line $y=0.94x+3.76$ ($S_{y,x}=6.33$ μg/L; $r=0.739$; $n=97$) and the regression line equation from the comparison of Abbott and HPLC-UV methods was $y=0.74x+4.12$ ($S_{y,x}=5.07$ μg/L; $r=0.793$; $n=97$). Mean difference and SD for the Bland-Altman plot were –1.8 μg/L and 17.4 μg/L respectively for the Roche method and 2.5 μg/L and 15.1 μg/L respectively for Abbott. Statistical analysis (Chi-square test) of frequency distribution among different vitamin D status categories (<10 μg/L severe deficiency, 10–20 μg/L deficiency, 20–30 μg/L insufficiency and >30 μg/L sufficiency) showed that the frequency distribution obtained with the Abbott method was significantly different from that of the HPLC results, in contrast to the Roche results frequency distribution which did not differ significantly. Also, statistical analysis of agreement between the three methods for each vitamin D status category showed that the results of both Roche and Abbott methods were significantly higher than HPLC ($P=0.005$ for Roche, $P=0.0407$ for Abbott), and in the sufficiency category the Abbott method significantly under estimated the concentration of 25(OH)D compared to HPLC results ($P<0.0001$). Median population values of 25(OH)D and iPTH were 16.7 μg/L and 76.6 ng/L, respectively. ANOVA analyses showed a significant ($P<0.05$) decrease in iPTH and Ca^{2+} concentrations across the 25(OH)D concentration categories. Stepwise multiple linear regression analyses indicated an independent correlation of iPTH with the 25(OH)D concentration ($\beta=-0.179$, $P=0.003$). Also, one-way ANOVA with the Student-Newman-Keuls test demonstrated that the 25(OH)D concentrations measured in summer and autumn were significantly ($P<0.001$) higher compared to those determined in winter and spring.

Despite the acceptable imprecision and inaccuracy of both examined methods, results obtained with them did not correlate well with HPLC-UV ($r<0.9$), which was used as a reference. Regardles of this fact, the methods showed satisfactory ability to classify patients into vitamin D status categories, which is important for the diagnosis of vitamin D deficiency and therapy follow-up. About two-thirds (68,5%) of the examined population had vitamin D deficiency (25(OH)D<20 μg/L) and only 8% had sufficient 25(OH)D concentrations (>30 μg/L).

LEVELS OF 25-OH VITAMIN D IN HEALTHY POSTMENOPAUSAL WOMEN. COMPARISON OF TWO DIFFERENT METHODS

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The main role of vitamin D in humans is its involvement in the regulation of calcium (Ca) absorption. Vitamin D is considered to be a part of the etiopathology leading to osteoporosis. There is no consensus in the literature concerning the level of vitamin D deficiency. A generally accepted minimum level of 25-OH-vitamin D (25OHVitD), the major storage form of Vitamin D in the human body, for maintaining healthy bones is 20 ng/mL. There are significant differences between commercial kits in the measurement of 25OHVitD concentrations. The aim of this study was to determine the levels of 25OHVitD in healthy postmenopausal women and to investigate the possible correlation with the levels of parathyroid hormone (PTH) and Ca in these women.

In 149 postmenopausal women, mean age 55±6.4 (43–78) years, 25OHVitD was measured with the electro-chemiluminescence »ECLIA« method (Vitamin D3 25-OH) on the Roche Cobas e-411 analyzer. The detection limit of the method is 4 ng/mL (10 nmol/L) and the precision estimated with the CV% is 9.9%. Forty-three (43) of the postmenopausal women were taking supplements containing Ca and vitamin D while the remaining 106 were not taking any supplement. In 80 of the postmenopausal women 25OHVitD was also measured with the new Roche method (Vitamin D total) on the Cobas e-411 analyzer.

The mean 25OHVitD in women who were not taking any supplement was 19.5±7.1 (4.4 –38.8) ng/mL, while in those receiving supplements it was 22.5± 8.4 (4.0–45.8) ng/mL. The difference was statistically significant (p=0.03). No significant correlation was found between 25OHVitD and PTH or Ca levels. Also, no significant correlation was noticed between 25OHVitD levels and the age or BMI of the women studied. The linear regression analysis between the new (Vitamin D total) and the old Roche method (Vitamin D3 25-OH) gave the equation: New Roche = 1.18*(old Roche) + 9.6.

In the present study, the mean concentration of 25OHVitD in postmenopausal women, measured with the Vitamin D3 25- OH Roche method, was found to be near the limit of deficiency (20 ng/mL). Nutritional supplements showed to slightly improve the levels of 25OHVitD in postmenopausal women. The new Roche method for Vitamin D total gives higher values compared to the old Roche method for Vitamin D3 25-OH, especially in the lower part of the reference range.

THE LOW SERUM CONCENTRATIONS OF 25-OH VITAMIN D IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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Recent studies have suggested an immunoregulatory role for vitamin D. An association between vitamin D deficiency and the development of rheumatoid arthritis (RA) has been implied. Its role in modulating RA disease activity has not been studied.

The study objectives were to evaluate the plasma level of 25-hydroxyvitamin D [25(OH)D] in RA patients and to explore the effects of serum vitamin D levels on clinical outcome in patients with early RA.

Serum levels of 25(OH)D were investigated in 73 patients with early rheumatoid arthritis (mean age 35.7±11.4 years, mean arthritis duration 5.1±4.3 months) versus 80 normal controls (mean of age 34.2±10.7 years). Serum levels of 25(OH)D were tested using the enzyme-linked immunosorbent assay (ELISA). The 25(OH)D concentrations were expressed as nmol/L. The results were statistically analyzed by Student t test and a $p<0.05$ (95% confidence intervals) was considered statistically significant. Correlation analyses were performed using Spearman's rank correlation, and differences between the groups were calculated with Fisher's exact test and two-tailed t-test for continuous variables.

The serum values of 25(OH)D were found low in 46 of 73 the patients with RA (63%) vs. normal controls ($p<0.01$). Eight out of 46 patients had 25(OH)D at baseline. Serum values of 25(OH)D in early RA showed a direct correlation with high disease activity ($r=0.61$; $p<0.01$). Levels of 25(OH)D correlate with high serum CRP levels ($r=0.59$; $p<0.01$). The mean serum concentration of 25(OH)D in early RA patients was 28.34±8.7 nmol/L as compared to that in normal controls, 52.42±13.4 nmol/L ($p<0.001$).

All early RA patients showed a marked deficiency of 25(OH)D. The low level of 25(OH)D had negative consequences on the clinical outcome.

DOES VITAMIN D REALLY AFFECT MUSCLE STRENGTH AND BALANCE IN KNEE OSTEOARTHRITIS?

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The aim of the study was to examine the effect of serum active vitamin D (1,25(OH)D) levels and creatinine clearance (CrCl) on balance ability and muscle strength in patients with knee osteoarthritis. Patients with radiographic knee osteoarthritis (OA) (Kellgren-Lawrence grade 2–3) with knee pain and age-matched healthy women were enrolled in the study. The intensity of pain was measured by the visual analog scale (VAS). Clinical balance tests performed were Berg balance scale (BBS), short physical performance battery test (SPPBT) and tandem walking. Balance ability was also measured by the kinesthetic ability trainer 3000 (KAT) with static and dynamic protocols. Isokinetic strength measurements of both knee flexor/extensors and both ankle dorsiflexor/plantar flexors were assessed using the Cybex Norm isokinetic dynamometer. The 1,25 (OH)D levels and CrCl were determined in all study participants. In the OA group, pain-VAS and tandem walking time were significantly higher than in the control group. In the OA group, the BBS scores, SPPBT-scores, mean peak torque values of knee extensor, knee flexor, ankle dorsiflexor and 1,25 (OH)D levels were significantly lower than in the control group. There was a positive correlation between the 1,25 (OH)D levels and mean peak torque values of knee extensor at 60o/sec, ankle dorsiflexor at 30o/sec, and ankle plantar flexor at 30o/sec. There was no statistically significant correlation between the 1,25 (OH)D levels and BBS scores, static and dynamic balance indexes measured on KAT. Decline in balance ability and muscle strength is common in patients with knee osteoarthritis. Vitamin D deficiency may contribute to the decrease in muscle strength seen in osteoarthritis or immobilization as a consequence of knee osteoarthritis.