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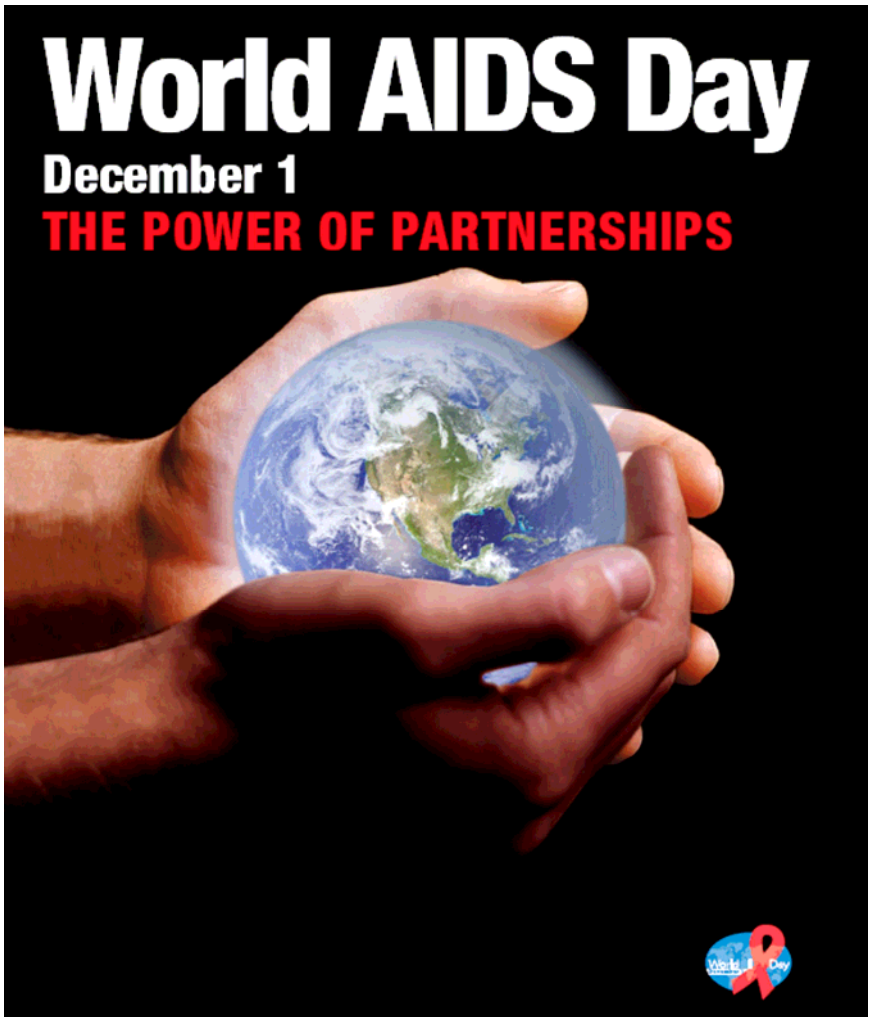

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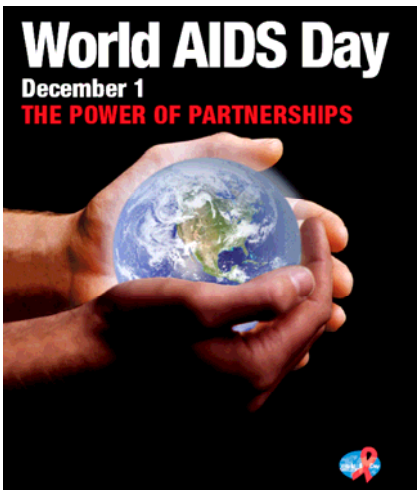

Prvi decembar 2008. godine obeležen je širom sveta kao 20. rođendan Svetskog dana borbe protiv AIDS-a koji je ustanovljen 1988. godine na Svetskom samitu ministara zdravlja posvećenom programima za prevenciju ove bolesti. Od tada, svake godine širom sveta sprovode se zajedničke kampanje vezane za HIV/AIDS (vidi Uvodnik).

On December 1st 2008 the 20th Anniversary of the World AIDS Day was celebrated. The concept of the World AIDS Day originated from the World Summit of Ministers of Health on Programmes for AIDS Prevention in 1988. Since then, each year joint campaigns on HIV/AIDS take place worldwide (see Editorial).

Poštovani čitaoci i saradnici,

Godina 2008, od koje ćemo se uskoro oprostiti, ostaće upamćena kao jedan od najznačajnijih datuma u istoriji našeg časopisa. Kao što vam je poznato, ove godine Vojnosanitetski pregled uvršten je u dve prestižne baze naučne publicistike - Science Citation Index Expended (poznat i kao SciSerach®) i Journal Citation Reports/Science Edition zahvaljujući čemu je postao pripadnik porodice najuticajnijih naučnih časopisa sveta. Ovaj uspeh je ostvarenje želja generacija autora i saradnika Vojnosanitetskog pregleda koji su svojim kontinuiranim, samopregornim radom doprineli ovom velikom međunarodnom priznanju.

U nadi da će se saradnja na daljoj afirmaciji časopisa nastaviti i ubuduće redakcija i izdavač Vojnosanitetskog pregleda svim svojim dosadašnjim, kao i budućim autorima, urednicima, recenzentima i vernim čitaocima žele srećnu i uspešnu nastupajuću 2009. godinu, dobro zdravlje i raspoloženje!

Dear Readers and Colleagues,

Rushing to its end, the year 2008 will surely remain remembered as the one of the most significant date within the history of Vojnosanitetski pregled. In this year, as you certainly know, Vojnosanitetski pregled has been indexed by the two eminent bases of scientific publications, Science Citation Index Expended (known as SciSerach®) and Journal Citation Reports/ScienceEdition, thus joining the family of the most influential scientific journals worldwide. This success is 'a wish came true' of numerous generations of authors and associates in Vojnosanitetski pregled who by their permanent and unselfish work contributed a lot to this important international recognition.

In hope to go on cooperating in further affirmation of Vojnosanitetski pregled, Editorial Staff and the Publisher wish a Happy New Year to all the regular and future authors, editors, reviewers, as well as to our true readers!

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД
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U V O D N I K / E D I T O R I A L

Dvadeset godina „svetskog rata“ protiv AIDS-a. Ima li izgleda za pobedu?

Twenty years of “the World War” against AIDS. Is there a chance for victory?

Silva Dobrić

Vojnomedicinska akademija, Institut za naučne informacije, **Beograd**

Od 1988. godine, 1. decembar obeležava se širom sveta kao Svetski dan borbe protiv AIDS-a (AIDS je akronim od engleskog izraza **a**cquired **i**mmuno**d**eficiency **s**yndrome – **s**indrom stečene **i**muno**d**eficijencije, kod nas poznat po skraćenici SIDA). Ove godine taj dan slavi svojevrsni jubilej – 20 godina obeležavanja, odnosno 20 godina neprekidne borbe sa, prema mnogima, najvećom pošasti savremenog sveta.

Od samog početka, koncept obeležavanja Svetskog dana borbe protiv AIDS-a imao je za cilj dobijanje podrške celokupne svetske javnosti za razvoj programa prevencije širenja HIV (virus humane imunodeficijencije) infekcije, kao i obrazovanje i podizanje svesti o problemima vezanim za ovu bolest. Na globalnom nivou obeležavanje ovog dana služi za međusobno potpomaganje i unapređenje zajedničke borbe protiv HIV/AIDS-a.

Svake godine Svetski dan borbe protiv AIDS-a obeležava se pod nekim sloganom koji određuje osnovni cilj i karakter kampanje koja će se sprovoditi širom planete te godine, premda i svaka zemlja, u okviru globalne kampanje može da promoviše svoje ciljeve za tekuću godinu. Glavni, zajednički slogan ovogodišnjeg Svetskog dana borbe protiv AIDS-a jeste: *Stop AIDS. Keep the Promise – Lead – Empower – Deliver* [1], dok je naša zemlja, u okviru ove globalne kampanje pažnju usmerila na mlade sa porukom - Moj izbor: koristim kondom i prihvatam drugačije od sebe [2].

Šta je učinjeno tokom proteklih 20 godina kontinuirane borbe protiv HIV/AIDS? Prema rečima dr Petera Piota, izvršnog direktora UNAIDS-a (Joint Unated Nations Programme on HIV/AIDS), u ovom periodu uspostavljeno je snažno zajedništvo na svetskom nivou u borbi protiv ove bolesti zahvaljujući kojem je danas smanjen i morbiditet i mortalitet od AIDS-a. Međutim, još uvek smo daleko od prave „pobede“.

Prema procenama Svetske zdravstvene organizacije i UNAIDS-a u periodu od 1981. godine, kada je AIDS prepoznat kao nova bolest, pa do 2007. godine, od njega je umrlo više od 25 miliona ljudi širom sveta, a procenjuje se da sada živi oko 33 miliona osoba zaraženih HIV-om [3]. Uprkos sve široj dostupnosti lekova protiv HIV/AIDS-a, pogotovo u poslednjih 5–10 godina, što je rezultovalo i značajnim smanjenjem smrtnosti od ove bolesti, ipak su podaci od 2 miliona umrlih od AIDS-a tokom 2007. godine, od čega 13,5% dece mlađe od 15 godina, i dalje duboko zabrinjavajući. Kao i ranije, i danas HIV infekcija, uglavnom pogađa narkomane koji drogu uzimaju intravenskim putem i osobe koje imaju nezaštićene homoseksulne i hetroseksulane odnose, pogotovo sa korisnicima intravenskih narkotika.

Podsharska Afrika još uvek je najteže pogođen region sveta u kome živi 2/3 svetske populacije zaražene HIV-om, od čega su 60% žene. U prošloj godini, na ovom području registrovano je 1,9 miliona novoinfiociranih HIV-om, a od svih obolelih od AIDS-a koji su umrli u 2007. godini, čak njih 75% je iz ove oblasti.

Prema procenama s kraja 2007. godine, u regionu Azije 5 miliona osoba bilo je zaraženo HIV-om, od čega se njih 380 000 vode kao novoregistrovani slučajevi.

Ruska Federacija i Ukrajina vodeće su zemlje istočne Evrope, odnosno centralne Azije, po broju zaraženih HIV-om. Samo tokom 2007. godine u ovom regionu zabeleženo je 110 000 novoinficiranih slučajeva i 58 000 umrlih od AIDS-a.

Region severne Amerike, zapadne i centralne Evrope karakteriše porast broja osoba inficiranih HIV-om što je, smatra se, rezultat produženja životnog veka ovih osoba zbog široko dostupne anti-HIV terapije, ali i porasta broja novoregistrovanih slučajeva. Krajem 2007. godine u ovom regionu živelo je 2 miliona osoba inficiranih HIV-om, od čega su njih 81 000 registrovani kao novodijagnostikovani slučajevi.

Region centralne Evrope, u kome se nalazi i Srbija, region je u kome je i do sada bila najniža stopa zastupljenosti HIV infekcija. U većini zemalja ovog regiona nezaštićeni hetroseksualni odnos glavni je put transmisije virusa, dok je u zemljama poput Češke, Mađarske, Slovenije i Hrvatske to nezaštićen seksualni odnos među muškarcima. Treba napomenuti da je u ovom regionu zabeleženo smanjenje broja novodijagnostikovanih HIV infekcija kod intravenskih korisnika droge.

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Prema podacima Instituta za javno zdravlje Srbije „Dr Milan Jovanović Batut“, objavljenih na sajtu Ministarstva zdravlja Republike Srbije, u našoj zemlji od 1985. godine do, zaključno 20.11.2008. godine registrovano je 2 287 HIV pozitivnih osoba (87 novoregistrovanih u ovoj godini), od kojih je 1 432 već obolelo od AIDS-a (34 u ovoj godini), a 936 umrlo (16 u ovoj godini). Nadalje, 67 osoba inficiranih HIV-om umrlo je od bolesti i stanja koja nisu specifična za AIDS, tako da danas u Srbiji žive 1 284 osobe zaražene ovim virusom. Od 1999. godine dolazi do postepenog opadanja broja obolelih, a taj će se trend, prema procenama stručnjaka, zadržati i u narednom periodu. Istovremeno, zabeleženo je i smanjenje broja umrlih osoba. Smatra se da su ovi podaci rezultat primene kombinovane visokoaktivne antitretrovirusne terapije koja je od 1997. godine kod nas besplatna i široko dostupna.

Najveći broj HIV pozitivnih osoba u našoj zemlji (90%) registrovan je u regionu centralne Srbije i to, uglavnom, u Beogradu (70%). U skladu s tim je i podatak da je od 87 novootkrivenih osoba inficiranih HIV-om tokom ove godine njih 60% iz Beograda.

Slično svetskim i evropskim podacima i kod nas među osobama inficiranim HIV-om i obolelim od AIDS-a dominiraju muškarci, uzrasta 25–39 godina (osobe sa novodijagnostikovanom HIV infekcijom), odnosno 30–49 godina (oboleli i umrli od AIDS-a). Među decom HIV infekcija je retka (3%), dok je među mladima uzrasta 15–24 godine nešto češća (13%).

Najčešći način prenošenja HIV-a u Srbiji u proteklom periodu jeste korišćenje zajedničkog pribora za injektovanje droge (43%), zatim nezaštićeni seksulani odnos (36%), prenos infekcije sa majku na dete (1,5%), dok za 11% obolelih, uglavnom muškaraca, nije naveden način zaražavanja (pretpostavlja se da se radi o nezaštićenom homoseksulanom odnosu koji, verovatno, zbog predrasuda koje u našem društvu postoje prema takvom odnosu, muškarci ne navode kao razlog inficiranja). Od 1987. godine inficiranje putem transfuzija krvi i primene krvnih derivata praktički je svedeno na minimum i zbog obaveznog testiranja na HIV kome su podvrgnuti svi davaoci krvi, kao i davaoci tkiva i organa.

Upadljiv je porast broja seksulanog prenosa HIV-a, posebno u poslednjih 10–15 godina (sa 15% u 1991. na 51% u 2005. godini), pa ne čudi što je Ministarstvo zdravlja Republike Srbije odlučilo da svoje aktivnosti usmeri upravo na podizanje svesti o potrebi korišćenja sredstava zaštite tokom seksualnog odnosa. Preduzete mere i aktivnosti biće neophodno sprovoditi i ubuduće jer ne treba zaboraviti da u Srbiji, uprkos niskoj prevalenciji HIV infekcije, i dalje postoji nestabilna epidemiološka situacija kada je HIV/AIDS u pitanju. To se, pre svega, odnosi na nepovoljne socijalno-ekonomske uslove, postojanje rizičnih grupa i izloženost migracijskim tokovima iz istočne Evrope i centralne Azije, regiona s velikom zastupljenošću HIV/AIDS-a. Dakle, rat protiv AIDS-a se nastavlja. Verujemo, sa još većim uspehom nego do sada!

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U V O D N I K / E D I T O R I A L

HIV/AIDS i nacionalna bezbednost

HIV/AIDS and national security

Vesna Šuljagić

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Razvijanjem savremenog društva vojna populacija postala je jedan od njegovih najvećih i istorijski najznačajnijih sektora. Zbog toga, prevencija i kontrola različitih bolesti, pre svega zaraznih, u ovoj populaciji od ogromnog je značaja za društvo u celini. Pojavom i pandemijskim širenjem HIV/AIDS-a, pogotovu u najteže pogođenim regionima sveta, poput Afrike i Azije, došlo je do izražaja da ova bolest, pored toga što uništava osnovne strukture neke države (pojedinca, porodicu, ekonomske i socio-političke institucije), utiče i na one koji su odgovorni za zaštitu institucija sistema, a to su vojska i policija.

Slobodno možemo reći da su AIDS i globalna nesigurnost deo začaranog kruga. Stoga, Peter Piot, izvršni direktor UNAIDS-a, ističe da iako na prvi pogled terorizam i AIDS predstavljaju po značaju neuporedive faktore nestabilnosti u svetu, AIDS pandemija značajnije utiče na njeno širenje, te bi trebalo uvećati napore u prevenciji pandemijskog širenja ove smrtonosne bolesti.

Tokom poslednjih 20 godina, tačnije od pojave prvog slučaja HIV/AIDS-a na našim prostorima, Vojnomedicinska akademija, kao jedna od najuglednijih zdravstvenih i vojnih institucija, konstantno učestvuje u dijagnostici i terapiji bolesnika obolelih od ove bolesti, kao i u aktivnostima u vezi sa prevencijom HIV/AIDS-a u vojnoj populaciji.

Poseban napredak na polju prevencije napravljen je tokom proteklog trogodišnjeg perioda, 2006–2008. godine. To je posledica, pre svega, realizacije saradnje saniteta naše vojske sa sanitetom vojske SAD, koja je započeta na skupu o prevenciji HIV/AIDS-a u vojnim populacijama, održanom u Moskvi septembra 2004. godine, u organizaciji ministarstava odbrane Ruske Federacije i SAD-a. U radnom delu ovog skupa aktivno učešće uzeli su i predstavnici saniteta tadašnje Vojske Srbije i Crne Gore (SCG), koji se bave problemom HIV/AIDS-a. Nakon prvih ostvarenih kontakata, našoj vojsci ponuđena je stručna i materijalna pomoć iz Sektora za prevenciju HIV/AIDS Ministarstva odbrane SAD (*Department of Defense of the United States, HIV/AIDS Prevention Program*). Inače, sredstva iz ovog programa namenjena su i trenutno su angažovana na preventivnim aktivnostima u vojsci 71 države, tačnije u preventivne aktivnosti uključeno je 6 815 200 vojnika širom sveta.

U januaru 2005. godine Vojska SCG, prvi put prijavila se svojim projektom „Prevencija i kontrola HIV/AIDS-a u Vojsci SCG“ na Konkurs Sektora za prevenciju HIV/AIDS Ministarstva odbrane SAD za dodelu sredstava za fiskalnu 2006, a Vojska Srbije konkurisala je projektima i za fiskalnu 2007. i 2008. godinu. Projekti su prihvaćeni i novčana sredstva odobrena, a najveći deo planiranih aktivnosti realizovan je.

Obavljeno je usavršavanje naših stručnjaka na *University of California San Diego, San Diego State University*, *Naval Medical Center San Diego, California, Defens Institute for Medical Operation, San Antonio, Texas.*

Deo sredstava utrošen je za nabavku laboratorijske opreme, za nabavku testova za dijagnostiku HIV/AIDS-a, za nabavku računarske opreme, kao i za štampanje priručnika za edukatore, edukativnih i propagandnih postera, kao i propagandnog materijala za vojnike (brošure, olovke, kalendari, kondomi).

Tokom 2007. godine snimljen je i edukativni film „Prevencija i kontrola HIV/AIDS-a u Vojsci Srbije“, koji je namenjen edukaciji pripadnika naše vojske, ali zbog svoje komunikativnosti, jednostavnosti i pristupačnosti može se koristiti i u edukaciji svih zainteresovanih za ovu, još uvek, neizlečivu i tešku bolest, protiv koje se možemo izboriti samo preventivnim radom.

Multidisciplinarna stručna komisija VMA, u sastavu epidemiolog, infektolog, mikrobiolog i neuropsihijatar, do sada je održala četiri predavanja za edukatore, psihologe i doktore medicine Vojske Srbije, čiji zadatak je da stečeno znanje o različitim aspektima ove bolesti aktivno prenose svim pripadnicima vojske.

Znamo da je prevencija primarna i zbog toga želimo da smanjimo rizike od nastanka HIV/AIDS.

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Rešavam na vreme

Grip

Grippostad C
ATC R06AB54

jedna kapsula **Grippostad C** sadrži:

- 200 mg paracetamola
- 25 mg kofeina
- 2.5 mg hlorfenamin maleat
- 150 mg askorbinske kiseline

Grippostad C je lek iz grupe kombinovanih preparata namenjenih za ublažavanje simptoma prehlade i gripa.
Predstavlja fiksnu kombinaciju četiri aktivne supstance:
Paracetamol ublažava bol (analgetik) i normalizuje telesnu temperaturu (antipiretik)
Kofein pojačava analgetičko dejstvo paracetamola i ublažava sedaciju
Hlorfenamin smanjuje kijanje i curenje iz nosa, olakšava disanje na nos
Vitamin C povećava otpornost organizma i smanjuje intenzitet simptoma prehlade i gripa.

Odraslima i deci starijoj od 12 g preporucuju se po dve kapsule sa dosta tečnosti ujutru, u podne i uveče, a sa povlačenjem simptoma po jedna kapsula 3 puta dnevno.
Pakovanje: kutija sa 10 kapsula u blisteru.

Pre upotrebe pažljivo pročitajte uputstvo. Za više informacija konsultujte se sa Vašim lekarom ili Vašim apotekarom. Čuvati van domašaja dece.

MOJAapoteka Hemofarm

ORIGINAL ARTICLES UDC: 616.137.8/9-007.272-08

SMART Control stents in femoropopliteal region

SMART *Control* stentovi u femoropoplitealnom regionu

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Abstract

Introduction/Aim. Occlusive disease of lower limb arteries have been so far traditionally best treated with bypass surgery, but we want to find minimally invasive approach that should be at least as good as conventional surgery, and hopefully better. The aim of this study was to evaluate SMART Control stents (Cordis, J&J) in Trans Atlantic Society Consensus (TASC) B and C femoropopliteal lesions during one-year follow-up. **Methods.** Retrospective nonrandomized analysis included forty arteries in consecutive 40 patients who were stented with SMART Control stents. Primary patency at 12-month verified with Duplex Ultrasound and Acute Brachial Index (ABI) as well as freedom from Target Vessel Revascularization (TVR) were primary endpoints. **Results.** Primary technical success at stent implantation was 100%. Mean ABI values were preprocedurally 0.50, postprocedurally 0.83, at one month 0.86, at six months 0.84, at one year 0.78. After one year 39 stents were patent (97.5%). **Conclusion.** Excellent performance of the stent from technical point of view and a midterm results in vessel patency, as well as the absence of need for TVR were achieved. Yet, life expectancy in this cohort group of patients demands longer follow up data to draw a definite sustained positive conclusion.

Key words:
femoral artery; arterial occlusive diseases; stents; prognosis.

Apstrakt

Uvod/Cilj. Okluzivne bolesti arterija donjih ekstremiteta tradicionalno su do sada najbolje lečene *bypass* hirurškom intervencijom, ali mi želimo da pronađemo minimalno invazivni pristup koji bi bio barem toliko dobar kao konvencionalna hirurgija, a možda i bolji. Cilj ovog rada bila je evaluacija SMART *Control* stentova (Cordis, J&J) kod *Trans Atlantic Society Consensus* (TASC) B i TASC C femoropoplitealnih lezija tokom jednogodišnjeg praćenja. **Metode.** Ovom retrospektivom nerandomizovanom studijom obuhvaćeno je četrdeset arterija kod uzastopnih 40 bolesnika kod kojih su postavljeni SMART *Control* stentovi. Primarna prolaznost, 12 meseci posle, verifikovana dupleks ultrazvukom i *Ancle Brachial Index*-om (ABI), kao i odsustvo potrebe za revaskularizacijom ciljne arterije (*Target Vessel Revascularisation* – TVR) bili su primarni konačni ciljevi. **Rezultati.** Primarni tehnički uspeh procedure ugradnje stentova bio je 100%. Srednja ABI vrednost bila je preproceduralno 0,50, postproceduralno 0,83, posle jednog meseca 0,86, posle šest meseci 0,84, a posle jedne godine 0,78. Posle jedne godine 39 stentova bilo je prolazno (97,5%). **Zaključak.** Analiza je pokazala odlično ponašanje stenta sa tehničkog gledišta, odlične srednjoročne rezultate prolaznosti arterija, kao i odsustvo potrebe za TVR. Ipak, očekivani životni vek kod ove populacije bolesnika zahteva duže praćenje u cilju donošenja stabilnih pozitivnih zaključaka.

Ključne reči:
a. femoralis; arterije, okluzione bolesti; stentovi; prognoza.

Introduction

Well known factors influencing difficulties in any treatment of femoropopliteal arterial segment include different external and internal forces: proximity of major flexing points, large muscle masses around it, lack of high diastolic flow, etc. Apart from relatively recent phylogenetic origin, there are almost no collateral vessels along the entire course of the artery. From the very beginning of the endovascular approach to this territory, there were great many controversies about the issue of their true clinical benefit. Only recently, new papers on clinical trials about nitinol selfexpandable stents in femoropopliteal region really showed a substantial improvement in patency rates.

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The aim of this study was to evaluate the safety, efficacy and performance of nitinol slotted tube SMART Control stents (Cordis, J&J) in patients presented with Trans Atlantic Society Consensus (TASC) type B and type C distal femoral and proximal popliteal artery lesions during one-year follow-up [1].

Methods

A retrospective nonrandomized analysis included the collected data from 40 arteries in the consecutive 40 patients. Primary patency at 12 months verified with Duplex Ultrasound and freedom from Target Vessel Revascularization (TVR) were final goals and primary endpoints. Acute Brachial Index (ABI) was measured before and immediately after the procedure, at 30 days, six months and one year. A drop of at least 0.15 in ABI was considered a secondary endpoint. A peak systolic velocity (PSV) of 150 cm/s or less was considered a success. A raise in PSV above 230 cm/s was indicated as a hemodynamically significant (> 50%) restenosis and also a secondary endpoint.

Antegrade approach with 6Fr sheath was performed in 27 of the patients. Crossover access was performed in 13 patients utilizing a long 7Fr Vista Brite (Cordis J&J) 45 cm arterial sheath. Lesions were approached with either soft or stiff Terumo hydrophilic 0.035" angled guide wire supported with straight or multipurpose 5Fr diagnostic catheter, and if not long (260 cm) one Terumo guide wire was available at the moment, after passing the lesion, 150 cm long one would be changed for an exchangeable 260 cm (Amplatz superstiff) guide wire. All the lesions were predilated with balloon dilatation catheters of 4 mm or 5 mm. Intentional subintimal recanalisation was not included in the study. Every patient received Heparin 100 IU per kg of body weight before the moment of the lesion traversing. Activated Clotting Time (ACT) was routinely monitored. The preferred ACT value to be reached was 250 sec. If postdilatational angiogram revealed less than 30% residual stenosis, without angiographic signs of dissection, those lesions were left alone and no stent implantation was considered an option. Every other outcome precluded stenting with SMART Control stent (Cordis J&J). Crossover placement of the stent through the sheath seemed to pass with no troubles in all the cases nevertheless the acuteness of the aortic bifurcation angle. Occasionally slight pullback of the sheath was necessary to straighten and pass through the kink on the sheath in the case of acute aortic bifurcation angle. Early sheath removal was always performed with either manual compression hemostasis (5 patients), Angio Seal (St Jude) (17 patients), or STARCLOSE (ABBOTT) (18 patients) closure device. Stent visibility was fair. Manouverability and handling of the stent in the lesion was good. Postdilatation was performed in all the cases with a balloon matched to the artery diameter or max 10% larger and only within the stent struts. Every patient was prepared with tienopiridine derivate, clopidogrel or ticlopidin, continued for three months and aspirin 100 mg indefinitely.

Categorical variables are expressed as the number and percentage of the patients. Continuous variables are presented as an average ± SD, if appropriate. Stent patency rates were calculated on the basis of color-coded duplex sonography findings using Kaplan-Meyer survival analysis.

Results

There were 40 patients among who 31 were males and 9 females, mean age of who was 64.7 years ranging from 42 to 77 years. Considering risk factor distribution, 14 (35%) patients were diabetics, 30 (75%) had hyperlipidemia, 24 (60%) were hypertensive, and all of them were smokers, 35 (87.5%) active, and 5 (12.5%) former ones (table 1).

Table 1
Demographic data of 40 patients before stent implantation

Characteristics of patients	n (%)
Total number	40 (100)
Sex	
male	31 (77.5)
female	9 (22.5)
Patients with	
diabetes mellitus	14 (35)
lipid disorder	30 (75)
hypertension	24 (60)
Smokers	
active	35 (87.5)
former	5 (12.5)
Age (yrs), mean (range)	64.7 (42–77)

According to Rutherford et al.[2], class II (moderate claudications) had 2 patients (5%), class III (severe claudications) had 26 (65%), class IV (ischemic rest pain) had 11 (27.5%) and class V (minor tissue loss) 1 patient (2.5%) (table 2).

Table 2
Rutherford class patients distribution

Rutherford class	n (%)
Class II (moderate IC*)	2 (5)
Class III (severe IC*)	26 (65)
Class IV (rest pain)	11 (27.5)
Class V (minor tissue loss)	1 (2.5)

* Intermittent claudications

Lesion severity distribution was as follows: Trans Atlantic Society Consessus (TASC) B lesion type in 14 (35%) patients, and TASC C lesion type in 26 (65%) patients, while 27 (67.5%) lesions were total occluded.

Full lesion length coverage with stent struts was achieved in 38 (95%) arteries. The remaining were longer, more or less diffusely diseased arteries.

Lesion mean length was 80.65 ± 14.35 mm with mean arterial diametar of 5.45 ± 0.3 mm. Mean stent length was 90.5 ± 11.97 mm. The number of patent crural runoff vessels was 1.625 ± 0.42 mm (table 3).

Only the arteries stented with single stent were enrolled in the study. All stents were 7 mm (9) or 8 mm (31) in diameter. Average stent length was 90.5 ± 11.97 mm ranging

Table 3

Lesion morphology issues in 40 patients

Lesion morphology	Values
TASC* B [n(%)]	14 (35%)
TASC* C [n(%)]	26 (65%)
Occlusion [n(%)]	27 (67.5%)
Full stent coverage [n(%)]	38 (95%)
Lesion length (mm) ($\bar{x} \pm SD$)	80.65 ± 14.35
Artery diameter (mm) ($\bar{x} \pm SD$)	5.83 ± 0.96
Number of patient runoff vessels ($\bar{x} \pm SD$)	1.625 ± 0.42

* TASC – Trans Atlantic Society Consensus

from 80–100 mm. Average stent diameter was 7.7 ± 0.42 mm. All vessels were stented after suboptimal Percutaneous transluminal angioplasty (PTA) result (residual stenosis > 30% or dissected wall) (figures 1 and 2).

locities (PSV) in popliteal artery were 150 cm/sec and 170 cm/sec respectively, which drove us to the crural disease progression as a cause of ABI failure. The worst clinical outcome appeared in the patient who could hardly

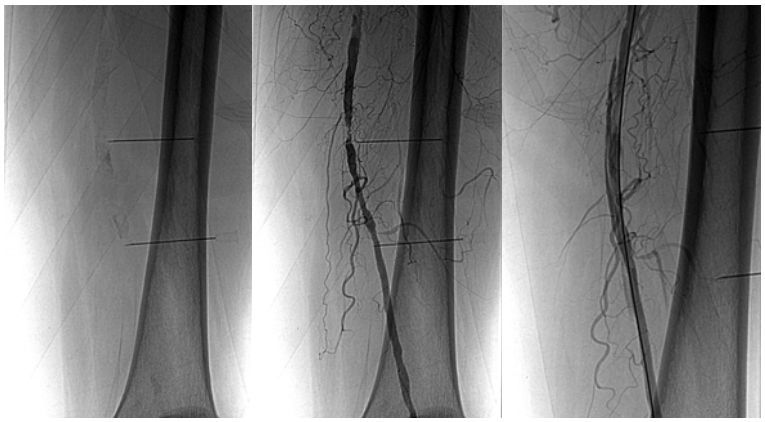

Fig. 1 – Calcified, occlusive, dissected lesion after percutaneous transluminal angioplasty

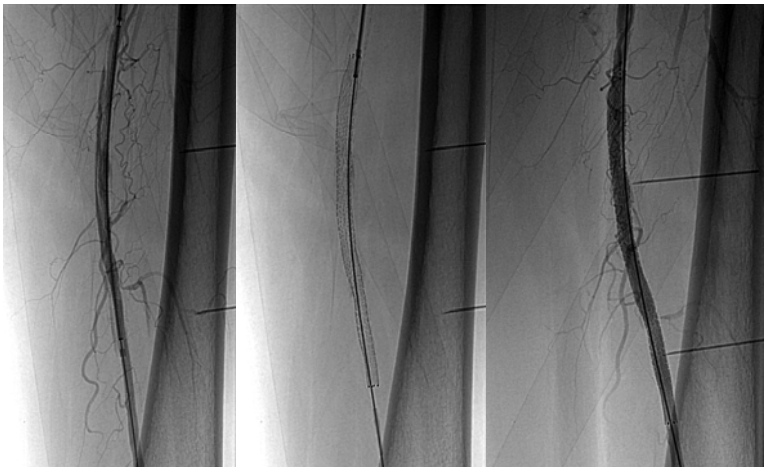

Fig. 2 – Calcified, occlusive, dissection lession after percutaneous transluminal angioplasty (healed with stent implantation)

Mean ABI value raised from preprocedural 0.50 to postprocedural 0.83, and 0.86, 0.84 and 0.78 at one, six and twelve months respectively (Table 4). Only two patients presented with drop in ABI of more than 0.15 (0.64 and 0.59 respectively) after six months but without signs of major clinical deterioration. Also, routine duplex controlled examinations revealed that their peak systolic velocities (PSV) in popliteal artery were 150 cm/sec and 170 cm/sec respectively, which drove us to the crural disease progression as a cause of ABI failure. The worst clinical outcome appeared in the patient who could hardly walk but the control revealed ABI of 0.92. The reason of this complication was aggravated hiparthrosis in the ipsilateral leg.

At one year check up one stent was unpatent in a diabetic patient with heavily compromised crural runoff (ABI = 0.38). All other stents did not show duplex signs of significant PSV rise.

Table 4
Acute brachial index (ABI) during follow-up period

Time after stenting procedure	ABI value (mean)
Preprocedural	0.50
Postprodedural	0.83
30 days	0.88
6 months	0.84
12 months	0.78

One procedural complication was groin hematoma, which required surgical evacuation due to inadequate hemostasis after crossover approach (manual hemostasis) but fortunately did not jeopardize the ipsilateral extremity.

Discussion

Literature data inconsistency was historically predominant feature of endovascular surgery in femoropopliteal region. Until recently, actually until newer generation of selfexpandable nitinol stents showed up in the field, there was strong hesitation in vascular surgical community when referring this cohort of patient population to endovascular repair was an issue.

Mewissen [3] in his very important study evaluated safety and efficacy of selfexpanding SMART nitinol stents in patients with chronic limb ischemia (CLI) demonstrating type B or C TASC lesions in the femoropopliteal (FP) arterial segment. In the series of 137 lower limbs with chronic limb ischemia, secondary to TASC A (n = 12) or TASC B, C (n = 125) lesions in the femoropopliteal artery were treated with Cordis SMART selfexpanding nitinol stents. The mean lesion length was 12.2 cm. The technical success was 98%. Within the follow-up period (mean, 302 days), 24 limbs were diagnosed with hemodynamic stent failure. The primary stent patency rates were 92%, 76%, 66%, and 60% at 6, 12, 18, and 24-months, respectively. These results truly represent a breakthrough in SFA revascularization strategies.

Lugmayr et al. [4] evaluated effectiveness of nitinol stents (Symphony, Boston Scientific) in patients with lesions in the superfitial femoral and popliteal arteries assessing midterm results in 54 extremities in 44 patients for treatment of short, less than 6 cm, complex stenoses (n = 32) and occlusions (n = 22). The mean duration of follow-up was 27 months. The primary 1 year patency rate was 87%, and 1 year secondary patency rate was 91%. The primary 3-year patency rate was 76%, and the secondary patency rate was 87%. The study was very nicely conducted and long term followed up, but the issue was only a portion of real life problems within territory of SFA. The point is that not a vast majority of patients are presented with such a short diseased artery segments. This could also be the drawback of our study, although our lesions were slightly longer.

It seems that concern about eventual stent fractures could not be justified in the scenario of relatively shorter lesions stenting, less than 10 cm, requiring single stent mostly [5]. Still, our study did not definitely last long enough to test this hypothesis. We will test all the stents for fractures during further follow-up period.

Systematic stenting versus selective use of Palmaz stents was not approved as presented in the paper by Becquemin et al. [6], though some other authors challenge this statement within the nitinol stents [7, 8]. Our opinion is that we are closer to the selective stenting until much more data show clear benefit, taking into account a high money value for nitinol stents, as well. SIROCCO I & II trials caused slight disappointment in the world of endovascular surgery. These trials could not transfer brilliant outcomes of DES from coronary teritory, but at the same time they expressed excellent performance of nitinol BMS indeed [9]. This drives us to the fact that SFA and especially its biomechanics itself is actually the limiting factor much more than simple extrapolating the restenosis process from other vascular territories, e.g. coronary.

What about diffuse disease or very long lesions, TASC D? Well, we ought to be honest and accept the fact that apart from eventual possibility to perform endovascular treatment of these lesions, the reasonable approach is still surgical one, although some advocate that approach to any kind of SFA disease [10]. Especially in the lesions requiring multiple long stents [11], we must count on stent fractures and, moreover, extremely modulated biomechanics along the course of the whole artery, particularly true for diabetic population according to the paper of Sabeti et al [11, 12].

One could postulate that we should not comment on the issue of long lesions, and that might be true, but in our institution, vascular surgeons still avoid endovascular approach to long SFA lesions.

Our results are encouraging and correlate with other most recently cited papers [13]. Considering the approach, either antegrade or contralateral crossover have their pros and cons. It is our opinion that regular utilization of closure devices in these procedures should be mandatory in which case the antegrade approach should be the easier, less cumbersome and safer, at least due to lower ionizing radiation for the operator, and generally more acceptable.

Another issue in our series is important. A vast majority of arteries accepted stents of 8 mm diameter. For most of them, it was between 20 and 25% or even 30% oversizing the artery lumen according to QVA (Quantitative Vessel Analisys). Although some patients claimed certain discomfort during the first month after the procedure, very few showed any clinical problem in patency yet. Still, we will test this in a 24-month control having in mind the concern of some authors that this could possibly lead to stent fracture due to chronic strain and fatigue of the material [14, 15].

There are, of course, some drawbacks of this study. First, the lack of randomization we are quite aware of. Second handicap is the one of ABI at rest, particularly in diabetic patients, but it seemed easy for routine orientation control, followed by duplex examination. The fact that the vast majority of stents are patent according to duplex PSV of 230 cm/s does not mean that at least some of them has already been restenosed, yet, not significantly. Since we did not perform any kind of morphological control (angiography or computerized tomography, e.g.) we do not really know that proportion. Therefore, it seems to be another drawback.

Conclusion

We achieved 100% patency rate in the intermediate 6-month follow up and 97.5% in a 12-month follow-up. More important is what will be the result in 24- or maybe 48- or 60-month follow-up. Further clinical studies of SFA stenting will give us more information like what kind of stents, what design, what force resistance should they express in different parts of adductor channel for instance, etc. At the same time, additional molecular, biochemical, and pathological studies are needed in order to understand the particularity of restenosis in SFA. Also, considering life expectancy in this population, we need sustained high patency rates for a longer periods.

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Primena mehaničke ventilacije kod pedijatrijskih bolesnika

Use of mechanical ventilation in pediatric patients

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Apstrakt

Uvod/Cilj. Osnovni ciljevi mehaničke ventilacije (MV) u pedijatriji podrazumevaju doprinos potpunom izlečenju akutnog poremećaja ili uspostavljanju stabilnosti prethodno dugotrajnije izmenjenog zdravstvenog stanja. Mehanička ventilacija danas se primenjuje kod 16–46% bolesnika koji se leče u odeljenjima pedijatrijske intenzivne nege. Cilj rada bio je uvid u zastupljenost oboljenja i patoloških stanja i ishod MV u zavisnosti od prethodnog zdravstvenog stanja pedijatrijskih bolesnika. **Metode.** Retrospektivnom analizom bilo je obuhvaćeno 476 pedijatrijskih bolesnika kod kojih je posle prvog meseca života bila neophodna primena MV. Svi bolesnici bili su svrstani u dve grupe: grupa A koju je činilo 157 prethodno zdrave dece, dok je grupu H činilo 319 dece kod kojih je MV bila neophodna usled akutnog pogoršanja postojeće hronične bolesti. U obe grupe dominirali su bolesnici iz mlađih uzrasnih kategorija, a najčešći razlog za primenu MV bila su akutna i hronična neurološka oboljenja. **Rezultati.** Od ukupnog broja pedijatrijskih bolesnika umrlo je 178 ili 37,4% bolesnika, od čega je u grupi A umrlo 17 ili 10,9% bolesnika, a u grupi H 161 ili 50,5% dece ($p < 0,01$; RR 4,85; CI 3,1–7,6). Ukupno trajanje MV u obe grupe iznosilo je 7 525 dana i to 1 345 (15%) dana u grupi A, odnosno 4 567 (85%) dana u grupi H. U grupi A MV prosečno je trajala 7,48 dana (± 9,23), a grupi H 21,8 dana (± 57,96) sa statistički visoko značajnom razlikom ($p < 0,001$). **Zaključak.** Mehanička ventilacija značajno doprinosi povoljnom ishodu pedijatrijskih bolesnika sa različitim akutno nastalim patološkim stanjima i oboljenjima. Jasna predominacija hronično obolele dece koja su zahtevala MV usled akutnog pogoršanja bolesti sa značajnim angažovanjem svih resursa otvara nove, složene probleme iz domena organizacije pedijatrijske intenzivne nege i terapije.

Ključne reči:
disanje, mehaničko; pedijatrija; akutna bolest; hronična bolest; preživljavanje.

Abstract

Bacground/Aim. Major aims of mechanical ventilation (MV) in pediatrics mean the contribution to complete recovery of acute disorder or to establishing stability of previously long-term changed health condition. MV is used today in 16–46% of patients treated in pediatric intensive care units. The aim of this paper was to get insight into the presence of the disease and pathologic conditions and outcome of MV regarding previous health condition of pediatric patients. **Methods.** This retrospective study included 476 pediatric patients (beyond neonatal age) who underwent mechanical ventilation (MV). On the basis of previous health status the patients were classified in two groups: the group A consisted of 157 children with no previous chronic disease leading to MV and the group H comprised of 319 children who received MV due to worsening of previous chronic disease. **Results.** In both groups of pediatric patients there was significant predominance of younger age patients. Acute and chronic neurological disorders were the most frequent conditions requiring use of MV. Out of a total number (476) of the patients, 178 patients (37.4%) died. In the group A 17 patients (10.9%) died, while in the group H mortality rate was significantly higher (161 or 50.5% patients died; $p < 0.01$; RR 4.85; CI 3.1–7.6). Total duration time of MV in all the patients was 7 525 days, with 1 345 days (15%) accounted for the group A and 4 567 days (85%) for the group H. Mean MV duration was 7.48 (± 9.23) days for the patients in the group A which is significantly shorter in comparison to mean 21.8 (± 57.96) days for the group H patients ($p < 0.001$). **Conclusion.** These results point out significant contribution of MV to better outcome in pediatric patients with different acute disorders. Clear dominance of chronically ill children requiring mechanical ventilation due to acute worsening of their condition implies new complexity of problems regarding organization of pediatric intensive care and treatment.

Key words:
respiration, artifical; pediatrics; acute disease; chronic disease; survival.

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Uvod

Začeci šire primene produžene veštačke potpore kod pedijatrijskih bolesnika datiraju iz prve polovine prošlog veka kada je mehanička ventilacija (MV) bila značajan elemenat lečenja dece sa poliomijelitisom [1]. Potom, nagli razvoj neonatologije tokom 60-tih godina XX veka u znatnoj meri zasnivao se na iznalaženju i usavršavanju novih metoda MV. Zahvaljujući tome, kao i sticanju iskustva i povećanoj raspoloživosti savremene opreme, MV se danas primenjuje kod 16–46% bolesnika koji se leče u odeljenjima pedijatrijske intenzivne nege [2, 3]. Uprkos tome, za razliku od brojnih radova posvećenih različitim aspektima primene MV kod novorođene dece, medicinska literatura se u znatno manjoj meri bavi problematikom MV kada se radi o pedijatrijskim bolesnicima iz starijih grupa [3]. U analizi razloga za ovu pojavu svakako treba istaći poteškoće koje iskrsavaju usled velikog uzrasnog raspona koji obuhvata pedijatrijska populacija. Osim toga, usled, neretko, istovremenog zahvatanja različitih organskih sistema, često nije moguće izdvojiti vodeći uzrok poremećaja koji su neposredni razlog za primenu MV. Kada se svemu dodaju i sve slobodnije indikacije za MV u pedijatriji, moguće je objasniti zašto je samo nešto više od trećine pedijatrijskih bolesnika „pogodno“ za uključivanje u prospektivna klinička ispitivanja posvećena MV [2]. Sve navedeno ima za rezultat da se pojedinačne retrospektivne studije uglavnom bave komplikacijama MV, bez selektivnosti u odnosu na razloge za primenu i ishod MV [1]. Posebno treba naglasiti da, prema našem uvidu u postojeće baze podataka, u domaćoj medicinskoj literaturi nedostaju radovi posvećeni problematici MV kod pedijatrijskih bolesnika.

Polazeći od prethodnih razmatranja, naša studija je imala za glavni cilj uvid u zastupljenost oboljenja i patoloških stanja i ishod mehaničke ventilacije kod pedijatrijskih bolesnika zavisno od prethodnog zdravstvenog stanja. Takođe, u cilju sagledavanja iskorišćenosti opreme i opterećenosti osoblja uporedno je analizirano trajanje MV u slučaju kada je potpora disanja bila potrebna kod prethodno zdrave dece, kao i u slučaju akutnih pogoršanja postojećeg hroničnog oboljenja.

Cilj rada bio je uvid u zastupljenost oboljenja i patoloških stanja i ishod MV u zavisnosti od prethodnog zdravstvenog stanja pedijatrijskih bolesnika.

Metode

Retrospektivna analiza obuhvatila je bolesnike smeštene u Odeljenju pedijatrijske intenzivne nege i terapije (OPINT) u periodu od 1.1.2000. do 31.12.2006. godine kod kojih je bila neophodna primena MV. U studiju bili su uključeni bolesnici uzrasta > 30 dana, a analiza nije obuhvatila decu sa primarnim hirurškim oboljenjima, kao ni bolesnike kod kojih su pri prijemu bili prisutni znaci moždane smrti. Iz analize bili su isključeni i bolesnici kod kojih je pri prijemu bila neophodna kardiopulmonalna reanimacija, a smrtni ishod je nastupio unutar 24 h od prijema.

Naše odeljenje je organizaciona i prostorna celina tercijarne pedijatrijske ustanove sa ukupno 12 mesta za bolesnike van neonatalnog uzrasta. Za MV koriste se respiratori Babylog, tipovi 1,2 i 8 000 plus, Savina i UV 2 (proizvođač firma Dräger), kao i Servo ventilatori tipovi 300, 900 i 900 C (proizvođač firma Siemens).

Apsolutna indikacija za započinjanje MV kod naših bolesnika bila je respiratorna insuficijencija (hipoksemija sa paO_2 < 6,7 kPa mmHg; hiposaturacija hemoglobina kiseonikom – SaO_2 < 90%; hiperkapnija sa $PaCO_2$ > 6,7 kPa). Osim toga, MV bila je primenjena i u slučaju opstrukcije gornjih disajnih puteva, kao i kod bolesnika sa znacima septičkog šoka i uz unos velike količine kristaloidnih i koloidnih rastvora.

U zavisnosti od prethodnog zdravstvenog stanja bolesnici su bili svrstani u dve grupe: grupa A koju su činila prethodno zdrava deca i grupa H koja je obuhvatila decu sa postojećim hroničnim oboljenjem.

Dobijeni podaci obrađeni su metodama deskriptivne i analitičke statistike uz primenu softverskog paketa SPSS v. 12.0.

Rezultati

U posmatranom sedmogodišnjem periodu u OPINT bilo je lečeno ukupno 5 857 bolesnika od kojih je 3 875 (66,2%) bilo starije od 30 dana. U ovoj grupi MV primenjena je kod 604 (15,6%) bolesnika od kojih je kriterijume za analizu ispunjavalo 476 bolesnika. Bez prethodnih oboljenja bilo je 157 ili 32,98% dece (grupa A), dok je kod 319 ili 67,01% prethodno postojalo hronično oboljenje različite prirode (grupa H). U obe grupe postojala je prevaga bolesnika muškog pola bez značajnosti razlike između grupa (tabela 1).

Tabela 1

Raspodela bolesnika po polu

Pol	Grupa A	Grupa H
	n (%)	n (%)
Muški	94 (59,9)	185 (58,0)
Ženski	63 (40,1)	134 (42,0)
Ukupno	157 (100,0)	319 (100,0)

* χ^2–0,153

Nevezano za osnovni razlog, MV najčešće je primenjivana kod bolesnika u prvoj godini života. U svakoj sledećoj uzrasnoj kategoriji zastupljenost bolesnika na MV bila je manja, a sve međugrupne razlike imale su visoku statističku značajnost (λ^2 166,8; $p < 0,001$) (tabela 2).

Tabela 2

Raspodela bolesnika po uzrastu

Uzrast bolesnika	Bolesnici	*p*
	n (%)	
30 dana–12 meseci	221 (46,4)	
2–6 godina	142 (29,8)	< 0,001
7–14 godina	80 (16,8)	
> 14 godina	33 (6,9)	
Ukupno	476 (100,0)	

Prema podacima navedenim u tabeli 3, u grupi A primena MV najčešće je bila neophodna ukoliko se radilo o akutnim oboljenjima centralnog ili perifernog nervnog sistema, respiratornim poremećajima i septičkim šokom, a razlika u odnosu na učestalost ostalih akutno nastalih poremećaja (trovanja, oboljenja kardiovaskularnog sistema – KVS) imala je visoku statističku značajnost ($\chi^2 = 110,9$; $p < 0,01$). Usled

primarnih neuroloških poremećaja umrlo je tri bolesnika sa meningoencefalitisom i jedan bolesnik sa poliradikuloneuritisom. U grupi oboljenja respiratornog sistema umrlo je jedno dete sa pneumonijom. Septički šok je izazvao mortalitet od 11,1%, a kod bolesnika sa akutnim trovanjima smrtni ishod nastupio je u dva slučaja (po jedno dete sa trovanjem natrijum valproatom i pečurkama). Usled miokarditisa (grupa oboljenja KVS) umrlo je jedno dete. U grupu označenu kao „ostalo" svrstana su po dva bolesnika sa kasnom hemoragijskom bolešću novorođenčeta i hemolitičko-uremijskim sindromom, kao i pojedinačni bolesnici sa teškim oblicima dehidracije, fulminantnim hepatitisom (umrlo), akutnim nediferentovanim poremećajem metabolizma (umrlo), zadesnog utopljenja (preživelo), pokušaja suicida vešanjem, kao i troje dece kod kojih uzrok respiratorne insuficijencije zbog brze progresije do smrtnog ishoda nije utvrđen (tabela 3).

U tabeli 4 navedena su najčešća hronična oboljenja i patološka stanja kod kojih je primenjena MV (podgrupa H1). U odnosu na grupnu zastupljenost postoji statistički visoko

Tabela 3

Akutna oboljenja kod kojih je bila neophodna mehanička ventilacija (grupaA)

Osnovno oboljenje	Broj bolesnika n (%)	Umrlo n (%)
Nervni sistem	46 (29,1)	4 (8,7)
meningitis-encefalitis	28	
poliradikuloneuritis	8	
ostalo	4	
Status epilepticus (prvi napad)	6	
Respiratorni sistem	41 (26,0)	1 (2,4)
pneumonija	20	
epiglotitis	10	
bronhiolitis	4	
apneja	3	
subglotisni laringitis	2	
ostalo	2	
Septički šok	34 (22,5)	4 (11,1)
Trovanja	11 (7,0)	2 (18,2)
lekovi*	4	
organofosfati	3	
kiseline/baze/rastvarači	2	
pečurke	2	
Kardiovaskularni sistem	6 (3,4)	1 (16,7)
miokarditis	4	
poremećaji ritma	2	
Ostalo	19 (12,0)	5 (26,3)
Ukupno	157 (100,0)	17 (10,9)

*opijati 2, antiepileptici 2

Tabela 4

Najčešća hronična oboljenja koja su zahtevala mehaničku ventilaciju (podgrupa H 1)

Osnovno oboljenje	Broj bolesnika n (%)	Umrlo n (%)
Neurološko	98 (35,0)	44 (44,9)
neuromišićne bolesti*†	41 (41,8)	20 (48,7)
psihomotorna retardacija	32 (32,6)	15 (46,9)
status epilepticus (lečena epilepsija)	22 (22,5)	7 (31,8)
SSPE‡	3 (3,1)	2 (66,7)
Kardiovaskularni sistem	79 (28,2)	33 (41,8)
urođene srčane mane	69 (87,3)	28 (40,6)
kardiomiopatija	10 (12,7)	5 (50,0)
Hematoonkološko	51 (18,2)	31 (60,8)
tumori	35 (68,6)	22 (62,9)
akutne leukoze	12 (23,6)	8 (66,7)
hemofilija	2 (3,9)	–
Respiratorni sistem	40 (14,3)	25 (62,5)
cistična fibroza	21 (52,5)	17 (80,1)
bronhopulmonalna displazija	8 (20,0)	2 (25,0)
pneumopatija-nediferencirana	5 (12,5)	5 (100,0)
astmatski status	4 (10,0)	–
laringomalacija	2 (5,0)	–
Imunodeficijencije	12 (4,3)	6 (50,0)
Ukupno	280 (100,0)	139 (49,6)

*broj bolesnika: spinalna mišićna atrofija – 16; različite urođene miopatije – 21; polineuropatija – 4; †6 bolesnika na hroničnoj kućnoj mahaničkoj ventilaciji; ‡subakutni sklerozirajući panencefalitis

značajna dominacija hroničnih neuroloških, kardiovaskularnih i hemato-onkoloških oboljenja ($\chi^2 = 201,5$; $p < 0,01$). Pored ukupne, tabelarni prikaz sadrži zastupljenost i smrtnost pojedinih oboljenja unutar svake grupe (tabela 4).

Tabela 5 prikazuje ređa i neklasifikovana hronična oboljenja kod kojih je bila neophodna primena MV.

Diskusija

Osnovni ciljevi MV i u pedijatriji podrazumevaju doprinos potpunom izlečenju akutnog poremećaja ili uspostavljanju stabilnosti prethodno dugotrajnije izmenjenog zdravstvenog stanja [3–5]. Shodno tome, naše bolesnike svrstali smo u

Tabela 5

Ređa i neklasifikovana hronična oboljenja koja su zahtevala mehaničku entilaciju (podgrupa H 2)

Oboljenje	Broj bolesnika (n)	Umrlo (n)
Multiple anomalije	11	5 (45,4%)
Nefrotski sindrom	3	3
Hiperlaktatemija	3	0
Obesitas-hipoventilacija	2*	0
Dijabetička ketoacidoza	2	0
Hemosideroza	2	2
Hronični juvenilni artritis	2	1
Mitohondropatije	2	2
Policistični bubrezi	1	1
Hronična bubrežna insuficijencija	1†	
Lowe sindrom	1	1
Down sindrom	1	1
Pierre-Robin sindrom	1	0
Morbus Gaucher	1	1
Deficit alfa-1 antitripsina	1	1
Metabolička alkaloza (neodređena)	1	0
Rahitis	1	1
Glutarna acidurija	1	1
Hepatička insuficijencija (ciroza)	1	1
Morbus Randy-Osler-Weber	1	0
Ukupno	39	21 (53,8%)

*na hroničnoj kućnoj mehaničkoj ventilaciji; †prevedeno u drugu ustanovu

Od 476 bolesnika umrlo je 178 ili 37,4%. Stopa mortaliteta u grupi A iznosila je 10,9% (umrlo je 17 od 157 bolesnika), a u grupi H (zbirno podgrupa H1 i H2) stopa mortaliteta iznosila je 50,5% (umrlo je 161 od 319 bolesnika). Razlika stope mortaliteta između grupe A i grupe H imala je visoku statističku značajnost ($\chi^2 = 76,97$; $p < 0,01$; RR 4,85; CI 3,1–7,6).

Ukupno trajanje MV iznosilo je 7 804 dana, od čega 1 173 dana ili 15% vremena za bolesnike iz grupe A, a 6 634 dana ili 85% od ukupnog vremena za bolesnike grupe H. U grupi A MV je najduže trajala 61 dan, dok je u grupi H gornja granica trajanja MV iznosila 730 dana sa statistički visoko značajnom razlikom (tabela 6).

dve osnovne grupe. Prvu su činila prethodno zdrava deca sa akutno nastalim poremećajima, a drugu grupu deca kod koje je prethodno postojalo oboljenje ili patološko stanje hronične prirode. Inače, slično rezultatima drugih studija koje su se bavile ovom problematikom, u obe grupe naših bolesnika postojala je prevaga bolesnika muškog pola, a MV su najčešće zahtevala deca u prvoj godini života [1–3].

Povećana dostupnost postupaka iz domena pedijatrijske intenzivne terapije i kod nas je za rezultat imala porast broja bolesnika kod kojih se primenjuje produžena veštačka potpora disanja. Primera radi, u 1982. godini u našem odeljenju MV je primenjivana kod 5% bolesnika, dok se aktuelni rezultati nalaze u rasponu prosečne primene MV i u sredinama

Tabela 6

Trajanje mehaničke ventilacije (MV)

Oznaka grupe	Trajanje (dani)			
	Raspon	x̄±SD	M	*p*
A	1–61	7,48 ± 9,23	6,0	< 0,01*
H	1–730	21,8 ± 57,61	8,0	

*Mann-Whitney *U* test

A – prethodno zdrava deca sa akutnim oboljenjem

H – deca sa postojećim hroničnim oboljenjem

sa razvijenijim sistemima zdravstvene zaštite [1–4]. Ta pojava se može objasniti i naporima da bezbednost bolesnika i očekivani povoljni efekti na ishod prevaziđu srazmerno visok jatrogeni potencijal MV [3, 4]. Osim toga, domaći autori za primenu MV preporučuju i elektivne kriterijume pored teške respiratorne nedovoljnosti kao apsolutne indikacije. Tada je MV deo složene celine neurointenzivne i terapije septičkog šoka ili se započinje i na osnovu ukupne procene stepena rizika nastanka disajnog zamora [6]. Određenu osobenost pedijatrijske patologije predstavljaju različiti, najčešće infektivni, uzroci opstrukcije gornjih disajnih puteva kada je MV deo obezbeđenja „sigurnog disajnog puta“ [1, 2].

Slobodnijom primenom MV u slučaju akutnog oboljevanja prethodno zdrave dece, mogu se objasniti visoka stopa preživljavanja od 89,1% u celoj grupi, kao i dominantna zastupljenost neuroloških i respiratornih poremećaja. Kod tih bolesnika srazmerno često primenjivana je MV iz elektivnih indikacija u slučaju težeg poremećaja svesti (meningitis-meningoencefalitis), preteće respiratorne nedovoljnosti (poliradikuloneuritis), odnosno u cilju obezbeđenja i održavanja prolaznosti disajnih puteva. Na trećem mestu su bolesnici sa septičkim šokom najčešće u sklopu sistemske meningokokne infekcije. Kod ove dece, takođe, postignuta je prihvatljiva redukcija stope mortaliteta na 11,1%. Te rezultate smo ranije objavili ističući značaj celovitog pristupa sa energičnom nadoknadom cirkulatornog volumena i istovremenim započinjanjem MV, invazivnim hemodinamskim monitoringom i inotropnom stimulacijom [7]. Značajnu osobenost septičkog šoka kod pedijatrijskih bolesnika predstavlja izražena hipovolemija čija korekcija neretko zahteva unos velikih količina kristaloidnih i/ili koloidnih rastvora u srazmerno kratkom vremenskom intervalu [8]. Ukoliko se, uprkos tome, održava cirkulatorna insuficijencija, postojeća nedovoljna oksigenacija tkiva umanjuje se dodatno i kritično usled povećanog utroška kiseonika od strane respiratorne muskulature. Istovremeno, povećana propustljivost kapilarne mreže i hipoalbuminemija mogu dovesti do plućnog edema. Shodno tome, u lečenju dece sa meningokokcemijom, kao standardni postupak preporučuje se elektivna endotrahejna intubacija i započinjanje MV uvek kada se inicijalnim intravenskim unosom tečnosti 40–60 ml/kg ne postigne korekcija šoka [8, 9]. I prema rezultatima drugih autora, očuvanje normalne gasne razmene MV, takođe, doprinosi povoljnom efektu savremene medikamentne terapije kod dece sa akutnim kardiovaskularnim poremećajima (miokarditis, akutni poremećaji ritma) [10, 11]. Osim toga, MV je integralni deo kompleksne simptomske terapije teških akutnih trovanja različite etiologije koja imaju znatnu zastupljenost u našem OPINT [12].

Značajan napredak u lečenju brojnih hroničnih pedijatrijskih oboljenja omogućio je kvalitetan život nemalom broju dece. Uprkos tome, kod ovih bolesnika postoji stalni rizik od akutnih pogoršanja sa potrebom hospitalizacije, a ponekada i intenzivne terapije sa primenom MV. Nedavno objavljeni rezultati opsežne studije pokazuju da više od 12% hospitalizovanih pedijatrijskih bolesnika u SAD ima hronični poremećaj zdravstvenog stanja. Osim toga, stalni porast broja dece koja zahtevaju arteficijelnu potporu i, u vezi s tim, tendencija povećanja bolničkih kapaciteta namenjenih intenzivnoj nezi i terapiji, neposredno se pripisuju činjenici da je čak kod 2/3 dece MV indikovana usled akutnog pogoršanja hronične bolesti [5]. Pored sličnosti u kvantitativnom odnosu akutnih i hroničnih bolesnika kod kojih je primenjena MV, naši rezultati su i po strukturi morbiditeta slični zapažanjima drugih autora da postoji značajna tendencija porasta zastupljenosti bolesnika sa hroničnim neurološkim, odnosno neuromišićnim, respiratornim i hematoonkološkim oboljenjima [3, 5].

Istraživanja koja su se bavila stavom pedijatrijskih intenzivista prema primeni MV kod hronično obolele dece, pokazuju naglašenu sklonost da se bez obzira na udaljenu prognozu osnovne bolesti, akutna pogoršanja moraju lečiti svim raspoloživim metodama intenzivne terapije [13]. U prilog ovakvom stavu govore i ohrabrujući rezultati primene MV kod bolesnika sa neuromišićnim oboljenjima [14]. Ne dovodeći u sumnju profesinalnu i etičku opravdanost navedenog pristupa, međutim, treba istaći da i naši rezultati o značajno dužem trajanju MV i nepovoljnijem ishodu kod dece sa hroničnim oboljenjima neminovno otvaraju pitanja opterećenja osoblja, kao i prostornih, odnosno mogućnosti vezanih za raspoloživu opremu [15]. Još pre dve decenije u Velikoj Britaniji uočen je problem dugotrajne MV sa mogućnošću „blokiranja“ respiratora i uskraćivanja prijema dece sa izglednijim povoljnim ishodom [16, 17]. U cilju razrešenja ovih složenih problema danas se predlažu dve međusobno komplementarne mere. Prva se odnosi na povećanje hospitalnog standarda ustanovljavanjem odeljenja tzv. intermedijarne nege u kojima bi se obezbedilo kontinuirano kliničko praćenje i vitalnih funkcija bolesnika koji neposredno ne zahtevaju najsloženije postupke iz domena intenzivne terapije [3]. Ideja je da se time u odeljenima intenzivne nege smanji broj „lakših“ bolesnika čime bi se povećale ukupne kadrovske, prostorne i mogućnosti optimalnog korišćenja raspoložive opreme za zbrinjavanje bolesnika sa najsloženijim poremećajima zdravstvenog stanja. Alternativna mogućnost odnosi se na organizovanje MV u kućnim uslovima [18]. I prema prvim sopstvenim iskustvima ovakav pristup namenjen je pre svega deci sa neuromišićnim oboljenjima, bronhopulmonalnom displazijom i obezitas-hipoventilacionom sindromom [3, 19, 20].

Retrospektivnost naše studije neminovno je nosila ograničenja vezana, pre svega, za poteškoće da se u potpunosti objektiviziraju poremećaji koji su bili neposredan razlog za primenu MV, kao i da se dosledno primene selekcioni kriterijumi za krajnju analizu. To je moglo da ima za posledicu uključivanje i bolesnika sa terminalnim poremećajima i, shodno tome, znatno veću smrtnost u grupi hroničnih bolesnika (50,5%) u odnosu na podatke koji se mogu naći u medicinskoj literaturi prema kojoj gornja granica smrtnosti u uporedivim kategorijama iznosi 25% [1–3, 5]. Pored navedenog, srazmerno mali broj bolesnika u pojedinim kategorijama unutar grupa A i H, nije dozvolio pouzdanu statističku analizu stope mortaliteta u zavisnosti od osnovnog uzroka akutnog, odnosno hroničnog mobiditeta.

Zaključak

Uprkos navedenim ograničenjima, na osnovu rezultata našeg istraživanja možemo zaključiti da je MV integralni deo

intenzivne terapije pedijatrijskih bolesnika sa značajnim doprinosom povoljnom ishodu različitih akutno nastalih patoloških stanja i oboljenja kod prethodno zdrave dece.

Prema našim rezultatima vodeći razlog za primenu MV kod pedijatrijskih bolesnika jesu akutna i hronična oboljenja centralnog i perifernog nervnog sistema.

Slično tendencijama u drugim sredinama, MV je značajno češće neophodna kod dece iz mlađih uzrasnih kategorija, uz sve izraženiju predominaciju pedijatrijskih bolesnika kod kojih MV indikuju akutna pogoršanja prethodno hronično izmenjenog zdravstvenog stanja. U tom slučaju beleže se znatno nepovoljniji rezultati lečenja uz nesrazmerno veći utrošak vremena i angažovanje osoblja i raspoložive opreme. Brojni problemi vezani za ove pojave mogu se ublažiti drugačijom organizacijom hospitalnog zbrinjavanja i korišćenjem mogućnosti primene MV u kućnim uslovima.

U cilju iznalaženja načina za efikasnije i kvalitetnije zbrinjavanje pedijatrijskih bolesnika koji uz ostale mere iz domena intenzivne terapije zahtevaju i primenu MV, neophodna su dalja klinička i epidemiološka istraživanja prospektivnog karaktera.

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Matrix metalloproteinases (MMP-1, -8, -13) in chronic periapical lesions

Metaloproteinaze matriksa (MMP-1, -8, -13) kod hroničnih periapeksnih oboljenja

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Abstract

Background/Aim. Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of degrading almost all extracellular matrix and basement membrane components in many destructive pathological processes, such as chronic inflammation and bone-destructive lesions. The aim of this study was to determinate the correlation between concentration of collagenases (MMP-1, -8, -13) in chronic periapical lesions and their dimension calculated with software predilection through X-ray. **Metods.** Chronic periapical tissues were collected by periapical surgery from 60 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lesions, 10 cysts). Ten normal pulps used as controls were obtained by extirpation of the pulp of impacted third molars after their surgery. For rapid analysis of MMP-1, -8, -13 collagenase activities in the examined material Chemicon Collagenase Activity Assay Kit were used. From the X-ray trough software predilection (Image Tool3 Program) of the volume of chronic periapical tissue, correlation between concentration of MMPs in the periapical lesions and their dimension was confirmed. **Results.** Different concentrations of collagenases (MMP-1, -8 and -13) in chronic periapical process from different inflammation types showed different activity of MMPs. The obtained results showed the highest values of collagenases concentration (MMP-1, -8, -13) in chronic diffuse lesions (5.39 ng/ml). Low values of concentration of MMPs accompanied less serious lesions, whereas chronical periapical lesions of large dimension had high concentration of MMPs, which was proportional to progression of the lesion and destruction of bone tissue. **Conclusions.** This study confirmed the destructive role of collagenases (MMP-1, -8 and -13) in inflammation process, which directly depends on the concentration of MMPs in pathologically changed tissue.

Key words:
periapical diseases; matrix metalloproteinases; collagen.

Apstrakt

Uvod/Cilj. Matriks metaloproteinaze (MMPs) su proteolitički enzimi koji razgrađuju većinu komponenata ekstraćelijskog matriksa i bazalne membrane u mnogim destruktivnim patološkim procesima, kao što su hronično zapaljenje i destruktivne lezije kostiju. Cilj rada bio je određivanje korelacije između koncentracije kolagenaza (MMP-1, -8, -13) u hroničnim periapeksnim lezijama i njihovih dimenzija izračunatih na osnovu softverske predlekcije rendgenskog snimka. **Metode.** Hronično periapeksno tkivo sakupljeno je oralnohirurškim intervencijama na 60 zuba sa klinički i radiografski verifikovanim raznorodnim hroničnim periapeksnim lezijama (20 granuloma, 20 difuznih periapeksnih lezija, 10 cista). Deset normalnih pulpi korišćeno je kao kontrolna grupa koja je dobijena ekstirpacijom pulpe trećeg molara nakon hirurške intervencije. Za brzu analizu aktivnosti MMP-1, -8, -13 kolagenaza u ispitivanom materijalu korišćen je *Chemicon Collagenase Activity Assay Kit*. Snimanjem X-zracima, sa prethodno utvrđenim parametrima (*Image Tool3* program) zapremine hroničnog periapeksnog tkiva, potvrđena je korelacija između koncentracije MMPs u periapeksnim lezijama i njihovih dimenzija. **Rezultati.** Kolagenaze MMP-1, -8, -13 dobijene iz različitih tipova zapaljenja u hroničnim periapeksnim procesima pokazale su različitu aktivnost. Najviše kolagenaza MMP-1, -8, -13 ustanovljene su u hroničnim difuznim lezijama (5,39 ng/ml). Niske koncentracije MMPs bile su udružene sa malim lezijama, dok su hronične periapeksne lezije velikih dimenzija imale visoke koncentracije MMPs, proporcionalne sa proširenošću lezija i stepenom destrukcije koštanog tkiva. **Zaključak.** Kolagenaze MMP-1, -8 i -13 u zapaljenskom procesu imaju destruktivnu ulogu koja direktno zavisi od njihove koncentracije u patološki promenjenom tkivu.

Ključne reči:
zub, periapeksne bolesti; matriks metaloproteinaze; kolagen.

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Introduction

Matrix metalloproteinases (MMPs) are a family of host-derived enzymes responsible for degradation of most extracellular matrix (ECM) proteins during organogenesis, growth and normal tissue turnover, wound healing, tooth morphogenesis and tooth eruption [1]. Expression and activity of MMPs in adult tissues are normally quite low, but increase significantly in many destructive pathological processes, such as chronic inflammation and bone-destructive lesions [2].

This group of 23 human enzymes is classified into collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs, mainly based on the substrate specificity and molecular structure [3].

Matrix metalloproteinases activity is controlled by changes in the delicate balance between the expression and synthesis of MMPs and their major endogenous inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) [4].

Based on structure and substrate specificity, MMPs are divided into five subgroups: collagenases, gelatinases/type IV collagenases, stromelysins (including matrilysin and metalloelastase), membrane-type MMPs, and others [5].

Collagenase-1 (MMP-1), collagenase-2 (MMP-8) and collagenase-3 (MMP-13) comprise a collagenase subfamily capable of initiating degradation of native fibrillar collagen types I, II, III, V and IX [6].

Collagenase-1 most effectively cleaves collagen type III. MMP-1 appears to be constitutively synthesized and secreted by fibroblasts and macrophages, and it is the most often associated collagenase with normal tissue remodeling. MMP-1 is currently shown to be produced by a variety of other cells such as osteoblasts and odontoclasts [7].

Collagenase-8 (MMP-8) is the most effective collagenase in initiating type I collagen degradation. Its main cellular source is polymorphonuclear leukocytes (PMNs), and the enzyme thus plays a key role in tissue destruction during inflammatory diseases [8].

Collagenase-13 (MMP-13) expression was originally documented in human breast cancer, and MMP-13 prefers type II collagen [9]. In normal physiology, MMP-13 is highly expressed in developing bone and cartilage [10]. Moreover, MMP-13 is expressed during many pathological conditions associated with excessive degradation of the extracellular matrix (ECM), such as osteoarthritic cartilage, oral mucosal epithelium during chronic inflammation and odontogenic keratocysts [11–13].

In normal conditions, the degradation and synthesis of ECM components is in balance, so that collagenases are expressed at very low levels, if at all, but their production and activation is rapidly induced whenever active tissue remodeling is required.

With respect to other literature findings that underline the role of collagenases (MMP-1, -8, -13) in chronic periapical process, the aims of this study were: quantitative measurements of tissue levels of collagenases (MMP-1, -8, -13) in chronic periapical process with enzyme method; determination of the dependence between collagenases (MMP-1, -8, -13) with the degree of tissue destruction of examination material (periapical tissue), as well as, character and differences between periapical lesions; and determination of the correlation between concentration of collagenases (MMP-1, -8, 13) in chronic periapical process and their dimension calculated with software predilection through X-ray.

Methods

This study included 50 patients, both male and female, investigated in the School for Dental Medicine (Clinic for Oral Surgery) in Skopje. Laboratory analyses were done in the School of Natural Sciences and Mathematics (Institute of biology) in Skopje.

On the basis of anamneses data, clinical intraoral and extraoral inspection and after detailed analysis of X-rays, diagnosis and indications for realizing oral surgery intervention was set up.

In each patient with detailed anamneses and extensive clinical investigation, the presence of subjective symptoms (pain, perusable sensibility) and objective symptoms (swelling, eventual exudation from the root canal and existing of fistula) were registered.

Using X-rays, condition of the periapical tissue was estimated in order to confirm bone resorption, the absence of lamina dura and existence of chronic periapical lesion. From X-ray to software predilection (Image Tool3 program) of the volume of chronic periapical tissue, correlation between concentration of MMPs in the periapical lesions and their dimension was confirmed.

Examination material was collected on the basis of clinical diagnosis after completely realized anamneses and clinical investigation with the analysis of radiological changes.

Chronic periapical tissues were collected in periapical surgery from 60 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lessions, 10 cysts). Ten health pulps used as controls were obtained by extirpation of it from impacted third molars after their surgery. The examination material was frozen at –80 ºC as soon as possible and stored till analysis, but not longer than six months.

For quantitative analysis of MMP-1, -8, -13 collagenase activities in all of the examination material (chronic periapical tissues and health pulp tissue) Chemicon Collagenase Activity Assay Kit (ECM710) was used.

Chemicon Collagenase Activity Assay Kit was designed so as to achieve quick, convenient and sensitive evaluation of MMP-1, -8 and -13 collagenase activities in a 96-well microplate format. Biotinylated, native triple helical type I collagen was used as a substrate and was cleaved from the activated MMP-1, -8, -13 enzymes.

Each sample was macerated in phosphate-buffered saline – PBS (1.5 ml) and then homogenized in Eppendorf-Centrifuge, 10.000 g for 10 min. The supernatant was used for the analysis. In the homogenized mixture with Bradford micromethod using series of five standards of bovine-serum albumin and than measuring the absorbance on 450 nm with spectrophotometer, concentration of total proteins was de-

termined. With interpolation from the standard curve, concentration of the proteins in samples was measured.

A microplate reader (Anthos ht III) was used to measure the absorbance at 450 nm. Values of the absorbance of each standard were corrected according to protein concentration.

By adding 100 μl of stop solution to each well, the bright yellow convert to bright blue colored product and the enzyme reaction was stoped.

The MMPs concentration of each sample was normalized versus concentration of proteins in each sample. Standard curve was designed with software program Curve Expert 1.3. With interpolation of the values, MMP-1, -8, -13 collagenase concentrations were calculated.

Comparison of the values to determine the significant difference between the specimens of the examination material was performed using descriptive and analytical statistical methods from program Stat Soft Statistic 6.0.

Results

Different concentrations of collagenases MMP-1, -8 and -13 in chronic periapical lesions from different inflammation types showed different activities of MMPs. The obtained results showed the highest values of the concentration of collagenases (MMP-1, -8, -13) in chronic diffuse lesions (5.39 ng/ml) (Table 1).

Concentration of MMPs in all of the samples was < 0.09 ng/ml (Figure 1).

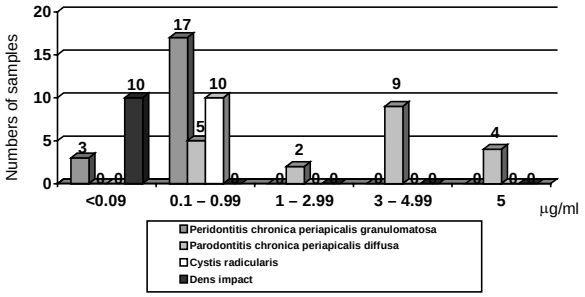

Fig. 1 – Concentration of matrix metalloproteinases in the samples of examinated material

Small values of concentrations of MMPs accompanied smaller lesions, whereas chronical periapical lesions of large dimension had higher concentration of MMPs, which was proportional to the progression of the lesion and destruction of bone tissue (Table 2).

There was a strong linear connection between the level of the lesions and concentration of MMPs in the patients with the diagnosis of *Parodontitis periapicalis chronica granulomatosa* ($R^2 = 0.868$, ANOVA F = 118.702), which means that 86% from the variable dates for the concentration of MMPs was due to variable data for the level of the lesion and the opposite (Figure 2).

Table 1

Concentration of matrix metalloproteinases (ng/ml) in chronic periapical lesions and normal pulp tissue

Clinical diagnosis	n	min	max	median	$\bar{x}$ ±SD
Parodontitis periapicalis chronica granulomatosa	20	0.05	0.95	0.44	0.46 ± 0.29
Parodontitis periapicalis chronica diffusa	20	1.15	5.39	4.12	3.63 ± 1.46
Cystis radicularis	10	0.10	0.64	0.19	0.25 ± 0.16
Dens impacta	10	0.00	0.02	0.01	0.01 ± 0.009

Table 2

Concentration of metalloproteinases (MMPs) in examination material and radiografic values of chronic periapical lesions

MMP (ng/ml)	Radiografic values (mm^2)						Total
	< 0.299	0.3–0.499	0.5–0.699	0.7–0.899	0.9–1.999	> 2	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
< 0.09	1 (33.3)	2 (66.7)	/	/	/	/	3 (6)
0.1–0.99	8 (25)	12 (37.5)	1 (3.1)	3 (9.4)	7 (21.9)	1 (3.1)	32 (64)
1–2.99	/	/	1 (50)	/	/	1 (50)	2 (4)
3–4.99	/	/	/	5 (55.6)	2 (22.2)	2 (22.2)	9 (18)
> 5	/	/	2 (50)	/	/	2 (50)	4 (8)
Total	9 (18)	14 (28)	4 (8)	8 (16)	9 (18)	4 (8)	50 (100)

In the samples of the patients with chronic periapical granuloma, the values of collagenases (MMP-1, -8, -13) concentration in most cases (85%, 17 specimens) were 0.1–0.99 ng/ml. Collagenases concentration in the diffuse periapical lesions was 3–4.99 ng/ml (nine samples, 45%). Concentration of the MMPs in all 10 samples (100%) with clinical diagnosis *cystis radicularis* was 0.1–0.99 ng/ml. In the control group, the smallest concentrations of MMPs were registered.

There was a strong linear correlation between the level of the lesions and concentration of MMPs in the patients with the diagnosis of *parodontitis periapicalis chronica diffusa* ($R^2 = 0{,}922$, ANOVA F = 211.414), which means that 92% from the variable dates for the concentration of MMPs was due to variable data for the level of the lesion and *vice versa* (Figure 3).

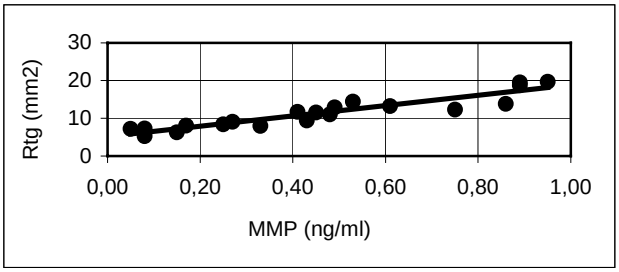

Fig. 2 –Correlation of the lesion and concentration of matrix metalloproteinases in *parodontitis chronica periapicalis granulomatosa*

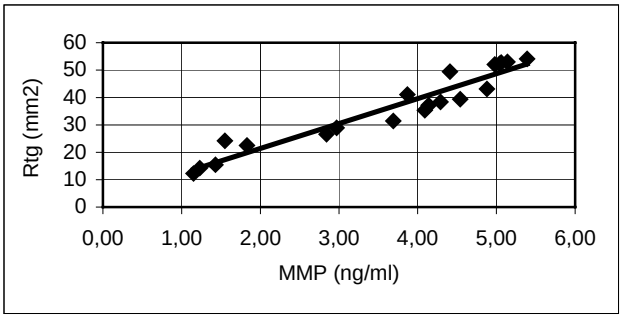

Fig. 3 – Correlation of lesion size and concentration of matrix metalloproteinases (MMPs) in *parodontitis chronica periapicalis diffusa*

The correlation line of dependence between the level of the lesion and concentration of matrix metalloproteinases in the patients with the diagnosis of *Cystis radicularis* had approximately equal values of the level of lesions, independently from the concentration of MMPs ($R^2 = 0.259$, ANOVA F = 4.144; Figure 4).

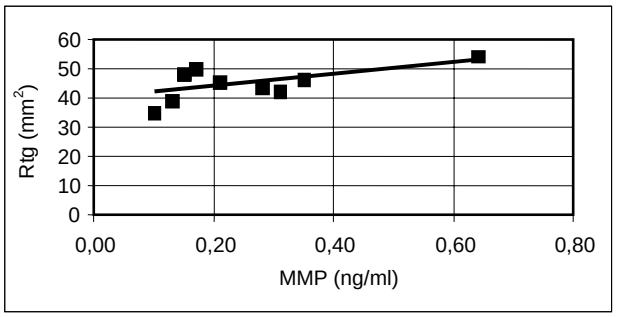

Fig. 4 – Correlation of the lesion size and concentration of matrix metalloproteinases in *cystis radicularis*

Discussion

Matrix metalloproteinases form a family of structurally related, but geneticly different endopeptidases which expression and activity in normal tissue are very low instead of significant increasing during pathological conditions that leads to unfavorable tissue destruction [14].

Degradation and synthesis of ECM components in normal, health tissues are in constant balance, so to maintain this concision, collagenases are expressed at very low levels and the enzyme activity is exactly controlled [15].

Our results confirmed the presence of small concentration of collagenases (MMP-1, -8, -13) in pulp tissues of impacted third molars. The measured concentration of MMPs in health pulp tissues of impacted third molar varied from the minimal value of 0.00 ng/ml to the maximal of 0.02 ng/ml.

Vu and Werb [16] showed the participation of MMPs in tissue remodeling and many other cell functions. According to these authors, MMPs adapt cellular behavior, for example, by inducing cell migration in normal growth and tissue remodeling, such as wound healing and angiogenesis.

The results from quantitative enzyme method in our study demonstrated that the concentrations of collagenases (MMP-1, -8, -13) in chronical periapical lesions were significantly higher than those of the control group ($p < 0.05$). Our results also confirmed the high significant difference ($p < 0.05$) among chronic periapical processes with different clinical diagnosis, which showed different activity of collagenases (MMP-1, -8, -13).

Results in the study of Tjäderhane et al. [17] have demonstrated that MMPs have a role in periapical lesion formation, because MMP inhibition significantly increase the lesion level. Accordingly, MMPs may be involved in defensive reactions against microbes in dental pulp or periapical area. These authors confirmed that the increase of the lesion level might be due to more rapidly advanced pulp infection.

Statistical analysis made with Pearson Chi-Square test showed that there was a high statistical significance between concentration of MMPs and radiographic values of chronic periapical lesions ($\chi^2 = 49.496$; df = 20; $p = 0.000$, means $p < 0.001$).

In the study of Leonardi et al. [18] expression pattern of MMP-13 demonstrated that it was involved in the conversion of periapical granuloma with epithelium into radicular cyst. According to these authors, this property was related to the ability of MMP-13 to influence not only the migration of epithelial cell but also the invasion of granulomatous tissue.

Wahlgren et al. [19] revealed with immunohistochemical analysis and *in situ* hybridization that plasma cells expressed MMP-8 and MMP-13 focally in periapical granulomas, odontogenic cysts and malignant plasmacytomas. These authors confirmed that MMP-8 and MMP-13 from plasma cells could participate in bone organic matrix destruction at sites of chronic inflammation and neoplastic growth. They also demonstrate that MMP-13 was more frequently expressed than MMP-8 in plasma cells of strongly recurring keratocysts and malignant plasmacytomas.

According to Jnakowska-Antczak et al. [20], metalloproteinases can be one of important factors decided about kinetics of periapical bone destruction and may act on periapical bone regeneration after apitectomy and radiectomy.

Results of the study of Wahlgren et al. [2] indicate that MMP analysis from periapical exudates could be used to indicate and monitor inflammatory activity and the success of treatment in teeth with periapical lesions.

Conclusion

According to the obtained results we can conclude that MMP-1, -8, -13 actively participate in tissue destruction and granulation tissue formation in chronic periapical lesions. This study opens a new opportunity for chronic periapical lesions diagnostics and monitoring of inflamed tissue condition, based on destructive role of collagenases (MMP-1, -8, 13) in inflammation process, which is directly dependent on thair concentration in pathologicly changed tissue.

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Epidemija trihineloze u vojničkom kolektivu

An outbreak of trichinellosis in a military unit

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Apstrakt

Uvod/Cilj. Krajem decembra 2001. godine izbila je epidemija trihineloze u vojničkom kolektivu. Cilj rada bio je da se ukaže na mogućnosti i posledice infestacije vojničkog kolektiva u mirnodopskim uslovima i time podigne svest sanitetskih organa, starešina i samih vojnika o značaju i prevenciji iste. **Metode.** Korišćen je deskriptivni i analitički epidemiološki model radi pronalaženja izvora infestacije, kao i utvrđivanja puteva prenošenja. **Rezultati.** Epidemija je izbila kao posledica konzumiranja kontaminiranih dimljenih svinjskih kobasica, koje su unete spolja, iz građanstva, u vojnički kolektiv. Od ukupno 38 eksponiranih osoba, 21 lice obolelo je i hospitalizovano. Najčešći simptomi kod obolelih bili su povišena temperatura (76,2%), bolovi u mišićima i zglobovima (76,2%), otok očnih kapaka (42,8%), otok lica (19%) i proliv (14,3%). Test indirektne imunofluorescencije bio je pozitivan kod 14,3%, a ELISA test kod još 28,6% obolelih. Eozinofili bili su povišeni kod 85,7% obolelih, vrednosti IgE bile su povećane kod 28,6% obolelih, a vrednosti CPK bile su povišene kod 61,9% bolesnika. Kod 17 eksponiranih neobolelih laboratorijske analize bile su negativne na trihinelozu. **Zaključak.** Stroge mere kontrole (veterinarski pregled i odobrenje) pri unosu nedovoljno termički obrađenog svinjskog mesa (suhomesnatih proizvoda) u jedinice/ustanove Vojske Srbije moraju biti obavezne.

Ključne reči:
trihinoza; vojne jedinice, vojnici; epidemije; epidemiologija.

Abstract

Background/Aim. In December 2001, an outbreak of trichinellosis spreaded in a military unit. The aim of this paper was to show possibilities and consequences of trichinellosis infestations in military units during peace time, as well as to improve knowledge and awareness of medical corps personnel, commanders and soldiers about this disease. **Methods.** A descriptive and analytical epidemiological models were used to find out a source of outbreak and to identify the ways of its transmission. **Results.** This outbreak was caused by the contaminated raw smoked sausage which had not undergone health inspection and brought from civilians to a military unit. Thirty-eight persons were exposed, twenty-one affected and hospitalized. The most frequent symptoms reported were fever (76.2%), myalgia (76.2%), palpebral edema (42.8%), face edema (19.0%) and diarrhea (14.3%). Test for indirect immunofluorescence was positive in 14.3% and ELISA test was positive in 28.6% of the patients. Eosinophilia was present in 85.7% of the affected. IgE values were increased in 28.6% and CPK values were increased in 61.9% of the diseased. All of the 17 exposed undiseased had negative laboratory analyses for trichinellosis. **Conclusion.** We propose intensifying health education and continuing the implementation of duly supervised and evaluated self-check programs. A well-tuned, fast-reacting epidemiological monitoring system has to be obligatory.

Key words:
trichinosis; military units; soldiers; disease outbreaks; epidemiology.

Uvod

Trihineloza je zoonoza uzrokovana crvolikim parazitom iz roda *Trichinella spp., T. spiralis.* Rasprostranjena je širom sveta [1]. Procenjuje se da je oko 11 miliona ljudi u svetu infestirano trihinelom. Bolest ima naročito zabrinjavajuće razmere na Balkanu, u Rusiji, Baltičkim Republikama, nekim delovima Kine i u Argentini [2].

Što se tiče okolnih zemalja, u Grčkoj *T. spiralis* retko se javlja kod čoveka [3]. U Hrvatskoj broj infestacija kod ljudi drastično je opao kao rezultat obavezne trihineloskopske kontrole [4]. U Rumuniji broj obolelih od trihineloze porastao je gotovo 17 puta u periodu između 1983. i 1993. godine [5]. U Srbiji, tokom poslednjih 10 godina, trihineloza je doživela ekspanziju iz tri endemska regiona (Srem, Mačva i Negotinska Krajina) u susedne regione. Istovremeno, broj infestacija

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kod ljudi porastao je tri do pet puta u poređenju sa periodom 1980–1990. [6]. U SR Jugoslaviji u periodu 1991–2000. godina bilo je ukupno registrovano 5 197 obolelih od trihineloze, a broj obolelih godišnje kretao se od 198 u 1991. godini do 804 u 1997. godini. Prosečna godišnja incidencija za posmatrani period iznosila je 4,9 na 100 000 stanovnika [7].

Više epidemija trihineloze u zemljama EU tokom poslednjih nekoliko godina izbilo je kao posledica konzumiranja svinjskog ili konjskog mesa uvezenog s prostora bivše SFRJ. U Britaniji opisana je epidemija trihineloze sa osmoro obolelih iz četiri domaćinstva koji su konzumirali svinjsku salamu poreklom iz severne Srbije [8]. Dva slučaja trihineloze (bračni par), od kojih jedan sa teškom kliničkom slikom, registrovana su u Danskoj kod osoba koje su nekoliko dana ranije konzumirale svinjetinu u Srbiji [9]. Velika epidemija trihineloze u Francuskoj izbila je konzumiranjem konjskog mesa uvezenog iz Jugoslavije kada je u dva regiona obolelo ukupno 395 osoba [10]. U Italiji su zabeleženi brojni sporadični slučajevi i epidemije trihineloze kao posledica konzumiranja sirovog ili nedovoljno termički obrađenog konjskog mesa uvezenog iz Jugoslavije i Poljske [11].

Domaća svinja najvažnija je karika u ciklusu zaražavanja čoveka. Svinje se mogu inficirati svinjskim otpacima koje čovek ostavlja iza sebe, proždiranjem pacova ili kanibalizmom (svinja-svinja). Obolevanje od trihineloze registruje se u toku cele godine, s tim što je najveći broj obolelih u periodu decembar– mart.

Cilj rada bio je ispitati kliničke i epidemiološke karakteristike epidemije trihineloze koja je izbila u vojničkom kolektivu u Svilajncu kao posledica konzumiranja dimljenih svinjskih kobasica.

Metode

U istraživanju epidemije korišćena je deskriptivna i analitička metoda. Od ukupno 38 osoba eksponiranih infestaciji trihinelom, obolela je 21 osoba muškog pola (18 vojnika, dvojica starešina i jedno civilno lice) u Garnizonu Svilajnac, tokom decembra 2001. i januara 2002. godine.

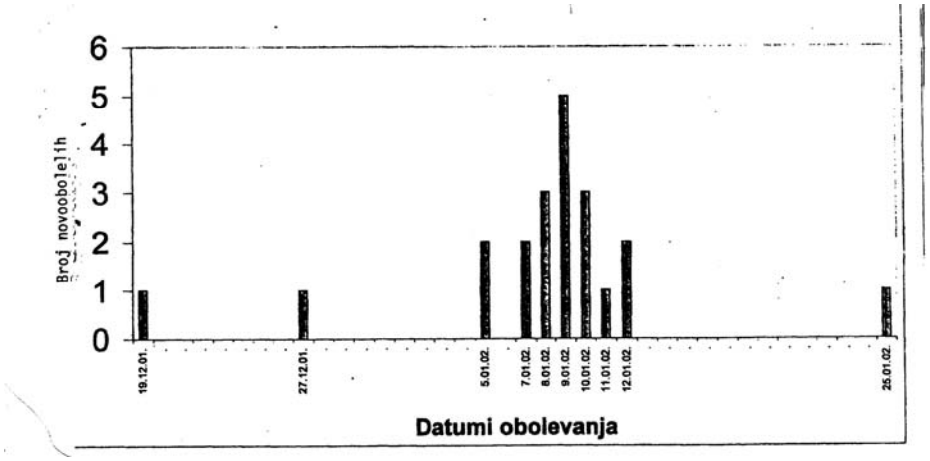
Tokom korišćenja analitičke metode uočene su kontrolne grupe koje su ukazale na način zaražavanja u ovoj epidemiji. Negativnu kontrolnu grupu predstavljale su starešine, civili i vojnici koji su kao i oboleli iz iste jedinice, ali nisu konzumirali zaraženo svinjsko meso i nisu oboleli. Pozitivnu kontrolnu grupu činile su osobe koje nisu pripadale jedinici zahvaćenoj epidemijom, ali su konzumirale zaraženu hranu i obolele su od trihineloze. Naime, više desetina osoba, koje su konzumirale suhomesnate proizvode iz mesarske radnje oca vojnika, koji je doneo kontaminiranu hranu u Garnizon Svilajnac, takođe infestiralo se gotovo istovremeno kada i vojnici.

Radi potvrde dijagnoze uzeta je krv obolelih i zdravih vojnika. Obavljena su serološka ispitivanja u Institutu za mikrobiologiju Zavoda za preventivnu medicinu Vojnomedicinske akademije (VMA). Za serološka ispitivanja korišćeni su imunoenzimski ELISA test i test indirektne imunofluorescencije (IIF test). Kao pozitivan nalaz infestacije *T. spiralis* smatran je titar 1:20 i viši.

Biohemijska i hematološka ispitivanja obavljena su u Institutu za medicinsku biohemiju VMA. Hematološka ispitivanja obuhvatila su određivanje broja eozinofila, a biohemijska ispitivanja obuhvatila su određivanje vrednosti enzima kreatin fosfokinaze (CPK) i imunoglobulina E (IgE).

Rezultati

Epidemija trihineloze u Garnizonu Svilajnac počela je 19.12.2001. godine pojavom proliva i mučnine kod jednog vojnika (slika 1). Tokom narednih dana broj obolelih postepeno se povećavao da bi do kraja epidemije 25.01.2002. godine obolelo ukupno 21 lice, i to 18 vojnika, dvojica starešina i jedno civilno lice na službi u vojsci. Prvi simptomi i



Sl. 1 – Dinamika obolevanja od trihineloze u Garnizonu Svilajnac

znaci kod obolelih javili su se znatno ranije, nego što se oboljenje zvanično registrovalo, jer bolest nije na vreme prepoznata i dijagnostikovana. Najkraća dužina inkubacije iznosila je četiri dana (1 oboleli), najčešća dužina inkubacije 17 dana (5 obolelih), a najduža inkubacija trajala je 31 dan (1 oboleli). Incidencija obolelih (21) u odnosu na eksponiranu grupu (38 osoba) iznosila je 55,2%.

U prikazanoj epidemiji trihineloze nije dokazano prisustvo uzročnika oboljenja u zaraženom mesu pošto je sva in-

kriminisana hrana pojedena pre dolaska ekipe za istraživanje epidemije sa VMA. Međutim, istovremena pojava epidemije trihineloze sa preko 300 obolelih u Zrenjaninu i okolini za koje je potvrđeno da su se zarazili mesom iz privatnih mesara odakle su poticale i dimljene kobasice koje su konzumirali oboleli iz Garnizona Svilajnac, mogla bi ukazati na izvor zaraze. U periodu od 15. do 23.12.2001. godine u jedinicu su uneta tri paketa, ukupne težine oko 15 kg, u kojima su se nalazile dimljene kobasice, pršuta i slanina, koje je vojnik, čiji je otac vlasnik pomenutih mesara, podelio i konzumirao sa drugim vojnicima i starešinama.

Sumnja na trihinelozu postavljena je na osnovu podataka iz epidemiološkog upitnika – anamneze (svi oboleli su u periodu inkubacije konzumirali inkriminisanu hranu unetu iz građanstva) i kliničke slike.

U Infektivnoj klinici VMA u periodu od 08.01.–20.02.2002. godine sa klinički jasno izraženim simptomima i znacima bolesti hospitalizovana je 21 osoba. U kliničkoj slici obolelih dominirao je opšti infektivni sindrom, a zastupljenost dominantnih simptoma i znakova prikazana je u tabeli 1.

hospitalizaciji obolelih, IIF testom potvrđena je dijagnoza trihineloze kod jednog obolelog, a ELISA testom kod još trojice obolelih. Serologija je ponovljena pošto nivo antitela može biti ispod nivoa detekcije u prvom uzorku. Za serokonverziju i stvaranje dovoljno visokog titra antitela za detekciju potrebno je da prođe u proseku dve nedelje od momenta infestacije. Zbog toga, posle dve nedelje od prvog uzorka uzet je drugi uzorak krvi, kada je IIF testom potvrđena dijagnoza trihineloze kod još dvojice, a ELISA testom kod još trojice obolelih. Ukupno, IIF testom potvrđena je dijagnoza trihineloze kod 14,3% obolela, a ELISA testom kod još 28,6% obolelih (ukupno 43%). Hematološkim ispitivanjima utvrđeno je da su eozinofili bili povišeni kod 18 obolelih (85,7%). Biohemijskim ispitivanjima utvrđeno je da su vrednosti IgE bile povećane kod šest obolelih (28,6%), a vrednosti CPK povišene kod 13 bolesnika (61,9%).

Kod 17 eksponiranih neobolelih sve izvedene laboratorijske analize bile su negativne na trihinelozu.

Tabela 1

Dominantni simptomi i znaci bolesti kod 21 obolelog lica u epidemiji trihineloze

Simptomi i znaci bolesti	Broj obolelih	Procenat obolelih
Povišena temperatura	16	76,2
Bolovi u mišićima i zglobovima	16	76,2
Otok očnih kapaka	9	42,8
Otok lica	4	19,0
Proliv, bolovi u stomaku	3	14,3

Povišena temperatura bila je dominantni znak kod svih obolelih, a kod 15 (71,4%) obolelih iznosila je preko 38 °C. Bolovi u mišićima i zglobovima jakog intenziteta bili su prisutni kod 16 (76,2%) obolelih. Otok očnih kapaka kao značajan i patognomoničan znak bio je prisutan kod devet (43%) bolesnika.

Dijagnoza trihineloze postavljena je na osnovu anamnestičkih podataka, kliničke slike i laboratorijskih nalaza. Bolesnici su lečeni mebendazolom i bronalom, kao i odgovarajućom simptomatskom terapijom. Lečenje je trajalo prosečno 17 dana. Prilikom otpusta svi su bili dobrog zdravstvenog stanja.

Od 15.01.2002. 15 vojnika i šest starešina koji su konzumirali inkriminisanu hranu, a bili bez simptoma, uzimali su mebendazol radi profilakse 10 dana, osim dvojice kojima je terapija prekinuta posle pet dana zbog pojave diareje, odnosno bolova u predelu jetre, kod vojnika koji je nekoliko meseci ranije preležao hepatitis. Toj grupi eksponiranih u dva navrata rađene su laboratorijske analize u VMA (24. i 31.01.2002). Četvorica od njih kasnije su, krajem januara i početkom februara, hospitalizovani kao oboleli od trihineloze u Infektivnoj klinici VMA.

Radi potvrde dijagnoze obavljena su serološka, biohemijska i hematološka ispitivanja kod 21 bolesnika i kod 17 neobolelih eksponiranih vojnika i starešina. Serološkim ispitivanjima potvrđena je dijagnoza trihineloze kod devetorice obolelih. U prvom uzorku krvi koji je uzet odmah po

Diskusija

Intenziviranje problema trihineloze u nekim zemljama objašnjava se boljim znanjem/informisanjem (ranije je često pogrešno dijagnostikovana kao grip), promenama navika konzumenata, povećanjem broja zaražene divljači, uvozom mesa iz zemalja u kojima je trihineloza endemska ili je insuficijentna veterinarska kontrola bilo zbog ljudskog faktora ili socijalnih dešavanja [2].

Obnavljanje i intenziviranje lanca infekcije *T. spiralis* kod domaćih životinja nastalo je kao prva posledica sloma državnih veterinarskih službi i državnih farmi u zemljama bivšeg Sovjetskog Saveza, Bugarskoj, Rumuniji, odnosno ekonomskih problema i rata (bivša Jugoslavija). To je rezultovalo naglim povećanjem broja slučajeva ove infekcije u čoporima svinja tokom devedesetih godina, s prevalencijom do 50% u selima Belorusije, Hrvatske, Latvije, Litvanije, Rumunije, Rusije, Srbije i Ukrajine [12]. Uloga divljači, kao izvora infekcije kod ljudi, značajno se povećala i u razvijenim i u zemljama u razvoju (Bugarska, Kanada, Litvanija, pojedine zemlje EU, Rusija, SAD).

U skandinavskim zemljama prevalencije silvatične trihineloze i trihineloze domaćih životinja prilično se razlikuju. Danska se smatra zemljom bez trihineloze domaćih životinja i sa vrlo retkim slučajevima silvatične trihineloze. U Švedskoj i Norveškoj trihineloza domaćih životinja je retkost, dok silvatična trihineloza nije. U Finskoj je zabeležen dramatičan

porast tokom poslednje dekade i silvatične trihineloze i trihineloze domaćih životinja. U Litvaniji, Letoniji i Estoniji trihineloza domaćih životinja je u porastu [13]. Problem trihineloze konjskog mesa ograničen je na Francusku i Italiju, jedine dve zemlje u kojima se jede sirovo konjsko meso i u kojima je u poslednjih 25 godina na taj način obolelo oko 3 000 ljudi [14]. Ovčetina i govedina zaražene trihinelom nađene su jedino u Kini [12].

Meksički autori navode prevalenciju antitela protiv trihinele u pojedinim regionima Meksika od 1 do 1,9% u opštoj populaciji [15]. Takvi nalazi sugerišu prisustvo endemske neregistrovane forme trihineloze kod ljudi i mogu biti od značaja za druge zemlje naročito u ruralnim i semi-ruralnim oblastima.

Trihineloza u SAD uglavnom se javlja u vidu sporadičnih slučajeva. Zbog toga izvori zaraze sporadičnih slučajeva moraju biti ozbiljno istraženi i shvaćeni. Od procenjenih 120 000 do 300 000 infestiranih osoba godišnje krajem šezdesetih u SAD, registrovano je otprilike samo stotinak obolelih, verovatno zbog teškoća u dijagnostikovanju oboljenja, smanjenog registrovanja/prijave oboljenja od strane lekara i velikog broja supkliničkih infekcija koji su se javile kao posledica konzumiranja niskoinfestiranog svinjskog mesa (sadrži manje od jedne larve *T. spirals* po gramu mesa) [16–18]. S druge strane, od uvođenja registrovanja slučajeva trihineloze 1947. godine u SAD broj obolelih svake godine je u kontinuiranom padu. Godišnje taj broj iznosio je oko 400 obolelih sa 10 do 15 smrtnih ishoda. Od 1991. do 1996. Centar za kontrolu bolesti u Atlanti registrovao je 230 obolelih i tri smrtna ishoda (u proseku 38 obolelih godišnje). Anamnestički podaci o konzumiranju zaražene/sumnjive hrane dobijeni su od 58% bolesnika. Najčešće inkriminisani izvor zaraze predstavljalo je svinjsko meso (60% bolesnika). Udeo bolesnika sa trihinelozom uzrokovan konzumiranjem svinjetine iz komercijalnih tokova u kontinuiranom je padu kao posledica više faktora (smanjenje prevalencije *T. spiralis* kod domaćih svinja, povećana upotreba zamrzivača i dobra termička obrada svinjskog mesa). Nasuprot tome, broj obolelih usled konzumiranja mesa divljači pokazao je blagi porast [19].

Na teritoriji bivše Jugoslavije, od ranije poznata endemska područja trihineloze su regioni južne Bačke i zapadnog Srema, što je potvrđeno i boravkom pripadnika Vojske Jugoslavije na ovim prostorima u vreme ratnih sukoba. U zimskom periodu 1991/92. godine registrovane su četiri epidemije trihineloze sa otkrivenih 48 obolelih, od kojih je jedan umro [7].

Zbog nekarakterističnih početnih simptoma (temperatura, mialgije) i doba kada se obično javlja (pozna jesen, zima) postoje teškoće u njenom pravovremenom prepoznavanju i lečenju, pa je zato potrebno i više opreza u kliničkom radu. Naročito, pojava novih *Trichinella species* (*Trichinella papuae* i *Trichinella murrelli*) i pojava bolesti kod ljudi izazvana *Trichinella pseudospiralis*, za koju se donedavno mislilo da se javlja samo kod životinja, zahtevaju promene u diferencijalno-dijagnoznom pristupu i kliničkom tretmanu trihineloze, jer je postojeće znanje bazirano uglavnom na slučajevima klasične *T. spiralis* infestacije [20]. Jedan od najvažnijih načina postavljanja dijagnoze trihineloze još uvek je tačan i precizan intervju sa konzumentom inkriminisane hrane. Ako intervju ukaže na podatke o skorašnjoj ingestiji nedovoljno termički obrađenog svinjskog mesa udruženoj sa pojavom karakterističnih znakova i/ili simptoma bolesti, onda je dijagnoza trihineloze prilično verovatna. Klinička slika bolesti može varirati od inaparentne forme do fulminantne, fatalne bolesti, zavisno od broja ingestiranih larvi. U tipičnim slučajevima, kada dođe do umnožavanja parazita u digestivnom traktu (enteralna faza trihineloze) javljaju se dijareja, povraćanje i drugi gastrointestinalni simptomi. Kada larve dospeju u krvotok (migratorna faza trihineloze) nastaje povišena temperatura, glavobolja, profuzno znojenje, mišićna osetljivost i bol, kao i edem očnih kapaka i lica. U težim slučajevima mogu biti zahvaćeni centralni nervni sistem, srce, pluća, a u najtežim dolazi i do smrtnog ishoda. Serološki testovi, hematološke i biohemijske analize mogu pomoći u dijagnozi.

Povišena temperatura i mialgije integralni su deo kliničke slike i javljaju se kod 90–100% obolelih [21, 22]. U našoj epidemiji taj procenat bio je manji i iznosio je 76,2%. Razlog tome bila je generalno blaža klinička slika hospitalizovanih. Zbirni podaci iz devet epidemija navode srednju učestalost diareje kod 16% obolelih [22]. Taj procenat je u slučaju naše epidemije bio nešto niži i iznosio je 14,3. Procenat bolesnika sa otokom očnih kapaka i/ili periorbitalne regije prema nalazima pojedinih autora izrazito je varijabilan. Kurup i sar. [21] navode učestalost periorbitalnog edema kod svega 4% obolelih, u većini drugih epidemija taj procenat se kretao od 15% do 90% [22]. Procenat bolesnika iz naše epidemije sa otokom očnih kapaka u skladu je sa rezultatima drugih autora (43%). Pojava kožnog osipa i kašlja kod obolelih prilično je varijabilna; većina autora navodi učestalost ovih znakova bolesti kod 15–65%, odnosno 5–40% bolesnika [21]. Pojava kožnog osipa i kašlja nije zabeležena kod naših bolesnika, verovatno zbog malog inokuluma larvi.

Eozinofilija je najraniji i jedan od najkarakterističnijih laboratorijskih nalaza kod trihineloze, prisutan kod i do 90% obolelih [23, 24]. Vrednosti broja eozinofila bile su povišene kod 18 (85,7%) obolelih u prikazanoj epidemiji. Povišen nivo CPK kod obolelih od trihineloze javlja se kao posledica upale mišićnog tkiva usled migriranja larvi [25, 26]. Prema podacima iz ranijih epidemija 35–100% infestiranih imalo je povišene vrednosti CPK [21]. U ovoj epidemiji povišen nivo CPK zabeležen je kod 13 (62%) obolelih, dok su vrednosti IgE bile povećane kod šest obolelih (28,6%).

Rezultati seroloških analiza inostranih studija (epidemija) veoma su različiti. Najniži procenat pozitivnih seroloških nalaza kod obolelih od trihineloze bio je 25%, odnosno 37% [27, 28]. Italijanski autori pak navode pozitivnost IIF testa od 94%, odnosno ELISA testa od 100% obolelih [29, 30]. Serološke analize u našoj epidemiji bile su pozitivne kod 43% obolelih. Pozitivnost seroloških testova kod obolelih zavisi od infestirane doze, promptnosti i adekvatnosti dijagnostikovanja i lečenja, imunološkog stanja obolelog, vrste, tj. reaktogenosti datog parazita. Time bi se mogle objasniti razlike u procentu obolelih sa pozitivnim serološkim nalazima.

Terapija trihineloze je kontroverzna. Neki autori preporučuju samo aspirin i mirovanje, ako je simptomatologija blaga, drugi preporučuju antihelmintike kao rutinsku proceduru da bi se sprečilo dalje stvaranje larvi [22, 31]. Kod naših

bolesnika klinička slika i tok bolesti bili su tipični i bez komplikacija. Tokom lečenja (primena antihelmintika mebendazola) svi oboleli pokazali su znake promptne rezolucije simptoma bolesti, klinički oporavak bio je brz i potpun.

Generalna strategija prevencije podrazumeva primenu mehanizama kojima se može sprečiti/prekinuti infestacija hrane i transmisija bolesti. U budućnosti, prevencija trihineloze u većoj meri će zavisiti od kontrole kontaminiranosti hrane životinjskog porekla. S veterinarske tačke gledišta, preporučljiva je upotreba senzitivnijih seroloških metoda (ELISA, IIF) za pregled zaraženosti životinjskog mesa na *T. spiralis* umesto važećeg mikroskopskog ispitivanja nekoliko grama mesa. Zdravstveno prosvećivanje opšte populacije od najveće je važnosti u prevenciji infestacije trihinelom. Znanje konzumenta o osnovnim principima bezbednosti hrane je važna, ali nedovoljna komponenta prevencije. Na farmama, uništavanje populacije glodara i sprečavanje kontakata između svinja i divljih životinja važne su strategije u sprečavanju humane trihineloze.

Ova epidemija je pokazala da moderna masovna proizvodnja može dovesti do velikih epidemija trihineloze kada je kontrola nedovoljna ili potpuno izostane. Poboljšan epidemiološki nadzor kombinovan sa epidemiološkim istraživanjem većih epidemija i sporadičnih slučajeva može identifikovati rezervoare/izvore zaraze i dovesti do razvoja specifične strategije prevencije. Lekari i veterinari koji rade u oblastima sa visokim rizikom od trihineloze treba da budu redovno informisani o pojavi takve bolesti i preduzetim merama. Problem može biti i to što lekari retko razmišljaju o trihinelozi, pripisujući često početne simptome drugim oboljenjima, na primer gripu ili virusnom gastroenteritisu. Jedini nalaz koji u takvim situacijama ukazuje na parazitozu je izražena eozinofilija, bez obzira da li je praćena dugotrajnom febrilnošću.

Zaključak

Do epidemije trihineloze među pripadnicima Vojske Srbije došlo je zbog konzumiranja kontaminiranih svinjskih suhomesnatih proizvoda koji su uneti iz građanstva u vojnički kolektiv.

Obolela je 21 osoba, a efikasno lečenje obolelih sprovedeno je mebendazolom.

Stalna prisutnost trihineloze na teritoriji naše zemlje, kao i redovna (svake zime) pojava velikih epidemija sa po više desetina, a u nekim slučajevima i više stotina obolelih, nameće obavezu intenzivne kontrole svinjskog mesa.

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Cardiovascular morbidity and mortality in patients treated with hemodialysis – epidemiological analysis

Kardiovaskularni morbiditet i mortalitet bolesnika na hemodijalizi: epdiemiološka analiza

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Abstract

Background/Aim. Cardiovascular diseases are the leading cause of death in patients treated with hemodialysis (HD). The annual cardiovascular mortality rate in these patients is 9%. Left ventricular (LV) hypertrophy, ischemic heart disease and heart failure are the most prevalent cardiovascular causes of death. The aim of this study was to assess the prevalence of traditional and nontraditional risk factors for cardiovascular complications, to assess the prevalence of cardiovascular complications and overall and cardiovascular mortality rate in patients on HD. **Methods.** We investigated a total of 115 patients undergoing HD for at least 6 months. First, a cross-sectional study was performed, followed by a two-year follow-up study. Beside standard biochemical parameters, we also determined cardiac troponins and echocardiographic parameters of LV morphology and function (LV mass index, LV fractional shortening, LV ejection fraction). The results were analyzed using the Student's *t* test and Mann-Whitney *U* test. **Results**. The patients with adverse outcome had significantly lower serum albumin ($p < 0.01$) and higher serum homocystein, troponin I and T, and LV mass index ($p < 0.01$). Hyperhomocysteinemia, anemia, hypertriglyceridemia and uncontrolled hypertension had the highest prevalence (86.09%, 76.52%, 43.48% and 36.52%, respectively) among all investigated cardiovascular risk factors. Hypertrophy of the LV was presented in 71.31% of the patients and congestive heart failure in 8.70%. Heart valve calcification was found in 48.70% of the patients, pericardial effusion in 25.22% and disrrhythmia in 20.87% of the investigated patients. The average annual overall mortality rate was 13.74%, while average cardiovascular mortality rate was 8.51%. **Conclusion**. Patients on HD have high risk for cardiovascular morbidity and mortality.

Key words:
cardiovascular diseases; morbidity; mortality; renal dialysis; risk factors; prevalence; hypertrophy, left ventricular; homocysteine.

Apstrakt

Uvod/Cilj. Kardiovaskularne bolesti su vodeći uzrok smrti bolesnika koji se leče hemodijalizom (HD). Jednogodišnja stopa kardiovaskularnog mortaliteta iznosi 9%, a od kardiovaskularnih poremećaja, kao uzročnici smrti najveću prevalenciju imaju hipertrofija leve komore (LK), ishemijska bolest srca i srčana slabost. Cilj rada bio je da se utvrdi prevalencija tradicionalnih i netradicionalnih faktora rizika od razvoja kardiovaskularnih komplikacija, prevalencija kardiovaskularnih komplikacija, kao i stopa opšteg i kardiovaskularnog mortaliteta bolesnika podvrgnutih HD. **Metode.** Ispitano je 115 bolesnika, koji su lečeni HD duže od šest meseci. Prvo, urađena je studija preseka, a zatim praćenje bolesnika u dvogodišnjem vremenskom periodu. Parametri ispitivanja, pored standardnih laboratorijskih analiza, uključili su srčane troponine i ehokardiografske parametre morfologije i funkcije LK: indeks mase LK, frakciono skraćenje LK, ejekcionu frakciju LK. Za statističku analizu dobijenih podataka korišćeni su Studentov *t* test i Mann-Whitney *U* test. **Rezultati.** Bolesnici sa nepovoljnim ishodom imali su visoko statistički značajno ($p < 0,01$) manje vrednosti albumina u serumu, kao i visoko statistički značajno ($p < 0,01$) veće vrednosti homocisteina, troponina I, troponina T i indeksa mase LK. Od ispitivanih faktora rizika najveću prevalenciju imali su hiperhomocisteinemija (86,09%), anemija (76,52%), hipertrigliceridemija (43,48%) i nekontrolisana hipertenzija (36,52%). Hipertrofiju LK imalo je 71,31%, a kongestivnu sistolnu srčanu insuficijenciju 8,70% bolesnika. Kalcifikaciju srčanih valvula imalo je 48,70%, perikardni izliv 25,22%, dok je poremećaj srčanog ritma imalo 20,87% ispitivanih bolesnika. Prosečna jednogodišnja stopa opšteg mortaliteta iznosila je 13,74%, a prosečna jednogodišnja stopa kardiovaskularnog mortaliteta 8,51%. **Zaključak.** Bolesnici na HD u visokom su riziku od razvoja kardiovaskularnog morbiditeta i mortaliteta.

Ključne reči:
srce, bolesti; morbiditet; mortalitet; hemodijaliza; faktori rizika; prevalenca; srce, hipertrofija leve komore; homocistein.

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Indtroduction

Cardiovascular diseases are the leading cause of death in patients on hemodialysis (HD). The annual mortality rate from cardiovascular disease in these patients is 9%. The major cardiovascular complications are left ventricular (LV) hypertrophy (LVH) (75%), ischemic heart disease (40%) and congestive hart failure (40%) [1]. High incidence of cardiovascular disease in patients on HD is related to high prevelence of traditional (hypertension, disturbed lipid metabolism, diabetes mellitus, cigarette smoking) and nontraditional (microinflammation, oxidative stress, hyperhomocysteinemia, secondary hyperparathyroidism) risk factors, which lead to increased atherosclerosis, plaque destabilization, myocardial fibrosis and valvular heart disease [2, 3].

Patients on hemodialysis are at higher risk for sudden cardiac death [4]. Hypertrophy of LV (present in 75% of patients on HD), fast electrolyte changes during HD (absence of potassium in dialysis solution), hyperkalemia, myocardial fibrosis and decreased coronary perfusion, all contribute significantly to the appearance of sudden cardiac death in these patients [4].

The aim of this study was to determine the prevalence of traditional and nontraditional (metabolic and hemodynamic) risk factors for the development of cardiovascular complications in patients on HD. Furthermore, we aimed to determine the prevalence of cardiovascular complications and overall and cardiovascular mortality rate in patients on regular HD.

Methods

This study was conducted at the Department of Hemodialysis, Clinic for Urology and Nephrology, Clinical Center of Kragujevac at Kragujevac, Serbia. The study included 115 patients (71 males and 44 females). All patients gave informed consent for participating in the study, according to the Declaration of Helsinki. All subjects were hemodynamically stable and on standard bicarbonate HD for over 6 months, with diuresis < 200 ml/24 h and had various primary renal diseases.

We investigated the following variables: body mass index (BMI), hemoglobin concentration, hematocrit (Hct), arterial blood pressure (BP), arteriovenous shunt blood flow (Q_{AV}), serum homocystein (tHcy), serum C-reactive protein (CRP), total cholesterol, low-density lipoproteins LDL-cholesterol, high-density lipoproteins HDL-cholesterol and triglycerides, lipoprotein (a), calcium, phosphate, calcium-phosphate product (calcium × phosphate) and serum intact parathyroid hormone (iPTH) concentration.

Laboratory analysis

Blood samples for laboratory analyses were drawn after 12 hours overnight fasting, before the dialysis session and heparin administration.

Serum urea was determined with complete enzymatic method (*urease-glutamate-dehydrogenase*), reference range being 3.5–7.5 mmol/l.

Hemoglobin concentration was measured by colorimetry, standard range was 110–180 g/l. Hematocrit was determined automatically with COULTER® A^C apparatus and from the formula: Hct(%) = (RBC × MCV)/10, where RBC – red blood cells and MCV – mean cell volume. Normal range was 0.35–0.60.

Serum albumin level was measured by photometric colour test with bromcresol green. The normal range was 38–46 g/l and concentration < 36 g/l suggested malnutrition.

Serum cholesterol was determined with enzymatic method (*cholesterol esterase –cholesterol oxidase*). Reference range was 3.37–6.48 mmol/l. Serum HDL lipoproteins concentration was measured by colorimetry. Reference range was 0.78–1.55 mmol/l. Serum LDL concentration was calculated from the formula: $C_{LDL} = C_{HOL\text{-}tot} - (TGL/2.2) - C_{HDL}$, where C_{LDL} is serum LDL concentration, $C_{HOL\text{-}tot}$ is total serum cholesterol, C_{HDL} is serum HDL concentration and TGL is serum triglycerides concentration. Reference range for LDL cholesterol was 2.39–4.08 mmol/l. Serum triglycerides were determined by enzymatic colorimetric method, normal range is 0–1.88 mmol/l. Serum lipoprotein (a) concentration was determined by the Behring Nephelometer System and N Latex Lp(a) reagent. Levels < 30 mg/dl were considered normal. Serum apolipoprotein AI (ApoAI) and apolipoprotein B (ApoB) levels were determined with N Antiserum to Human ApoAI (ApoAI) and N Antiserum to Human ApoB reagents respectively. Normal range for ApoAI was 1.25–2.15 g/l (women), 1.10–2.05 g/l (men), for ApoB was 0.55–1.25 g/l (women), 0.55–1.40 g/l (men). Reference range for apoB/apoAI ratio was 0.30–0.90 g/l for women and 0.35–1.00 g/l for men.

Serum calcium concentration was determined by photometric color test (Arseniko), reference range being 2.20–2.65 mmol/l. Serum phosphate was determined by photometric ultraviolet (UV) test, normal range being 0.80–1.45 mmol/l. Serum iPTH was determined by radioimmunoassay (IRMA). Normal iPTH concentration is 11.8–64.5 pg/ml for healthy individuals. Target levels for pateints on HD are below 200–300 pg/ml.

Homocystein concentration was measured by Fluorescence Polarization Immunoassay (FPIA) method. Levels > 15 μmol/l indicated hyperhomocysteinemia. Serum CRP was determined using the immunochemical nephelometric method, and calculated as mean value of two measurements in three months. Normal value was ≤ 5 mg/l.

Measurement of serum cardiac troponin T (cTnT) was based on electrochemiluminescence immunoassay technology (ElektroChemiLumineszenz ImmunoAssay – ECLIA method), by using the Roche Diagnostics troponin T kit. A level of > 0.1 ng/ml was considered positive for myocardial necrosis. Serum cardiac tropinin I (cTnI) was determined with ADV A × SYM cTnI immunoassay technology (*Abbott laboratories*). A level of > 0.15 ng/ml was considered positive for myocardial necrosis.

Echocardiography

The echocardiocraphic study was performed 15 to 20 hours after the dialysis session, in order to avoid end-diastolic LV diameter alterations induced by the interdialytic volume gain. All studies were performed on a SHIMADZU-2200 ultrasound machine, with a 2.5 megahertz (MHz) transducer probe, by a single experienced physician.

Left ventricular hypertrophy was determined by measuring the LV mass index (LVMi), which is normally ≤ 131 g/m^2 in men and ≤ 100 g/m^2 in women. Left ventricular mass index was calculated as follows:

$$\text{LVMi} = \frac{0.00083\,x\left[(LVEDD + IVSd + LVPWd)^3 - (LVEDD)^3\right] + 0.6}{BSA}\ \text{g/m}^2$$

Left ventricular end-diastolic volume index (iEDV), normally ≤ 90 ml/m^2 was quantified as:

$$\text{iEDV} = \frac{(LVEDD)^3\,x0.001047}{BSA}\ \text{ml/m}^2.$$

Abbreviations in the formulas stand for: IVSd - interventricular septal wall thickness in diastole (mm), LPWd – LV posterior wall thickness in diastole (mm), LVESD – LV end-systolic diameter (mm), LVEDD – LV end-diastolic diameter (mm), LVESV – LV end-systolic volume (ml), LVEDV – LV end-diastolic volume (ml), ET – ejection time (ms), BSA – body surface area (m^2).

Left ventricular hypertrophy was diagnosed when IVSd > 11 mm, LVPWd > 11 mm and LVMi > 131 g/m^2 for men and > 100 g/m^2 for women [5–7]. Concentric LVH existed when LV interventricuar septum thickness was > 11 mm in diastole, LV posterior wall thickness was > 11mm in diastole, LV internal diameter was < 4.7 mm in diastole, LVMi > 131 g/m^2 in males and > 100 g/m^2 in females, with normal LVFS and relative wall thickness (RWT) > 45% [5–7]. Determinants of LV eccentric hypertrophy were LVMi > 131 g/m^2 in males and > 100 g/m^2 in females, LV internal diastolic diameter > 57 mm, with normal LVFS and RWT ≤ 45% [5–7]. Left ventricular dilatation was diagnosed when internal LVEDD > 57 mm and iEDV > ml/m^2, with preserved systolic function and normal LVMi [5–7]. Echocardiographic findings of disturbed LV systolic function include LVFS ≤ 25% and LVEF ≤ 50% [5–7].

Arterial blood pressure was calculated as average value of twelve monthly measurements prior to laboratory and echocardiographic investigations. Hypertension exists when arterial BP is ≥ 140/90 mmHg in patients treated with antihypertensive drugs.

Arteriovenous shunt blood flow was determined with colour flow Doppler ultrasound, just before echocardiographic examination, on SHIMADZU-2200 machine, using the 7.5 MHz probe. Arteriovenous shunt blood flow was calculated as mean of three measurements on efferent vein, each performed 2–4 cm proximally to anastamosis. Target Q_{AV} for adequate dialysis is 300–800 ml/min.

Dialysis adequacy was assessed using Kt/Vsp index, calculated with Daugridas second-generation formula: $Kt/Vsp = -\ln(C_2/C_1 - 0.008 \times T) + (4 - 3.5 \times C_2/C_1) \times UF/W$, where C_1 stands for predialysis serum urea, C_2 – postdialysis serum urea (mmol/l), T – treatment time (h), UF – ultrafiltration (L), W – body weight after dialysis (kg). According to Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines delivered Kt/V should be ≥ 1.2.

Clinical definition of cardiovascular morbidity and mortality

Heart failure was characterized with presence of dyspnea and two of the following parameters: increased jugular venous pressure, rales, pulmonary hypertension or interstitial pulmonary edema confirmed on chest radiography [8].

Ischemic heart disease was defined as the presence of angina pectoris and/or a previous myocardial infarction. According to the American College of Cardiology and European Society of Cardiology myocardial infarction is characterized with spontaneous chest pain lasting over 20 minutes, accompanied by rise in cTnI level (cTnI ≥ 2 ng/mL), ST segment elevation ≥ 0,2 mV in leads V2–V3, or ≥ 0,1 mV in other leads, or presence of Q wave ≥ 1 mm and ≥ 30 ms wide in at least two consequent leads. *De novo* left bundle brunch block can also be a sign of acute myocardial infarction [8].

Infectious endocarditis was diagnosed based on the presence of either two major or one major and 3–5 minor Duke's criteria. The major criteria are: at least two positive blood cultures, new valvular regurgitation, positive echocardiogram (oscillating intracardiac mass on valve or supporting structures). The minor criteria include: patient predisposition, fever, vascular phenomenon, immunologic phenomenon, microbiological evidence (one positive blood culture) and echocardiographic finding consistent with infectious endocarditis but do not meet a major criterion as noted above [9, 10].

Causes of death in patients on HD were classified as cardiovascular events (acute myocardial infarction, congestive heart failure and sudden death) and non-cardiovascular events (infection/sepsis, neoplasm, unknown) [11].

Statistic analysis

Results were statistically analyzed with Student′s *t* test and Mann-Whitney *U* test. Values < 0.05 and < 0.01 were considered significant.

Results

Average age of 115 investigated patients was 53.30 ± 12.17 years, average time on dialysis 4.51 ± 4.01 years and average single pool modeling fractional clearance of body water of urea (Kt/Vsp) – Kt/Vsp 1.17 ± 0.23. General patients' data are shown in Table 1.

According to the results of a two-year follow-up period the patients were separated in two groups, 86 alive persons and 29 deceased. The patients with adverse outcome had significantly lower serum albumin levels and significantly higher serum tHcy, cTnT, cTnI and LVMi (Table 2).

Table 3 shows the prevalence of traditional risk factors for the development of cardiovascular complications in patients on HD. Hypertriglyceridemia (43.48%) and unregulated hypertension (36.5%) were the most prevalent risk factors in our group.

Table 1

General patients data

Variables	Outcome		Significance	*p*
	Alive (86 patients)	Deceased (29 patients)		
	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
Sex (male/female)	56/30	15/14	$\chi^2_{emp} = 1.647$	0.199
Age (years)	52.47 ± 12.04	55.76 ± 12.41	$t_{emp} = -1.264$	0.209
Body mass index (kg/m^2)	22.76 ± 3.20	22.13 ± 3.15	$t_{emp} = 0.925$	0.357
Time on dialysis (years)	4.50 ± 4.20	4.55 ± 3.45	$Z_{emp} = -0.612$	0.541
Dialysis adequacy-Kt/Vsp*	1.18 ± 0.21	1.12 ± 0.29	$t_{emp} = 1.206$	0.230

*Single pool modeling fractional clearance of body water of urea (KT/V)

Table 2

Basic patients parameters based on outcome during the two-year follow-up

Variables	Outcome		Significance	*p*
	Alive (86 patients)	Deceased (29 patients)		
	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
Systolic blood pressure (mmHg)	137.67 ± 20.39	139.48 ± 22.85	$t_{emp} = -0.401$	0.690
Diastolic blood pressure (mmHg)	83.95 ± 12.54	84.48 ± 14.10	$t_{emp} = -0.190$	0.849
Mean arterial pressure (mmHg)	101.86 ± 14.77	102.82 ± 16.19	$t_{emp} = -0.294$	0.769
Hemoglobin (g/l)	90.60 ± 14.74	86.90 ± 11.82	$t_{emp} = 1.227$	0.222
Hematocrit (%)	26.89 ± 4.44	25.98 ± 3.51	$t_{emp} = 0.997$	0.321
Total proteins (g/l)	73.38 ± 6.10	73.41 ± 6.49	$t_{emp} = 0.274$	0.784
Serum albumin (g/l)	41.31 ± 4.36	38.31 ± 4.71	$t_{emp} = 3.145$	0.002
Total cholesterol (mmol/l)	4.65 ± 1.11	4.50 ± 1.17	$t_{emp} = 0.653$	0.515
LDL*-cholesterol (mmol/l)	2.64 ± 0.82	2.56 ± 0.86	$t_{emp} = 0.422$	0.674
HDL†-cholesterol (mmol/l)	1.05 ± 0.28	1.07 ± 0.33	$t_{emp} = -0.284$	0.777
Triglycerides (mmol/l)	2.00 ± 1.18	1.78 ± 0.83	$t_{emp} = 0.968$	0.335
Lipoprotein (a) (g/l)	0.26 ± 0.26	0.28 ± 0.26	$Z_{emp} = -0.309$	0.757
Apolipoprotein A-I (mg/dl)	1.30 ± 0.26	1.28 ± 0.31	$t_{emp} = 0.405$	0.686
Apolipoprotein B (mg/dl)	1.05 ± 0.35	1.06 ± 0.30	$t_{emp} = -0.195$	0.845
C-reactive protein (mg/l)	5.17 ± 5.64	14.39 ± 13.89	$Z_{emp} = -1.150$	0.250
Homocystein (μmol/l)	22.25 ± 8.86	26.80 ± 8.42	$t_{emp} = -2.419$	0.017
Troponin T (ng/ml)	0.09 ± 0.12	0.30 ± 0.37	$Z_{emp} = -2.476$	0.013
Troponin I (ng/ml)	0.14 ± 0.46	0.35 ± 0.55	$Z_{emp} = -4.893$	0.0001
Left ventricular mass index (g/m^2)	138.02 ± 34.57	161.13 ± 53.61	$t_{emp} = -2.681$	0.008
End-diastolic volume index (ml/m^2)	98.46 ± 33.61	107.76 ± 37.18	$t_{emp} = -1.254$	0.212
Left ventricular fractional shortening (%)	33.01 ± 7.35	31.04 ± 8.70	$t_{emp} = 1.191$	0.236
Left ventricular ejection fraction (%)	68.86 ± 10.44	65.70 ± 12.79	$t_{emp} = 1.330$	0.186

*low-density lipoproteins; †high-density lipoproteins

Table 3

The prevalence of traditional risk factors for cardiovascular complications in patients on regular hemodialysis

Traditional risk factor	Percent of patients
Triglycerides (> 1.7 mmol/l)	43.48
Unregulated hypertension predialysis blood pressure (> 140/90 mmHg)	36.52
Total cholesterol (tChol > 5.2 mmol/l)	18.26
High density lipoproteins (HDL < 1.0 mmol/l)	20.87
Lipoprotein (a) [Lp(a) > 0.3 g/l]	29.57
Diabetes mellitus	11.30
Cigarette smoking	10.43
Body mass index (BMI > 25 kg/m^2)	4.35

Table 4 shows the prevalence of non-traditional risk factors for cardiovascular complications in patients on HD. Hyperhomocysteinemia and anemia had the highest prevalence (86.09% and 76.52%, respectively).

The prevalence of LV morphology alterations is shown in Table 5. Left ventricular hypertrophy was present in 71.31% (82) of the patients, while only 14.78% (17) had normal LV morphology. Disturbed systolic function, accompanied by symptoms and signs of congestive hart failure, was present in 8.70% of the patients, as shown in Table 6.

Valvular calcification was present in 48.70% (56) of the patients (Table 7), and pericardial effusion in 25.22% (29), as shown in Table 8.

Disrrhythmias were present in 20.87% (24) of the patients. Disrrhythmias due to impaired impulse generation (atrial fibrillation (4.35%), atrial flutter (0.87%), ventricular extrasystole (5.22%) and supraventricual extrasystole (0.87%) were more frequent than those involving impaired impulse conduction (complete right bundle branch block (1.74%), incomplete left bundle branch block (4.35%), sec-

Table 4

The prevalence of non-traditional risk factors for cardiovascular complications in patients on regular hemodialysis

Non-traditional risk factors	Percent of patients
Hemocystein (tHcy > 15 μmol/l)	86.09
Anemia (Hb < 110 g/l)	78.26
C reactive protein (CRP > 5 mg/l)	34.78
$Ca \times PO_4$ solubility product ($Ca \times PO_4 > 4.4$ mmol^2/l^2)	36.52
Phosphate ($PO_4 > 1.7$ mmol/l)	31.30
Secondary hyperparathyroidism (PTH > 300 pg/ml)	20.00
Arteriovenous shunt blood flow ($Q_{AV} > 1\,000$ ml/min)	9.57

Table 5

Echocardiographic assessment of left ventricular (LV) morphology in patients on regular hemodialysis

LV morphology	Percent of patients
Concentric LV hypertrophy	28.70
Eccentric LV hypertrophy	42.61
LV dilatation	13.91
Normal LV morphology	14.78

Table 6

Echocardiographic assessment of left ventricular (LV) systolic function in patients on regular hemodialysis

LV systolic function	Percent of patients
LV fractional shortening > 25% and LV ejection fraction > 50%	91.30
LV fractional shortening ≤ 25% and LV ejection fraction ≤ 50%	8.70*

*disturbed systolic function

Table 7

Echocardiographic assessment of heart valves calcification in patients on regular hemodialysis

Heart valve calcification (CVC)	Percent of patients
Mitral valve calcification	14.79
Aortic valve calcification	33.91
No CVC	51.30

Table 8

Echocardiographic assessment of pericardial effusion in patients on regular hemodialysis

Pericardiol effusion	Percent of patients
No pericardiol effusion	74.78
Present LVPW*	21.74
Present LVPW/RVAW†	3.48

*left ventricular posterior wall; †right ventricular anterior wall

ond degreee atrioventricular block (0.87%), as shown in Table 9.

Cardiovascular complications account for 62.7% of all deaths in patients on HD (Table 10). The average annual mortality rate in our patients was 13.74%, while the average annual cardiovascular mortality rate was 8.51% (Table 11).

Discussion

Patients on HD have 10–20 times greater risk of cardiovascular mortality compared to general population [12]. These patients are exposed to a number of traditional and nontraditional risk factors for cardiovascular complications [13, 14]. Traditional risk factors include hypertension, hyperlipidemia,

Table 9

Disrrhythmia in patients on regular hemodialysis

Disrrhythmia	Percent of patients
No dissrhythmia	79.13
Present disrrhythmia	20.87
- disturbed impulse generation (2/3 of the patients)	
- disturbed impulse conduction (1/3 of the patients)	

Table 10

Cause of death in patients on hemodialysis during two-year follow-up

Cause of death		Patients	
		n	%
Cardiovascular	sudden cardiac death *(Cardiac arrest cause ignota)*	5	17.24
	acute myocardial infarction	1	3.45
	pulmonary tromboembolism	2	6.90
	pericardial effusion	1	3.45
	disrrhythmia	3	10.34
	acute heart failure	3	10.34
	infectious endocarditis	1	3.45
	valvular heart disease	1	3.45
	cerebrovascular insult	1	3.45
Non-cardiovascular	pneumonia	2	6.90
	sepsis	3	10.34
	neoplasm	2	6.90
	gastrointestinal bleeding	3	10.34
	acute abdomen	1	3.45
Total		29	100.00

Table 11

Overall and cardiovascular mortality rate in patients on hemodialysis during two-year follow-up

Mortality	Mortality rate		
	1st year	2nd year	Average biennial
Overall mortality	14 (12.17%)	15 (15.31%)	13.74%
Cardiovascular mortality	9 (7.83%)	9 (9.18%)	8.51%

cigarette smoking, diabetes and obesity. Nontraditional risk factors encompass a number of hemodynamic and metabolic factors. Hemodynamic factors include anemia, sodium and water retention, and increased shunt blood flow. Metabolic risk factors are hyperhomocysteinemia, oxidative stress, microinflammation and disturbed calcium and phosphate metabolism [13–17]. Our patients had similar distribution of cardiovascular risk factors as reported in other studies in patients on HD: 86.09% had hyperhomocysteinemia, 76.52% had anemia, 43.48% had hypertriglyceridemia and 36.52% had uncontrolled hypertension [18–23].

Left ventricular hypertrophy is a strong predictor of cardiovascular morbidity and mortality in patients on HD [24]. Increase of LVMi by ≥ 1,0 g/m^2/month is associated with increased risk of cardiovascular complications [24]. Left ventricular hypertrophy was present in 71.31% of our patients on HD. Similar rate of LVH on HD was reported by other authors [25–27]. In patients with normal LV volume (iEDV ≤ 90 ml/m^2) and normal systolic function (LVFS > 25%, LVEF > 50%), LVMi > 120 g/m^2 and LVMi/iEDV > 2.2 g/ml are independently associated with late mortality (death > 2 years following start of HD treatment) [28, 29]. Timely detection of risk factors and adequate treatment enable regression of LVH in patients on HD [30].

Aortic valve calcification is present in 28–58% of treated with patients on HD, while mitral valve calcification was found in 24%, as reported in previous studies [31]. In our study group, aortal valve calcification was found in 33.91% of patients and mitral valve calcification in 14.79%. Aortic valve calcification is associated with high peak transaortic blood flow, values ≥ 2.5 m/s indicating aortic stenosis [31]. Patients' age, time on dialysis, hyperphosphatemia and high calcium-phosphate product significantly contribute to the development of aortic valve calcification [31]. The annual incidence of hemodynamically significant aortic stenosis in patients on HD is 3.3% [32]. Aortic valve calcification leads to aortic stenosis, LV pressure overload and concentric LVH [31, 32]. Long term LV pressure overload caused by aortic stenosis leads to progression of LVH from adaptive to maladaptive stage, development of cardiomyopathy, myocyte loss and heart failure [32]. Mitral valve calcification causes left atrial dilatation and atrial fibrillation (absolute arrhythmia) [33, 34]. Secondary hyperparathyroidism, hyperphosphatemia and high calcium-phosphate product are associated with calcification of heart valves, coronary arteries and increased cardiovascular mortality [33, 34].

Intermitent atrial fibrillation is present in 16% of patients on HD [35]. It usually appears during HD session and stops spontaneously without therapeutic intervention 2–3 hours after the HD session. Propafenone was used successfully in both acute atrial fibrillation and as a prophylactic agent in patients on HD [36].

The prevalence of symptomatic pericardial disease in patients treated with HD is 11.8–21%. Mortality rate due to pericardial disease is 1.5% [37]. Pericardial effusion was present in 25.22% of our patients, while 3.48% of them had clinically significant effusion. Risk factors for development of pericarditis and pericardial effusion in end-stage renal disease patients are uremia, volume overload, malnutrition, inadequate HD, uncontrolled secondary hyperparathyroidism, high calcium-phosphate product and infection [37].

Risk factors for heart arrhythmia in patients treated with HD are: ischemic heart disease, LVH, congestive heart failure, pericardial effusion and electrolyte dysbalance [38]. The

prevalence of atrial disrrhythmia in end-stage renal disease patients on HD is 6%, atrial fibrillation being the most common [38]. Ischemic heart disease, LVH and mitral valve calcification are the most often correlated with atrial fibrillation [38]. The prevalence of ventricular extrasystole in our study group was 5.22%, and the prevalence of persistent atrial fibrillation was 4.35%.

Congestive heart failure, ventricular disrrhythmias, sudden cardiac death and acute myocardial infarction account for at least 40% of cardiovascular deaths in patients on HD [39]. The annual overall mortality rate in patients on HD is 6–16% and the annual cardiovascular mortality rate is 9% [39]. Our results show that annual overall mortality rate is 13.74% and average annual cardiovascular mortality rate is 8.51%. These results correspond with the mentioned published data [39].

The strategy for lowering overall and cardiovascular mortality in patients on HD should include identification and selection of high-risk patients, permanent evaluation of dialysis adequacy, maintaining better hemodynamic stability and electrolyte balance, and constant evaluation of prescribed cardioprotective therapy [39–41]. The strategy for identifying patients at high risk for cardiovascular complications and cardiovascular mortality should encompass determination of serum cardiac troponins, electrocardiographic markers, such as length of QTc interval and QTc-interval dispersion, and echocardiographic markers (LVMi) [35, 40–43].

Early detection of high-risk patients enables timely implementation of adequate therapeutic strategy. The primary therapeutic strategy for lowering cardiovascular mortality rate in patients on HD should include: antiaggregation therapy, control of lipid metabolism disorders and hypertension, while secondary therapeutic strategy includes coronary revascularisation and controle of heart rhythm disorders [44–48].

Conclusion

Patients on HD have high risk for cardiovascular morbidity and mortality. Hiperhomocysteinemia, anemia, hypertriglyceridemia and uncontrolled hypertension had the highest prevalence of cardiovascular risk factors among all investigated. Echocardiographic assessment for cardiovascular status in patients on HD identifies those with increased risk of cardiovascular complications, LVH, congestive heart failure, and heart valve calcification. Establishing the most sensitive parameters for identifying patients at risk for cardiovascular complications enables successfull treatment.

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O R I G I N A L N I Č L A N C I UDC: 616.894-053.8:577.122]-07

Beta amiloidni i tau protein u cerebrospinalnoj tečnosti: biomarkeri Alchajmerove bolesti

Cerebrospinal fluid amyloid beta and tau protein: biomarkers for Alzheimer's disease

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Apstrakt

Uvod/Cilj. Simptomatsko lečenje Alchajmerove bolesti (AB) inhibitorima acetilholinesteraze doprinelo je važnosti postavljanja dijagnoze ove bolesti u početnom stadijumu. Posebno je istaknut značaj biomarkera u cerebrospinalnoj tečnosti (CST) u ranom otkrivanju AB: niskih vrednosti β amiloidnog proteina sa 42 aminokiseline ($A\beta_{42}$), povišenih vrednosti totalnog tau (T-tau), kao i fosforilisanog tau proteina (P-tau). Cilj ovog rada bio je određivanje nivoa $A\beta_{42}$, T-tau i P-tau proteina u CST obolelih od AB i zdravih, kontrolnih osoba, uporedive starosti, pola i obrazovanja. **Metode.** Lumbalna punkcija urađena je kod 63 bolesnika sa AB i 26 kontrolnih osoba koje su imale neku od ortopedskih operacija. Za određivanje $A\beta_{42}$, T-tau i P-tau u CST korišćeni su enzimski imunotestovi sa čvrstom fazom, tzv. sendvič ELISA (Innotest; Innogenetics, Belgija). **Rezultati.** Srednje vrednosti T-tau i P-tau proteina bile su značajno više u CST kod bolesnika sa AB u odnosu na kontrolnu grupu ($p < 0{,}001$), za razliku od niskih vrednosti $A\beta_{42}$ u grupi bolesnika sa AB ($p < 0{,}001$). Pokazano je značajno progresivno sniženje vrednosti $A\beta_{42}$, kao i značajno progresivno povećanje vrednosti T-tau i P-tau između tri podgrupe bolesnika sa različitom težinom AB i kontrolne grupe. **Zaključak.** Dobijeni rezultati ukazuju na značaj ovih biomarkera u CST kao dopunskog dijagnostičkog sredstva za AB, posebno u njenom ranom stadijumu.

Ključne reči:
alchajmerova bolest; cerebrospinalna tečnost; biološki pokazatelji; amiloid beta-protein; tau proteini.

Abstract

Background/Aim. Introduction of acetylcholine esterase inhibitors as a symptomatic treatment of Alzheimer's disease (AD) has additionally highlighted the importance of diagnostic markers in cerebrospinal fluid (CSF) for early AD diagnosis: low level of 42 amino acid form of amyloid-β peptide ($A\beta_{42}$), and levels of tau protein (T-tau) and phosphorylated tau protein (P-tau). The aim of this study was to diagnostic potential of CSF biomarkers T-tau, P-tau and $A\beta_{42}$ as biochemical markers for AD. **Methods.** Lumbar puncture was performed in 63 patients with AD and 26 control subjects who passed orthopedic surgery. The Innotest, ELISA sandwich test (Innogenetics – Belgium) was used for measuring the levels of T-tau, P-tau and $A\beta_{42}$. **Results.** The patients and the control group did not differ in age, education and sex. Mean levels of CSF T-tau and P-tau were significantly higher in the patients with AD ($p < 0.001$) compared to the control group, in contrast to significantely lower CSF $A\beta_{42}$ in AD group ($p < 0.001$). A significant progressive decrease of $A\beta_{42}$, as well as significant progressive increase of T-tau and P-tau was found among AD subgroups (according to MMSE staging) and controls. **Conclusion.** The obtained results suggest that these biomarkers may be supportive in the diagnosis of AD, especially in the early course of the disease and could be used in the routine clinical practice considering the approaching target therapeutics.

Key words:
alzheimer disease; cerebrospinal fluid; biological markers; amyloid beta-protein; tau proteins.

Uvod

Postavljanje dijagnoze Alchajmerove bolesti (AB) tokom života na nivou je verovatne AB i zasniva se na kliničkim kriterijumima Dijagnostičkog i statističkog priručnika za mentalne poremećaje i isključivanju drugih poznatih uzroka demencije (*National Institute of Neurological and Communicative Disorders and Stroke – Alzheimers Disease and Re-*

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lated Disorders Association, tj. NINCDS–ARDA kriterijumima) koje je postavio Nacionalni institut SAD za neurološka oboljenja, cerebrovaskularne bolesti i AB još pre 25 godina [1, 2]. Definitivna dijagnoza AB postavlja se tek patološkim ispitivanjem tkiva mozga dobijenog biopsijom ili autopsijom, što se smatra zlatnim standardom [2]. Iako korišćenje ovih kliničkih kriterijuma u tercijarnim centrima omogućava tačnost postavljene dijagnoze od 65 do 90%, mora se uzeti u obzir da se ovi podaci dobijaju od bolesnika u razvijenim fazama bolesti nakon nekoliko godina praćenja [3–5].

Iako trenutno ne postoji nijedan dovoljno specifičan i senzitivan biološki marker za postavljanje dijagnoze AB, kao najbolji kandidati u istraživanjima u protekle dve decenije izdvajaju se veličina hipokampalne i entorinalne atrofije na magnetnoj rezonanciji (MR) mozga, ispitivanje metabolizma glukoze i beta amiloidnih depozita pomoću pozitronske emisione tomografije (PET) i određivanje β amiloidnog proteina sa 42 aminokiseline ($A\beta_{42}$), ukupnog tau (T-tau), kao i fosforilisanog tau proteina (P-tau) u cerebrospinalnoj tečnosti (CST) [6–13]. Upravo je CST prihvaćen kao dobar izvor biomarkera, s obzirom da je u neposrednoj komunikaciji sa ekstracelularnim strukturama moždanog parenhima čije se promene odražavaju i u CST. Pretpostavljeno je da bi biohemijski markeri mogli da reflektuju patogenetske mehanizme AB tipa stvaranja beta amiloidnih depozita, degeneracije sinapsi i neurona, te hiperfosforilacije tau proteina formiranjem neurofibrilarne klubadi. Nedostatak bioloških markera bio je osnovna smetnja u razvoju novih terapijskih mogućnosti [14–16]. Upravo stoga, kao i zbog dokazane vrednosti, biomarkeri u CST ($A\beta_{42}$, T-tau i P-tau) uključeni su u nove dopune NINCDS-ARDA kriterijuma 2007. godine [17].

Cilj našeg rada bio je da se ispita značaj određivanja $A\beta_{42}$, T-tau i P-tau u CST u postavljanju dijagnoze AB u većoj grupi bolesnika.

Metode

U periodu od aprila 2006. do marta 2008. godine u Centru za poremećaje pamćenja i demenciju Instituta za neurologiju dijagnostikovano je 135 bolesnika sa verovatnom AB. Od ovog broja, 76 (56%) dalo je pristanak za dijagnostičku lumbalnu punkciju (LP) i uzimanje uzorka CST, a biohemijski obrađena su 63 uzorka. Dijagnoza AB postavljena je prema kliničkim DSM-IV kao i NINCDS-ADRDA kriterijumima [1, 2]. Težina demencije određivana je *Mini-Mental* skalom (MMSE), a trajanje bolesti procenjivano je na osnovu heteroanamnestičkih podataka o vremenu kada su se javili prvi simptomi [18].

Kontrolnu grupu činilo je 26 bolesnika koji su primljeni na Institut za ortopediju Kliničkog centra Srbije (KCS) zbog operacije kuka ili kolena u spinalnoj anesteziji. Nijedan od ovih bolesnika nije imao neurološka, psihijatrijska, maligna niti sistemska oboljenja (npr. reumatoidni artritis), dok su osobe sa MMSE skorom manjim od 28 isključivane iz kontrolne grupe.

Studiju je odobrio Etički komitet KCS, a bolesnici (grupa sa AB kao i kontrolna grupa) uključivani su u studiju po davanju pristanka (ili pristanka staraoca, u slučaju njihove nemogućnosti).

U prepodnevnim časovima, od 10 do 12 h (pre davanja spinalne anestezije u kontrolnoj grupi), rađena je LP u sedećem položaju, nakon prethodnog 30 minutnog sedenja. Uzorci CST (1 ml) uzimani su u polipropilenske epruvete, čuvani u ledu, a potom u roku od 2 sata vršeno je njihovo centrifugiranje i zamrzavanje na -80 ºC [19].

Za određivanje $A\beta_{42}$, T-tau i P-tau u CST korišćeni su enzimski imunotestovi sa čvrstom fazom (tzv. sendvič ELISA) *Innotest* $A\beta_{42}$, *Innotest* hTAU-Ag i *Innotest Phospho-tau* $_{(181P)}$ proizvođača *Innogenetics*, Belgija. Kod određivanja T-tau u testu koriste se antitela koja detektuju sve izoforme ovog proteina; za određivanje $A\beta_{42}$ u imunotestu prisutno je antitelo koje se specifično vezuje samo za formu Aβ od 42 amino-kiseline, dok je antitelo koje se koristi za određivanje P-tau proteina specifično za oblik tau proteina koji je fosforilisan na 181. aminokiselinskom ostatku – treonin 181 (P-tau 181) [20–22].

Za određivanje razlika između grupa korišćena je analiza varijanse (ANOVA) i *post-hoc* Scheffeova procedura, dok je za utvrđivanje postojanja korelacija upotrebljen Pearsonov koeficijent korelacije. Od neparametarskih testova, tamo gde je bilo neophodno korišćen je Mann-Whitney *U* test. Statističke analize rađene su u programu *Statistica release*.

Rezultati

Kontrolna grupa ortopedskih bolesnika bila je kognitivno očuvana (MMSE raspon od 28 do 30), dok je u grupi bolesnika sa AB MMSE raspon iznosio od 0 do 26. Drugi demografski podaci nisu se razlikovali između ove dve grupe (tabela 1).

Nivo $A\beta_{42}$ u CST kod bolesnika sa AB bio je statistički značajno snižen u odnosu na nivo ovog markera u CST kod kontrolne grupe, paralelno sa povišenim nivoima P-tau i T-tau i značajno nižim indeksom $A\beta_{42}$/P-tau (tabela 2).

Tabela 1

Osnovne demografske karakteristike bolesnika sa Alchajmerovom bolesti (AB) i kontrolnih osoba

Karakteristike bolesnika	Bolesnici sa AB	Kontrolna grupa	*p*
Broj	63	26	
Pol (M/Ž)	26/37 (41% M)	6/20 (23% M)	> 0,05
Starost (godine) ($\bar{x} \pm SD$)	66,7 ± 10,6	65,3 ± 11,9	> 0,05
Obrazovanje (godine) ($\bar{x} \pm SD$)	11,22 ± 3,54	11,31 ± 3,64	> 0,05
MMSE ($\bar{x} \pm SD$)	12,8 ± 7,7	29,2 ± 0,8	< 0,001

MMSE – *Mini Mental* skala

Tabela 2

Beta amiloidni protein sa 42 aminokiseline ($A\beta_{42}$), ukupni tau (T-tau) i fosforilisani tau protein (P-tau) u cerebrospinalnoj tečnosti kod ispitivanih grupa

Markeri u cerebrospinalnoj tečnosti	Bolesnici sa AB (n = 63) ($\bar{x} \pm SD$)	Kontrolna grupa (n = 26) ($\bar{x} \pm SD$)	*p*
$A\beta_{42}$ (ng/l)	443,4 ± 227,2	825,3 ± 247,7	< 0,001
T-tau (ng/l)	616,9 ± 436,1	246,9 ± 100,4	< 0,001
P-tau (ng/l)	147,5 ± 86,5	79,1 ± 42,5	< 0,001
$A\beta_{42}$/P-tau	4,7 ± 4,6	14,2 ± 8,9	< 0,001

AB – Alchajmerova bolest

Bolesnici sa AB bili su rasvrstani u tri podgrupe prema MMSE skoru: grupa AB I, raspon MMSE od 20 do 30, grupa AB II, raspon od 10 do 19 i grupa AB III, raspon od 0 do 9 (tabela 3). Kada je analiziran ceo uzorak, nivo $A\beta_{42}$ značajno se statistički razlikovao po ispitivanim podgrupama ($p < 0,001$). Najveće vrednosti ovog markera nalazile su se u CST kod bolesnika sa najvišim MMSE skorom, tj. u kontrolnoj grupi i grupi bolesnika sa početnom AB (AB I). Značajno su se razlikovale i vrednosti T-tau kao i P-tau proteina ($p < 0,001$), pa su se tako najveće vrednosti nalazile kod bolesnika sa najnižim MMSE skorom, tj. najtežom demencijom (AB III). Indeks $A\beta_{42}$/P-tau značajno je najveći u kontrolnoj grupi i grupi bolesnika sa početnom AB (AB I) ($p < 0,001$).

Post-hoc Scheffeova analiza, uprkos trendu, nije pokazala statistički značajnu razliku između podgrupa bolesnika sa AB u različitim stadijumima težine bolesti.

U grupi bolesnika sa AB nije pokazana značajna povezanost između godina starosti i pola, sa jedne, i markera u CST, sa druge strane. Nasuprot tome, u kontrolnoj grupi dobijena je značajna povezanost između godina starosti, P-tau i odnosa $A\beta_{42}$/P-tau, kao i pola i indeksa $A\beta_{42}$/P-tau (tabela 4).

Nije pokazana značajna korelacija između MMSE skora i $A\beta_{42}$, T-tau, P-tau i $A\beta_{42}$/P u grupi bolesnika sa AB, kao ni značajna korelacija između dužine trajanja AB i gore navedenih parametara u CST.

Tabela 3

Markeri iz cerebrospinalne tečnosti prikazani po pojedinačnim stadijumima Alchajmerove bolesti (AB) i u kontrolnoj grupi

Parametar	AB I (n = 10) ($\bar{x} \pm SD$)	AB II (n = 39) ($\bar{x} \pm SD$)	AB III (n = 14) ($\bar{x} \pm SD$)	kontrolna grupa (n = 26) ($\bar{x} \pm SD$)	*p*
Broj	10	39	14	26	< 0,001
MMSE	23,7 ± 2,9	14,1 ± 3,1	1,4 ± 2,8	29,2 ± 0,8	< 0,001
$A\beta_{42}$ (ng/l)	512,8 ± 240,1	442,7 ± 244,9	389,2 ± 151,4	825,3 ± 247,7	< 0,001
T-tau (ng/l)	589,6 ± 286,3	612,7 ± 469,7	646,6 ± 431,6	246,9 ± 100,4	< 0,001
P-tau (ng/l)	139,6 ± 85,4	152,9 ± 85,6	171,3 ± 96,3	76,1 ± 44,4	< 0,001
$A\beta_{42}$/P-tau	4,9 ± 4,8	4,6 ± 4,2	4,1 ± 4,5	14,2 ± 8,9	< 0,001

$A\beta_{42}$ – β amiloidni protein sa 42 aminokiseline; T-tau – ukupni tau protein; P-tau – fosforilisani tau protein; AB I, AB II, AB III – videti objašnjenje u tekstu; MMSE – *Mini Mental* skala

Diskusija

U našoj studiji bile su utvrđene snižene vrednosti $A\beta_{42}$, uporedo sa povišenim vrednostima T-tau i P-tau u CST kod bolesnika sa AB u odnosu na kontrolnu grupu. Nivoi ovih markera razlikuju se zavisno od stadijuma AB.

Srednje vrednosti $A\beta_{42}$, T-tau i P-tau u CST u našem uzorku u saglasnosti su sa nalazima u švedskoj i holandskoj populaciji, gde je korišćena ista metodologija [23–25]. S obzirom da su istraživanja pokazala različite vrednosti markera u CST u zavisnosti od doba dana kada je LP rađena, poštovano je vreme uzimanja uzoraka isključivo u periodu između 10 h i 12 h [26]. Uzorci CST uzimani su u polipropilenske epruvete zbog toga što se pokazalo da se pomenuti biomarkeri zadržavaju na zidu staklenih i polistirenskih epruveta, što je doprinosilo nepreciznosti rezultata [12, 27].

Tabela 4

Korelacija između godina starosti, pola i markera cerebrospinalne tečnosti u kontrolnoj grupi

Parametar	Aβ42	T-tau	P-tau	$A\beta_{42}$/P-tau
Starost	r* = -0,37	r = 0,46	r = 0,31	r = -0,63
p	> 0,05	> 0,05	< 0,05	< 0,001
Pol	r = -0,05	r = 0,37	r = 0,27	r = -0,44
p	> 0,05	> 0,05	> 0,05	< 0,05

$A\beta_{42}$ – β amiloidni protein sa 42 aminokiseline; T-tau – ukupni tau protein; P-tau – fosforilisani tau protein; * Pearsonov koeficijent korelacije

Naš rezultat, takođe, u saglasnosti je sa većinom objavljenih studija u kojima je pokazano da su vrednosti $A\beta_{42}$ do 50% niže kod AB u odnosu na kontrolnu grupu [13]. Samo u jednoj studiji izmereni su povećani CST nivoi $A\beta_{42}$ u AB, ali se to objašnjava metodološkim razlikama [28]. Novija istraživanja sa patohistološkom potvrdom AB ukazuju na značajnu korelaciju između niskih vrednosti $A\beta_{42}$ u CST i velikog broja amiloidnih plakova u hipokampusu i neokortikalnim strukturama, sugerišući na taj način da se sniženje $A\beta_{42}$ jednim delom može pripisati njegovom taloženju u amiloidne plakove [29].

Iako se niske vrednosti $A\beta_{42}$ mogu naći i u drugim vrstama demencija kao što je demencija sa Lewyjevim telima (DLT), frontotemporalna (FTD) i vaskularna demencija (VD), prosečna vrednost senzitivnosti ovog markera iz CST za AB iznosi 86%, u poređenju sa kontrolnom grupom starih osoba, dok je specifičnost 90% [13, 30, 31].

I naši rezultati potvrđuju da su vrednosti T-tau za 200–300% više kod obolelih sa AB u odnosu na kontrolnu grupu [13]. Smatra se da vrednosti T-tau u CST označavaju intenzitet opšteg neuronalnog oštećenja. Tako, na primer, prolazno povišenje T-tau u CST kod akutnog moždanog udara koreliše sa veličinom infarkta na kompjuterizovanoj tomografiji [32]. Posebno visoke vrednosti ovog markera merene su u CST kod obolelih od Jakob-Creutzfeldtove bolesti (CJB), koja se karakteriše intenzivnim propadanjem neurona [33]. Uz to, specifičnost ovog markera nije idealna jer su u najvećem broju studija sa VD, takođe, nađene visoke vrednosti T-tau [31, 34].

Što se tiče drugih tipova demencija nivoi T-tau u CST mogu biti normalni ili blago povišeni, kao što je to slučaj sa FTD i DLT [13, 17, 31, 35].

Stoga je u našoj studiji određivan i P-tau protein fosforilisan na poziciji treonina 181, a dobijeni rezultati u saglasnosti su sa rezultatima iz drugih studija [13, 22, 36, 37]. Smatra se da vrednosti P-tau u CST odslikavaju stepen fosforilacije tau proteina. Ovome idu u prilog i nalazi koji govore da nakon akutnog moždanog udara nema promene P-tau, iako se nivo T-tau značajno povećava [38]. Takođe, u CJB, P-tau je normalan ili blago povišen uprkos značajno povišenim vrednostima T-tau [39]. Ovi podaci sugerišu da P-tau nije samo marker neuronske degeneracije ili oštećenja, nego označava i stepen fosforilacije tau proteina, a samim tim moguće je i da ukazuje na količinu neuronskih klubadi u mozgu bolesnika sa AB. Specifičnost P-tau za AB u odnosu na druge vrste demencija veća je u poređenju sa ostalim parametrima ($A\beta_{42}$ i T-tau), s obzirom da se njegove normalne vrednosti nalaze kod VD, FTD, DLT, kao i CJB [13, 36, 37].

Zaključak

Određivanje biomarkera u CST ($A\beta_{42,}$ T-tau i P-tau proteina) kod bolesnika sa AB može da bude korisna metoda u rutinskoj kliničkoj praksi, posebno u razlikovanju AB u početnom stadijumu i normalnog starenja, a u svetlu novih, najavljenih terapijskih mogućnosti. Oni se ne smeju koristiti, međutim, kao jedinstven dokaz AB, već kao dopunsko dijagnostičko sredstvo postojećim kliničkim kriterijumima.

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The effects of polarized light therapy in pressure ulcer healing

Uticaj terapije polarizovanom svetlošću na zarastanje dekubitusne ulceracije

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Abstract

Background/Aim. Neglecting polarized light as an adjuvant therapy for pressure ulcers and methodology distinctions in the trials engaging polarized light are the reasons for many dilemmas and contradictions. The aim of this study was to establish the effects of polarized light therapy in pressure ulcer healing. **Methods.** This prospective randomized single-blind study involved 40 patients with stage I-III of pressure ulcer. The patients in the experimental group (E) were subjected, besides polarized light therapy, to standard wound cleaning and dressing. Standard wound cleaning and dressing were the only treatment used in the control group (C). A polarized light source was a Bioptron lamp. Polarized light therapy was applied for six min daily, five times a week, four weeks. The Pressure Ulcer Scale for Healing (PUSH) was used in the assessment of outcome. Statistic analysis included Mann Whitney Test, Fisher Exact Test, Wilcoxon Signed Rank test. **Results.** There were significant differences between the groups at the end of the treatment regarding the surface of pressure ulcer (E: 10.80±19.18; C: 22,97±25,47; $p = 0.0005$), rank of pressure ulcer (E: 5.90±2.48; C: 8.6±1.05; $p = 0.0005$) and total PUSH score (E: 7.35±3.17; C: 11.85±2.35; $p = 0,0003$). The patients in the experimental group had significantly better values of the parameters monitored than the patients in the control group. **Conclusion.** After a four-week polarized light therapy 20 patients with stage I–III ulcer had significant improvement in pressure ulcer healing, so it could be useful to apply polarized light in the treatment of pressure ulcers.

Key words:
pressure ulcer; therapeutics; physical medicine; phototherapy; wound healing; treatment outcome.

Apstrakt

Uvod/Cilj. Zanemarivanje polarizovane svetlosti kao pomoćne terapije za dekubitusne ulceracije i metode u trijažama izaziva dileme i kontradiktornosti. Cilj ovog rada bio je da se utvrde efekti primene polarizovane svetlosti na zarastanje dekubitusne ulceracije. **Metode.** Ova prospektivna randomizovana jednostruko slepa studija uključila je 40 bolesnika sa dekubitusnom ulceracijom faze I–III. Bolesnici eksperimentne grupe (E) bili su podvrgnuti, pored terapije polarizovanom svetlosti, i standardnom čišćenju i premazivanju rane. U kontrolnoj grupi (C) jedina primenjena terapija bilo je standardno čišćenje i premazivanje rane. Kao izvor polarizovane svetlosti korišćena je lampa Bioptron. Terapija polarizovanom svetlosti trajala je šest minuta dnevno, pet puta nedeljno, tokom četiri nedelje. Za određivanje efekata polarizovane svetlosti korišćena je skala zaceljivanja dekubitusnih ulceracija (*Pressure Ulcer Scale for Healing* – PUSH). Za statističku analizu primenjeni su testovi Mann Whitney, Fisher Exact i Wilcoxon Signed Rank. **Rezultati.** Nađena je značajna razlika između grupa na kraju tretmana. Naime, bolesnici eksperimentne grupe, kod kojih je osim standardne terapije primenjena i polarizovana svetlost, imali su značajno bolje zarastanje od bolesnika kontrolne grupe kod kojih nije primenjena polarizovana svetlost, u odnosu na površinu dekubitusne ulceracije (E: 10,80±19,18; C: 22,97±25,47; $p = 0,0005$), na grupu dekubitusne ulceracije (E: 5,90±2,48; C: 8,6±1,05; $p = 0,0005$) i ukupni skor PUSH (E; 7,35±3,17; C: 11,85±2,35; $p = 0,0003$). **Zaključak.** Posle četiri sedmice terapije polarizovanom svetlosti 20 bolesnika u fazi I–III dekubitusne ulceracije imalo je značajno bolje zaceljenje dekubitusnih ulceracija, te je poželjno primenjivati polarizovanu svetlost u lečenju dekubitusnih ulceracija

Ključne reči:
dekubitus; lečenje; medicina, fizikalna; fototerapija; rana, zarastanje; lečenje, ishod.

Introduction

Pressure ulcer, sometimes referred as decubitus ulcer or pressure sores, is a localized area of cellular necrosis [1, 2]. The National Pressure Ulcer Advisory Panel (NPUAP) in the United States, defines pressure ulcer as an area of unrelieved pressure resulting in ischemia, cell death, and tissue necrosis. It is usually localized over a bony prominence [3]. The most common sites of pressure ulcer formation are the ischium, the sacrum, the trochanter and the heel [4]. From the aspect of pathophysiology a local external pressure can cause hyperemia (skin redness), blue demarcation of the skin, necrosis and ulceration. The incidence of pressure ulcers varies widely by hospitalized population. In acute care hospitals the incidence ranges from 1

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to 29%, with prevalence of 3 to 69% [3, 5]. Within geriatric subpopulation, incidence rates as high as 24% with prevalence of 17, 4% [6]. These conditions are responsible for physical, social and vocational costs, as well as the economic cost of treating ulcer. A total cost of pressure ulcer treatment in the USA, for example, ranges from $ 1, 3 to $ 6 billion annually [3]. Medical providing of pressure ulcers implies their prevention and treatment. Prevention means skin inspection, skin care and pressure reduction modalities [2]. The measures of treatment are: education and nutrition of patients, prescribing the support surfaces, wound care in terms of its cleaning and dressing, surgery and the physical modalities as an adjuvant therapy [1–18].

Polarized light therapy, a kind of phototherapy, is a linearly polarized and polychrome light therapy. This light therapy contains a whole spectrum of visible rays, infrared A and B rays as well. Polarized light comes from refraction of common light through the specific laminated mirrors and admitted this light through photo filter system. Biologic effects of this physical modality are well known: enhancement of the cell membrane activities, acceleration of the production of the adenosine triphosphate (ADP) in mitochondria, return to normal cell membrane potential which was disturbed, stimulation of the regenerative processes. Additionally, fibroblast proliferation and deposition of collagen could be accelerated by this kind of physical therapy [19]. These, so-called cellular and subcellular polarized light effects are the base of systemic polarized light effects: improving microcirculation, diminishing inflammation, improving tissue oxygenation, enhancing of the wound healing, accelerating epithelialisation of wound and improving quality of early scar tissue formation [19]. Wounds and pressure ulcers befall to the most important indications for using polarized light therapy. There are no absolute contraindications for this kind of physical therapy [7].

Regarding pressure ulcers prevention and treatment, there are many dilemmas and contradictions. These are, connected with the physical modalities, so-called adjuvant therapies, which have long been described in the pressure ulcer literature. Among these therapies the authors suggest positioning and exercise therapy, hydrotherapy, ultrasound, pressure reduction measures, serial casting, low energy laser therapy, low-frequency current, electrical stimulation, hyperbaric oxygen therapy or new adjuvant therapies such as vacuum assisted therapy, normothermia and constant tension approximation [2, 3, 5, 6, 13, 20–26]. Of all the physical therapy options ultraviolet light is the most frequently recommended [3, 5–7, 13, 15, 16]. Today, physical therapy is an obligatory part of the protocols for the successful treatment of pressure ulcers [27]. In spite of that, dilemmas and contradictions exist and can be divided into three categories: unbelief in efficiency of physical therapy for the patients with pressure ulcers; negligence of polarized light as a relative new adjuvant therapy; methodology distinctions in the trials engaging of polarized light therapy [1–6, 8–10, 12–14, 19, 28–30]. All these facts give evidence of necessity for the replication of some studies, taking into account that a whole methodology and study design would be precise as more as possible. We are especially interested in wound characteristics, as the authors engaged with polarized light therapy were checking almost wound healing rate [19, 29].

The aim of our study was to establish the effects of polarized light therapy in the healing process of pressure ulcer.

Methods

We performed a prospective randomized single-blind study which involved 40 patients with several kinds and locations of pressure ulcers. Inclusion criteria were: 1) patients with stage I–III ulcer according the Pressure Ulcer Classification System; 2) absence of relative contraindications for using of polarized light; 3) absence of deterioration of a common disease or attack of new disease; 4) a patient's agreement to participate in the study [6, 7]. Before randomizing, subjects were excluded if: 1) they were previously in the study to treat their current pressure ulcer; 2) skin grafting was planned within one week; 3) nutrition was poor, as indicated by albumin levels below 3.0 g/dL; 4) presence of local or general infection, particularly the sacral (pylonidal) sinus or the sacral osteomyelitis; 5) necessity for drugs that can affect the skin and delay in healing, specially steroids, immunosuppressive agents, antineoplastic drugs and anticoagulants [11].

The patients who met inclusion criteria were randomly divided into the experimental (E) and the control group (C). The random divide was performed by the random numbers table [31]. The patients in the experimental group were treated using standard cleaning and dressing and polarized light therapy. The standard cleaning and dressing only were used in the control group. A linear polarized light source (Bioptron lamp) with the following technical characteristics was used: wavelength: 400–2000 nm; degree of polarization: > 95%; power density: 40 mW/cm^2; light energy: 2,4 J/cm^2. Polarized light therapy was performed for six min daily, at a distance of 10 cm, five times a week (Figure 1). Before the polarized light therapy, we splashed each wound by oxygen spray. All therapies were performed between 2 and 4 h p.m. The whole treatment lasted four weeks. All wounds were cleaned using 2% hydrogen peroxide. The standard dressing implied application of a gauze with normal saline (NaCl), then a dry gauze, next it a cotton wool and adhesive strip.

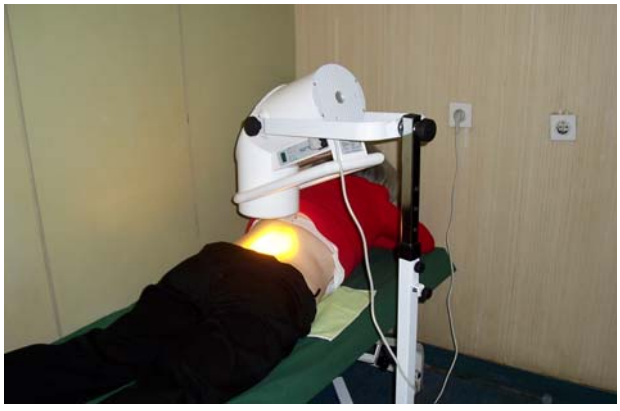

Fig. 1 – Polarized light therapy

The Pressure Ulcer Scale for Healing (PUSH) was used in assessment the effects of polarized light therapy. Toward this scale all wounds were described through the surface area measurement, exudates amount and surface appearance [32]. According to the statistical circumstances we divided these points into the surface of wounds, rank of wounds, exudates amount, tissue type and total PUSH score. Wound healing process was evaluated in a standard manner (centimeter ruler and some kind of callipers) by two independent blinded observers. Measurement was performed at the start and the end of the treatment.

Statistic analysis included Kolmogorov-Smirnov test, Shapiro-Wilk test, Mann Whitney Exact test, Exact Wilcoxon signed rank test and Fischers Exact test. Statistical significance was set up $p < 0.05$. The data were assessed by SPSS 10.0 for Windows.

Results

A total of 48 patients were recruited by a physiatrist, surgery specialist and physiotherapist. Out of them four patients refused to participate to the study. Two patients from the experimental group were withdrawn. One of them had deterioration of consciousness after stroke. Another was withdrawn because of anticoagulants drug administration. Two patients from the control group died in the second and third week of the treatment. A total of 40 patients participated to the study.

The groups were homogenous in terms of age and sex of patients, and duration of polarized light therapy. At the start of the treatment, there was no significant difference between groups regarding surface of pressure ulcer, rank of pressure ulcer and total PUSH score (Table 1).

The majority of the patients in both groups had pressure ulcers in the sacral area, the left hip and both heels (Table 2).

At the start of the treatment, there were significant differences between the groups regarding exudates amount and tissue type. Half of the patients in the experimental group (50%) had light exudates; the majority of the patients in the control group had no exudates (65%); epithelial tissue dominated in the experimental group (55%); in the control group half of the patients (50%) had completely covered wounds (Table 3).

Table 1

Subjects characteristics

Characteristics	Group		*p*
	Experimental (n = 20)	Control (n = 20)	
Age (years); ($\bar{x} \pm SD$)	61.85 ± 16.11	68.65 ± 19.87	0.06
Sex; n (%)			
– male	11 (55)	11 (55)	
– female	9 (45)	9 (45)	
Duration of a polarized light therapy (days); ($\bar{x} \pm SD$)	20.15 ± 3.57	21.0 ± 0	0.48
Surface of the pressure ulcers (cm^2); ($\bar{x} \pm SD$)	15.10 ± 17.61	19.15 ± 22.73	0.18
Rank of the pressure ulcers; ($\bar{x} \pm SD$)	7.40 ± 1.96	8.20 ± 1.51	0.20
Total PUSH* score of the pressure ulcers; ($\bar{x} \pm SD$)	10.65 ± 2.25	10.45 ± 2.74	0.79

* The Pressure Ulcer Scale for Healing

Table 2

Location of pressure ulcers

Location	Group			
	Experimental (n = 20)		Control (n = 20)	
	n	(%)	n	(%)
Low part of back	0	0	1	5
Righ-low part of back	1	5	0	0
Right buttock	1	5	0	0
Left buttock	1	5	1	5
Both buttocks	0	0	2	10
Sacral area	10	50	5	25
Right sacral-buttock area	1	5	0	0
Right iliac spine	0	0	1	5
Left hip	3	15	3	15
Right hip	0	0	1	5
Right heel	1	5	4	20
Left heel	2	10	2	10

Table 3

Exudate amount and tissue type of the pressure ulcers at the start of treatment

Characteristics	Group				*p*
	Experimental (n = 20)		Control (n = 20)		
	n	(%)	n	(%)	
Exudate amount					
– None	5	25	13	65	0.04
– Light	10	50	5	25	
– Moderate	5	25	2	10	
– Haevy	0	0	0	0	
Tissue type					
– Closed	2	10	10	50	0.01
– Epithelial	11	55	5	25	
– Granulation	7	35	4	20	
– Slough	0	0	1	5	

There were significant differences in the experimental group at the end of the treatment regarding the start of the treatment. A significant improvement was registered. Namely, surface of pressure ulcers, rank of pressure ulcers and total PUSH score were significantly smaller at the end of the treatment (Table 4).

There were significant differences in the control group at the end of the treatment as compared to the start of the treatment. A significant aggravation was registered. Namely, surface of pressure ulcers, rank of pressure ulcers and total PUSH score were significantly bigger at the end of the treatment (Table 5).

There were significant differences between the groups at the end of the treatment. The patients in the experimental group had significantly higer improvements in the surface of pressure ulcers, rank of pressure ulcers and total PUSH score than the patients in the control group (Table 6, Figures 2 and 3).

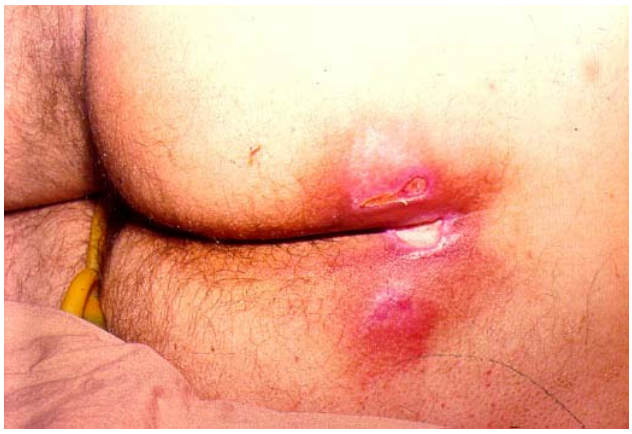

Fig. 2 – Pressure ulcus stage I–III before treatment with polarized light therapy

Table 4

Characteristics of the pressure ulcers at the start and the end of treatment in the experimental group

Characteristics	Start	End	*p*
Surface of the pressure ulcers (cm^2) ($\bar{x} \pm SD$)	15.10 ± 17.61	10.80 ± 19.18	0.01
Rank of the pressure ulcers ($\bar{x} \pm SD$)	7.40 ± 1.96	5.95 ± 2.48	0.0004
Total PUSH* score of the pressure ulcers ($\bar{x} \pm SD$)	10.65 ± 2.25	7.35 ± 3.17	0.0001

* The Pressure Ulcer Scale for Healing

Table 5

Characteristics of the pressure ulcers at the start and the end of treatment in the control group

Characteristics	Start	End	*p*
Surface of the pressure ulcers (cm^2) ($\bar{x} \pm SD$)	19.15 ± 22.73	22.97 ± 15.69	0.001
Rank of the pressure ulcers ($\bar{x} \pm SD$)	8.2 ± 1.51	8.6 ± 1.05	0.01
Total PUSH* score of the pressure ulcers ($\bar{x} \pm SD$)	10.45 ± 2.74	11.85 ± 2.35	0.003

* The Pressure Ulcer Scale for Healing

Table 6

Characteristic of the pressure ulcers between groups at the end of treatment

Characteristics	Group		*p*
	Experimental (n = 20)	Control (n = 20)	
Surface of the pressure ulcers (cm^2) ($\bar{x} \pm SD$)	10.80 ± 19.18	22.97 ± 15.69	0.0005
Rank of the pressure ulcers ($\bar{x} \pm SD$)	5.95 ± 2.48	8.6 ± 1.05	0.0005
Total PUSH* score of the pressure ulcers ($\bar{x} \pm SD$)	7.35 ± 3.17	11.85 ± 2.35	0.00003

*The Pressure Ulcer Scale for Healing

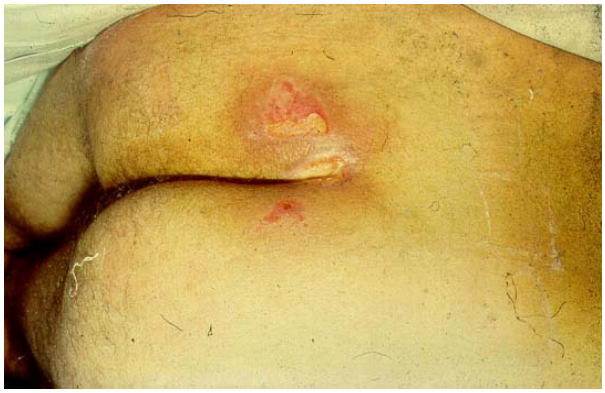

Fig. 3 – Pressure ulcus stage I–III after tratment with polarized light therapy

Discussion

Prevention and treatment of pressure ulcers is a serious clinical problem. Variations in patient's characteristics and ulcer management make difficult systematic clinical observation. There is a clear accord in requiring further research [29, 33]. Our study showed that patients with stage I–III ulcers, treated with polarized light therapy, had significant improvement after a 4- week treatment (Figures 2 and 3). Contrary to the control group, without polarized light, the patients in the experimental group had diminishing majority of PUSH tool parameters: surface of pressure ulcer, rank of pressure ulcer and total PUSH score. It is estimated that the PUSH tool will become the dominant wound healing tool in the future in the United States [32]. We could not compare other two PUSH parameters, exudates amount and tissue type because of a significant difference in these parameters between the groups at the start of the treatment (Table 3).

Our results could be ascribed to biological effects of polarized light therapy. Wound healing process has three successive stages: reaction, regeneration and remodeling [11]. Regeneration and remodeling are particularly important stages for pressure ulcer treatment. At regeneration stage capillaries bud and form new vessels; fibroblasts proliferate and secrete collagen, bacteria proliferate in dead tissue, macrophage activity increases, epithelial cells and myofibroblasts migrate, as well. On the contrary, in the remodeling stage, fibroblasts and macrophage activities are decreased, but the collagen starts to reorganize itself [7, 11]. Wound healing process is based on the vascular and cellular activity. Vasomotion is the periodic constriction and dilatation of small blood vessels. It is attributed to local metabolic needs, vascular myogenic responses and neurogenic controls. Pressure ulcer develops due to insufficient blood supply and removal of metabolites when pressure exceeds capillary blood pressure for a sufficient time [34]. Besides the fibroblast and macrophage activity, human wound-associated lymphocyte populations are modulated during a healing process [35]. A role of proteoglycans (glypican and syndecan) during the inflammation and cell proliferation in chronic ulcers was also established [36]. Protective function of human skin is well-known [17]. Additionally, significant time-dependent variation in cutaneous barrier was observed suggesting that there is a time-dependent variation in epidermal metabolism [37]. Because of that we performed the therapy always in the same time. Polarized light was found to trigger human cellular and humoral defences. It is considered that polarized light rearranges the polar heads of a lipid bilayer in the cell membranes. This is an area where enzyme reactions take place, catalyzed by proteins. Due to this interaction, structural changes may occur in cell membranes, in consequence of which the surface features and lipid protein connections can be modified [7]. The authors have reported different biological effects after polarized light irradiation, including stimulation of cell proliferation (especially in fibroblasts), release of growth factors and enhancement of collagen synthesis. Additionally, it establishes accelerated wound closure, increased wound epithelialisation and improved tensile strength of scars [19, 30]. Polarized light therapy has influence on the nerve structures. This can diminish pain receptors stimulation and improving endorphin production [7, 38].

The role of adjuvant therapies in pressure ulcer care has a long and controversial history. We also mention: positioning and exercise therapy, hydrotherapy, ultrasound, pressure reduction measures, special casting, low-energy laser therapy, ultraviolet therapy, low-frequency current, electrical stimulation, hyperbaric oxygen therapy [2, 3, 5, 6, 9, 10, 13, 14, 20–26]. When treating pressure ulcer, a clinician should always keep in mind that the main external precipitating factors are pressure, shearing forces, friction and moisture and therefore has to focus treatment on minimization of these factors [5, 39, 40]. Potential treatment complications exist for each therapy option in medical providing of pressure ulcer [5]. That applies to adjuvant therapies, as well. For example, in spite of serious recommendation for electrical stimulation [2, 6], Priebe [10] does not suggest this kind of adjuvant therapy in acute stage of pressure ulcer.

O`Conor [3] truly doubts in efficiency of ultrasound, but do not renounce it; however, McBrier et al. [41] demonstrated that therapeutic ultrasound may be detrimental to some of the pathways associated with skeletal muscle regeneration. Ultraviolet light is a well-known and powerful option for pressure ulcer adjuvant therapy, but it can affect intracellular redox state and increase the frequency of apoptosis in human melanocytes [6, 7, 9, 13, 15, 42]. There is no description of the potential treatment complications after polarized light therapy. In our study we did not record any side effect. Polarized light therapy was completely safe as a therapy option.

Deterioration in the control group is a very interesting fact. Namely, the patients from this group, who had only wound cleaning and dressing, showed significant deterioration in all of comparable PUSH parameters at the end of the treatment (Tables 5 and 6). We can wonder if cleaning and dressing were appropriate. There are some controversies about pressure ulcer cleaning and dressing. A protocol for pressure ulcer care based on a stage and amounts of wound care [12]. In this protocol, for example, normal saline is recommended as the best wound irrigant, but no hydrogen peroxide because of cytotoxic effects and damage of granulation tissue. In spite of that, Klipp et al. [6] recommended hydrogen peroxide, among antiseptic solutions, with a note on appreciation of toxicity index and appropriate dilution. Jovičić et al. [8] claim that there are no random control trials which proved that one kind of dressing was better than other. Opposite to that, Easton and O`Conor [3, 12] prefer occlusive or moisture-retentive dressing, using with success: films, hydrogels, hydrocolloids, copolymers and wound filters in the form of beads, gels, granules or pastes. Pressure ulcers in our patients were cleaned by 2% hydrogen peroxide and bringing a thin layer of appropriate fat. Dressing was performed by a gauze with 0,9% NaCl, dry gauze, cotton wool, and adhesive strip. This is a routine way of pressure ulcer care in our institution. It is possible that this kind of wound care was not appropriate. The questions are: how hydrogen peroxide in terms of its dilution and toxicity index was used; were wounds well protected with this kind of dressing; was the critical bacterial colonization of wounds a reason for such deterioration? Many authors emphasize the importance of careful cleaning of pressure ulcers and its dressing by the special kinds of bandage [2–6, 14].

Our results are partially comparable with the results of other authors. In the available literature we have not found many articles engaging with polarized light therapy. Karadag at al. [30] for example, experimentally confirmed the clinical results of Monstrey et al. [19] that polarized light therapy was effective in the treatment of burn wounds [19, 30]. Our results principly agree with the results of Iordanou et al. [28] and Verbelen [29], in spite of methodologic difference between our and their investigations. As compared to our study, Verbelen [29] did not carry out a blind clinical trial, his sample size was smaller, polarized light therapy, in terms of application, lasted longer (10 min), and his main assessment parameter was the frequency of appearance of pressure ulcer grade II. Similarly to our study, Iordanou et al. [28] investigated stage I – III ulcer and they took the characteristics of wound for the assessment parameters; but their polarized light therapy, in terms of whole program, was shorter (2-weeks) and their sample size was bigger. Beside this, Verbelen [29] engaged with prevention of pressure ulcer and Iordanou et al. [28] with treatment of pressure ulcer. These authors concluded that this kind of physical therapy could be efficient in medical care of pressure ulcer. We tried to partially replicate Iordanou et al. [28] investigation, improving their methodology and contributing to physiatrist's body of knowledge. Toward our results and the results of other authors, we consider that polarized light therapy need to be obligatory in pressure ulcer prevention and treatment.

This study has some advantages and shortcomings. The problem is in methodology, in other words, using a tool assessment in the wound healing process. Besides PUSH used in our study, in the world clinical practice there are still six most useful wound healing tools [32]. All of them have good and less good features. In the available literature on pressure ulcers and polarized light therapy we have not found the authors who use these wound healing tools [28–30]. Monstrey et al. [19], indeed, used a photographic method for assessment, but they observed burn wounds and we are not sure if they used the Photographic Wound Healing Tool (PWHT). The advantage of this study is the fact that the PUSH was used in our professional community first time. The PUSH, which has been used since 1997, incorporates three wound characteristics: surface area measurements, exudates amount, and surface appearance. It has a good validity which has been confirmed throughout two retrospective studies. The advantage of this assessment form is the possibility to note quickly any progress or degeneration of the wound. But its sensitivity in first version was not good enough. Some authors disputed its usefulness as assessment instrument for pressure ulcer. The PUSH tool does not include items that may be relevant for the treatment decision [11, 32]. We consider this as main shortcoming of our study. In further research of pressure ulcers and polarized light therapy, we should use other clinical tools for baseline and more comprehensive assessment.

Conclusion

The effects of polarized light as an adjuvant therapy for pressure ulcers were satisfactory. After a 4 week treatment, 20 patients with stage I-III ulcer showed a significant improvement in the wound healing process, so it could be useful to apply polarized light in the treatment of pressure ulcers.

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A K T U E L N E T E M E

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Mikrocirkulacijski poremećaji u ishemijsko/reperfuzijskom oštećenju jetre

Microcirculatory disorders in ischemic/reperfusion hepatic injury

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Key words:
liver; microcirculation; ischemia; reperfusion injury; endothelium; pathology.

Uvod

Ishemijsko/reperfuzijsko (I/R) oštećenje organa podrazumeva kompleksnu seriju različitih patofizioloških mehanizama koji dovode do ćelijskog oštećenja tokom i nakon perioda ishemije i reperfuzije tkiva [1]. Procesi ishemije i reperfuzije i posledično ćelijsko oštećenje značajni su u mnogim oblastima medicine. Takođe, I/R oštećenje tkiva klinički je značajna manifestacija u brojnim patološkim stanjima: cerebrovaskularni insult, infarkt miokarda, hemoragija praćena hipovolemijskim šokom, trauma i šok usled medicinskih dijagnostičko-terapijskih procedura i hirurških intervencija (npr. trombolitička terapija, koronarna angioplastika, transplantacija organa, kardiopulmonalni *bypass*, operacija aneurizme, hepatektomija itd).

Značaj u hirurgiji jetre

Ograničavanje perioda ishemije, odnosno vaskularna izolacija jetre, neophodan je postupak prilikom izvođenja većine hirurških procedura na jetri, naročito tokom operativnog lečenja ekstenzivne traume jetre, resekcije velikih (intra)hepatičkih tumorskih lezija i transplantacije jetre. Iako je blagovremena reoksigenacija tkiva ključna za očuvanje funkcijskog kapaciteta jetre i prevenciju postoperativne hepatičke insuficijencije, nakon ponovnog uspostavljanja dotoka krvi u jetru, odnosno reperfuzije, jetra je izložena dodatnom ošećenju koje pogoršava već postojeću ishemijsku leziju [2]. Stoga, I/R oštećenje hepatocita značajno vremenski ograničava bezbednu upotrebu vaskularne izolacije jetre tokom resekcionih zahvata. Stepen oštećenja hepatocita, nastalog usled I/R, zavisi delom od primarne lezije tokom ishemije, delom od sekundarnog oštećenja tokom reperfuzije, ali i od prethodnog strukturno funkcijskog stanja parenhima jetre i opšteg stanja bolesnika. S druge strane, postoje i stavovi da period reperfuzije nema izolovani, autonomni patogenetski značaj, tj. ne dovodi do ćelijskog oštećenja nezavisno od ishemije, već predstavlja samo nastavak prethodno započete inicijalne ishemijske lezije [3]. Pored lokalnog, parenhimskog oštećenja jetre, produžena ishemija i sledstvena reperfuzija jetre tokom resekcionih procedura dovode i do značajne sistemske organske disfunkcije, odnosno lezije udaljenih organa [4]. Sistemski efekti I/R jetre manifestuju se kao sindrom sistemskog inflamacijskog odgovora organizma (SIRS) i sindrom multiple organske disfunkcije (MODS) i, po pravilu, kritični su i, neretko, fatalni.

Pored ovoga, I/R oštećenje tokom transplantacije jetre jedan je od osnovnih uzroka primarne disfunkcije grafta, jedne od najozbiljnijih komplikacija kao i faktora morbiditeta i mortaliteta nakon ortotopske transplantacije jetre, za koju, sem retransplantacije, ne postoji efikasno lečenje [5, 6]. Mehanizam I/R oštećenja u osnovi primarne disfunkcije humanog hepatičkog alografta nije u potpunosti razjašnjen, ali je utvrđeno da važnu ulogu ima aktivacija Kupferovih ćelija i gubitak vitalnosti sinusoidnih endotelnih ćelija nakon čuvanja na hladnom i reperfuzije grafta jetre [7].

Incidencija primarne disfunkcije transplantovanog grafta jetre kreće se 2–23% i raste proporcionalno vremenu hladne ishemije grafta jetre (*cold ischemia storage time*) [5, 8]. Smatra se da je I/R oštećenje grafta jetre tokom transplantacije osnovni etiološki faktor rane disfunkcije grafta kod oko 10% bolesnika, kao i da dovodi do povećane incidencije epizoda i akutnog i hroničnog odbacivanja grafta [9]. Pored trajanja hladne ishemije jetre, osetljivost grafta jetre na I/R oštećenje zavisi i od drugih osobina donora (steatozna donorska jetra, ishemija donorske jetre usled hipotenzije, funkcijsko oštećenje donorske jetre izazvano lekovima, druge previđene bolesti donorske jetre itd) i operativnih faktora (neadekvatna

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operativna tehnika, redukovani portni i/ili hepatički arterijski dotok (*inflow*) krvi u jetru nakon završene transplantacije, produženo trajanje hladne i tople ishemije transplantovanog grafta jetre, itd).

U određenim okolnostima I/R oštećenje jetre ne predstavlja isključivo otežavajuću okolnost za hirurga koji se bavi patologijom jetre. Pokazalo se da je radiofrekventna ablacija malignoma jetre koji nisu pogodni za operaciju mnogo efikasnija kada se sprovodi uz korišćenje Pringleovog manevra [10]. Iako se to pripisuje smanjenom gubitku (odavanju) toplote, izraženi inflamacijski odgovor tokom perioda reperfuzije definitivno doprinosi promociji nekroze tumora.

Patogenetski značaj mikrocirkulacijskih poremećaja u ishemijsko-reperfuzijskom oštećenju jetre

Važna uloga odložene hepatičke mikrocirkulacijske perfuzijske insuficijencije do koje dolazi tokom ishemije, a naročito reperfuzije dobro je dokumentovana (tabela 1) [11–13].

Patofiziologija mikrocirkulacijskih poremećaja

Mikrocirkulacijski poremećaji tokom I/R jetre u najvećoj meri posledica su promena u endotelnim ćelijama koje se manifestuju poremećajima permeabilnosti, ekspresije citokina i adhezivnih molekula i mikrovaskularnog tonusa, pokretanjem inflamacijske i koagulacijske kaskade i konačnom kapilarnom okluzijom (fenomen *no-reflow*). Akumulacija leukocita dodatno ugrožava integritet endotelne barijere i povećava oksidativni stres, što dovodi do hiperpermeabiliteta i edema tkiva.

Azot-oksid i endotelin 1. Do disrupcije protoka krvi kroz hepatičke sinusoide dolazi usled kontrakcije zvezdastih (stelatnih) ćelija. S obzirom da ET-1 izaziva kontrakciju stelatnih ćelija zaključeno je da I/R oštećenje nastaje kao rezultat disbalansa između nivoa dva vazoaktivna antagonista: vazokonstriktora ET-1 i vazodilatatora azot-oksida (NO) [17]. Azot-oksid ima značajnu vazodilatatornu ulogu u mikrocirkulaciji i I/R oštećenju organa. Nastaje iz L-arginina delovanjem NO sintaze (NOS) koja u tkivima postoji kao induci-

Tabela 1

Mikrocirkulacijski poremećaji u tkivu jetre [13]

	Arteriole	Kapilari	Venule
Poremećaj	oštećena vazodilatacija	hipoperfuzija	povećana permeabilnost
Mehanizam	izmenjeni odgovor smanjena sinteza azot-oksida - deplecija prekursora i kofaktora sinteze - direktni efekti slobodnih kiseoničnih radikala	fenomen *no reflow* - adhezija trombocita i neutrofila kompresija - povećana hidraulična sprovodljivost - intersticijumski edem	adhezija neutrofila - povećana ekspresija adhezivnih molekula disrupcija zida - migracija leukocita oksidativni stres - stvaranje slobodnih kiseoničnih radikala
Posledica	povećana arterijska rezistencija	oštećena perfuzija i edem tkiva	hemodinamska nestabilnost

Utvrđeno je da je intenzitet mikrocirkulacijskih poremećaja u jetri proporcionalan trajanju perioda ishemije [11, 12]. Nastanak mikrocirkulacijskog oštećenja objašnjava se akumulacijom i adhezijom neutrofila i trombocita u hepatičkim sinusoidima, što dovodi do opstrukcije njihovog lumena. Ovaj alternativni mehanizam oštećenja jetre tokom I/R označava se kao fenomen izostanka reperfuzije *(*fenomen *no-reflow*). Postoje i dokazi koji ukazuju na to da začepljenje lumena sinusoida nije odgovorno za hepatičku I/R leziju. Utvrđeno je da neutrofili zaustavljeni u hepatičkim sinusoidama ne utiču na prekid sinusoidne perfuzije, tj. da je protok krvi kroz sinusoide ispunjene stagnantnim neutrofilima usporen, ali ne i zaustavljen [14, 15]. Smatra se da je verovatniji uzrok oštećenja hepatičke mikrocirkulacije povećano stvaranje vazokonstriktora i vazodilatatora i potencijalno izmenjena vaskularna osetljivost (sinusoida) jetre usled I/R i/ili inflamacije, što vodi lokalnom mikrocirkulacijskom perfuzijskom disbalansu i ishemijskom oštećenju [16]. U prilog takvom objašnjenju govori i dokazan korisni protektivni efekat različitih vazodilatatornih supstancija (NO, mizoprostol, itd), odnosno štetno delovanje vazokonstriktora (endotelin 1 – ET-1, tromboksan itd) tokom I/R.

bilna NOS (iNOS) i u konstitutivnim formama, kao što su endotelna NOS (eNOS) i neuronska NOS (nNOS) [18]. Azot-oksid ima različite funkcije uključujući i inhibiciju adhezije leukocita za endotelne ćelije sinusoida putem supresije adhezivnih molekula, inhibiciju agregacije trombocita, regulaciju mikrocirkulacije (vazodilataciju sinusoida) i inhibiciju kaspazne aktivnosti u prevenciji apoptoze [19, 20].

U ranoj fazi reperfuzije jetre koncentracija ET-1 raste i u plazmi i u hepatičkom parenhimu, što je povezano sa redukcijom protoka krvi kroz jetru [21, 22]. S druge strane, koncentracija NO niska je tokom prvih nekoliko časova reperfuzije, što je rezultat niskih intracelularnih nivoa nikotinamid adenin dinukleotid fosfata (NADPH) i kiseonika nakon perioda ishemije i oslobađanja velike količine arginaze koja razlaže L-arginin (prekursor neophodan za sintezu NO), što dovodi do deplecije L-arginina koja maksimum dostiže oko 30 minuta nakon početka reperfuzije [23, 24]. Utvrđeno je da aktivacija NOS (npr. tetrahidrobiopterinom, važnim koenzimom NOS) i povećanje ekspresije iNOS i eNOS dovodi do ublažavanja I/R oštećenja jetre [25, 26]. Smatra se da NO produkovan delovanjem eNOS ima hepatoprotektivna svojstva [27–29]. Hepatoprotektivno dejstvo aktivacije eNOS u I/R oštećenju nas-

taje posredstvom različitih mehanizama koji uključuju verovatnu vazodilataciju i redukovanu infiltraciju makrofagima [30].

Za razliku od eNOS, uloga iNOS još uvek je kontroverzna. Rezultati nekih studija ukazuju da aktivacija iNOS dovodi do redukcije I/R oštećenja organa [27, 31]. Međutim, suprotno ovim rezultatima pokazalo se da NO produkovan posredstvom iNOS ostvaruje štetna dejstva učešćem u oksidativnom stresu, tj. interakcijom sa anjonom superoksida koja vodi produkciji peroksinitrita, snažnog induktora ćelijske smrti [32]. Sam peroksinitrit pokazuje dvojako delovanje, odnosno moguće je da ima i hepatoprotektivno dejstvo putem redukcije leukocitne adhezije i infiltracije [33]. Ispitivanja na eksperimentnim modelima transplantacije jetre pokazala su da aktivacija iNOS u Kupferovim ćelijama i polimorfonuklearima može pokrenuti I/R oštećenje hepatocita [34]. Takođe, u eksperimentnom modelu I/R oštećenja steatozne jetre na pacovima utvrđena je pojačana aktivacija iNOS i ET-1, što ukazuje na njihovu važnu ulogu u regulaciji perfuzije sinusoida [35]. U skladu sa ovim rezultatima, inhibicija iNOS može imati korisne biološke efekte. Upotreba inhibitora iNOS (kao što su FK330 i ONO-1714) dovodi do redukcije aktivacije leukocita, hepatičke apoptoze i ukupnog I/R oštećenja jetre u eksperimentnom modelu transplantacije jetre na pacovima i svinjama [36, 37].

Teorija mikrocirkulacijske perfuzijske insuficijencije izazvane stvaranjem vazokonstriktora i vazodilatatora i mogućom izmenjenom vaskularnom osetljivošću potvrđena je brojnim eksperimentnim dokazima. Inhibicija sinteze vazodilatatora NO pogoršala je reperfuzijsko oštećenje, što se može sprečiti dodavanjem egzogenog NO i drugih vazodilatatora [38, 39]. Takođe, stimulacija produkcije NO L-argininom tokom reperfuzije umanjuje portnu hipertenziju i reperfuzijsko oštećenje [24]. S druge strane, dodavanje monoklonskog anti-ET-1 antitela ili antagonista receptora za ET-1 dovodi do poboljšanja hepatičkog mikrovaskularnog protoka krvi tokom reperfuzije, umanjuje oštećenje tkiva i poboljšava preživljavanje [40, 41].

Neutrofili i adhezivni molekuli. Utvrđeno je da su neutrofili odgovorni za tzv. kasnu fazu I/R oštećenja šest i više časova od početka reperfuzije (protektivni efekat neutropenije izostaje tokom prvih 5–6 h reperfuzije) [42]. Akumulacija neutrofila u jetri počinje već tokom prvih časova od početka reperfuzije. Intenzitet lokalne infiltracije neutrofilima zavisi od njihove ekstravazacije kroz vaskularni endotel i migracije kroz ekstracelularni matriks. Regrutacija i akumulacija neutrofila u jetri odvija se u dve faze: faza inicijalnog pripajanja i faza raspoređivanja neutrofila na sinusoidnom endotelu. To premeštanje neutrofila na mesta inflamacije regulišu brojni prethodno stvoreni i aktivirani akutni inflamacijski medijatori – hemoatraktanti. Među njima najznačajniji su pojedini faktori sistema komplementa, *tumor necrosis factor alpha* (TNF-α), interleukin 1 (IL-1), faktor aktivacije trombocita (*platelet activating factor* – pAF), *regulated on activation, normal T-cell expressed and secreted* (RANTES) citokin iz porodice interleukina 8 (IL-8), *monocyte chemoatractant protein-1* (MCP-1*), macrophage inflammatory proteins* (MIP-1 *alpha*, MIP-1 beta, MIP-2), hemokini i dr. Hemokine sekretuju tkivne ćelije, lokalni i akumulirani leukociti i endotelne ćelije aktivirane proinflamacijskim citokinima na mestima inflamacije. Jetra pokazuje sposobnost produkcije velike količine ovih hemokina. Svojom koncentracijom na mestu inflamacije sekretovani hemokini stvaraju lokalni gradijent od kojeg zavisi smer migracije neutrofila [43]. Pored ovog uticaja na lokalnu dijapedezu neutrofila, hemokini signalnim mehanizmom aktiviraju i adhezivne molekule od čije aktivnosti zavisi ekstravazacija i lokalna adhezija neutrofila za endotel hepatičnih sinusoida. Pored hemokina, ističe se i važna uloga fosfatidilserina (PS) u procesu dijapedeze i migracije neutrofila. U uslovima anoksije i reoksigenacije dolazi do postavljanja PS na spoljašnju stranu sinusoidnih endotelnih ćelija, što deluje hemoatraktantno na neutrofile i trombocite, odnosno privlači ih i promoviše njihovo vezivanje za mikrocirkulaciju oštećujući protok krvi [44]. Molekuli kao što je *diannexin* (homodimer humanog *annexina* V molekulske mase 73 kD) vezuje PS i smanjuje aktivaciju sinusoidnih endotelnih ćelija [45, 46]. On umanjuje lokalnu akumulaciju neutrofila (i trombocita) u jetri, indirektno suprimiše inflamacijski odgovor, redukuje apoptozu i štiti jetru od I/R oštećenja [47]. Pored toga, utvrđeno je i da proteaze matriksa olakšavaju kretanje neutrofila duž mikrocirkulacije. Npr. aktivnost metaloproteinaze matriksa 9 (MMP-9) visoko je povećana nakon I/R [48].

Za raspoređivanje neutrofila i njihovu čvrstu adheziju za sinusoidne endotelne ćelije odgovorne su brojne porodice različitih tzv. adhezivnih molekula [49]. Među njima su najznačajnije porodice selektina, integrina i imunoglobulina [49]. L-selektini poseduju receptore na neutrofilima i regulišu raspoređivanje neutrofila na endotelu sinousoida. E-selektini uglavnom su prisutni u sinusoidnim endotelnim ćelijama. Njihovu aktivnost regulišu proinflamacijski citokini TNF-α i IL-1 koji su odgovorni za akumulaciju i početnu slabu adheziju neutrofila za sinusoidne endotelne ćelije na mestima inflamacije. Nakon stvaranja ovih slabih inicijalnih veza, dalja adhezija neutrofila za endotelne ćelije hepatičnih sinusoida odvija se pod regulatornim delovanjem integrina koji u sadejstvu sa intercelularnim adhezivnim molekulom 1 (*intercellular adhesion molecule-1* – ICAM-1) i vaskularnim ćelijskim adhezivnim molekulom 1 (*vascular cell adhesion molecule-1* – VCAM-1) dovode do stvaranja čvrstih adhezija akumuliranih neutrofila za sinusoidne endotelne ćelije [43]. Pored adhezivnih molekula, u procesu adhezije neutrofila za sinusoidne endotelne ćelije važnu ulogu imaju i mehanički faktori: edem ćelije i lezija hepatičkih sinusoida tokom inflamacijskog odgovora, produkcija vazokonstriktora i smanjena deformabilnost (promenljivost i prilagodljivost oblika) neutrofila izloženih delovanju inflamacijskih medijatora [50–52].

Nakon završenog procesa adhezije za endotelne ćelije hepatičkih sinusoida sledi transmigracija neutrofila iz sinusoida u hepatički parenhim. Ovaj proces još uvek nije u potpunosti razjašnjen. Utvrđeno je da pravac transmigracije neutrofila određuje gradijent hemokina u parenhimu, a smatra se da i u ovom procesu transmigracije ključnu ulogu imaju adhezivni molekuli: β2-integrini, ICAM-1 i VCAM-1. Proinflamacijski citokini TNF-α, IL-1 i interferon gama (IFN-γ) identifikovani su kao snažni stimulusi transmigracije neutrofila u hepatički parenhim [53, 54]. Pored hemotaksičnog gradi-

jenta određenog parenhimskom koncentracijom hemokina, postoje indicije da transmigracija neutrofila iz hepatičkih sinusoida u parenhim zavisi i od drugih medijatora povezanih sa I/R oštećenjem koji mogu delovati kao hemoatraktanti za neutrofile (npr. neki produkti lipidne peroksidacije, faktori koji se oslobađaju iz parenhimskih ćelija nakon njihove apoptoze, itd.) [55, 56].

Po završenoj transmigraciji neutrofila sa endotela hepatičkih sinusoida u parenhim jetre, dolazi do adhezije neutrofila za parenhimske ćelije i sledstvenog oštećenja hepatocita. Utvrđeno je da je proces adhezije neutrofila za hepatocite takođe regulisan medijatorskim delovanjem adhezivnih molekula i to β2-integrina (LFA-1 i Mac-1) na neutrofilima i ICAM-1 na hepatocitima [57]. Takođe, vezivanje neutrofila za hepatocite zavisi i od prisustva niskih nivoa IL-8 [58]. Dokazano je da u patofiziološkom mehanizmu oštećenja hepatocita slepljenim neutrofilima učestvuju slobodni kiseonični radikali i proteaze, pri čemu dominantnu ulogu imaju proteolitički enzimi [59]. Najznačajnije među njima su kalcijum-zavisne proteaze koje aktiviraju slobodni kiseonični radikali i proinflamacijski citokini [60]. Nakon aktivacije, te proteaze dovode do proteolize citoskeletnih i membranskih proteina i posledičnog gubitka mitohondrijskog membranskog potencijala i povećanja permeabilnosti mitohondrijske membrane što dovodi do ćelijske smrti apoptozom ili nekrozom. Intracelularna aktivnost ovih proteaza značajno je povećana već 30 minuta nakon početka ishemije, a svoj maksimum dostiže 60 minuta od početka reperfuzije [61]. Patogenetska proinflamacijska uloga slobodnih kiseoničnih radikala u ovom mehanizmu sastoji se u inaktivaciji antiproteaza plazme [59], aktivaciji transkripcijskih faktora NF-κB i AP-1 koji regulišu transkripciju proinflamacijskih citokina, hemokina i adhezivnih molekula i aktivaciji intracelularne signalne transdukcije koja vodi ka ćelijskoj smrti [62]. Ova signalna transdukcija može se blokirati hormonima kao što su atrijski natriurezni peptid (ANP) i ciklični gvanozin monofosfat (cGMP) koji tako ispoljavaju protektivno delovanje na I/R oštećenje jetre [63]. Pored toga, neutrofili doprinose i razvoju oksidativnog stresa i stvaranju medijatora, kao što su leukotrien B4 i 12-hidroksieikozatetraenoična kiselina.

Zaključak

Ishemijsko/reperfuziono oštećenje jetre odigrava se posredstvom kompleksnih patofizioloških mehanizama koji uključuju oksidativni stres, proinflamacijski odgovor, aktivaciju imunskog sistema, koagulacijskog sistema, sistema komplementa i apoptozu hepatocita, što dovodi do mikrocirkulacijskih poremećaja. Iako autonomni, ti patofiziološki procesi deluju komplementarno i u različitom stepenu doprinose strukturnom i funkcijskom oštećenju hepatocita. Patogenetska dominacija svakog od ovih patofizioloških mehanizama varira i zavisi od dužine ishemije i prethodnog funkcijskog stanja tkiva jetre. Dalje detaljno rasvetljavanje ovih procesa neophodno je za uspešnu prevenciju razvoja I/R oštećenja jetre u različitim kliničkim okolnostima.

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Zašto je dvostruka apsorpciometrija X-zraka zlatni standard u dijagnostici osteoporoze

Why dual X-ray absorptiometry is the gold standard in diagnosing osteoporosis

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Ključne reči:
osteoporoza; denzitometrija; apsorpciometrija, fotonska; dijagnoza.

Key words:
osteoporosis; densitometry; absorptiometry, photon; diagnosis.

Uvod

Osteoporoza je koštano oboljenje koje karakteriše smanjena jačina kosti, što je predispozicija za povećan rizik od nastanka frakture [1]. Snižavanjem kvaliteta i kvantiteta kosti kod osteoporoze snižava se koštana čvrstina i, posledično, povišava rizik od fraktura.

Veliki broj radioloških tehnika korišten je ili se koristi u cilju dijagnostikovanja i praćenja osteoporoze. To su jednostruka i dvostruka apsorpciometrija fotona, jednostruka i dvostruka apsorpciometrija X-zraka, kvantitativna kompjuterizovana tomografija (KT), kvantitativni ultrazvuk i magnetna rezonancija (MR) visoke rezolucije.

Merenje mineralne gustine kosti (*Bone mineral density* – BMD) metodom dvostruke apsorpciometrije X-zraka (*dual energy X-ray absorptiometry* – DXA) smatra se zlatnim standardom za dijagnozu osteoporoze.

Cilj ovog rada bio je da isticanjem tehničkih karakteristika, razlika u radijacionim dozama radiološke opreme i razlika u stepenu preciznosti merenja koštane gusitne i mogućnostima snimanja više različitih regiona od interesa navedemo značaj dvostruke apsorpciometrije X-zraka (DEXA ili DXA) kao zlatnog standarda u dijagnostikovanju osteoporoze.

Osteodenzitometrijske metode

Prvi koraci u razvoju osteodenzitometrije učinjeni su uz pomoć merenja energije gama zraka metodama jednostruke i dvostruke foton apsorpciometrije. Rezultati dobijeni ovim metodama bili su prihvatljivi, ali se pokušalo izbeći gama zračenje, zbog čega su razvijeni aparati sa merenjem apsorpcije X-zraka. Uporedo sa ovim glavnim tokom razvoja opreme za osteodenzitometriju razvijani su i drugi dijagnostički aparati. Tako su radiološki skeneri sa digitalnom slikom postali prihvatljivi za razvoj merenja u postprocesingu slike, pa se osteodenzitometrija razvijala i na skenerima za KT, ultrazvuka i MR.

Prve su razvijane metode osteodenzitometrije jednostruke apsorpciometrije fotona (*single photon absorptiometry* – SPA) i dvostruke apsorpciometrije fotona (*dual photon absorptiometry* – DPA). Komercijalna upotreba SPA počela je još 1963. godine. Izvori zračenja su gama zraci, kod SPA 125 J fotona energije 27,3 keV, a kod DPA 135 Gd fotona energije od 40 i 100 keV. Za merenje energije propuštenih fotona koristi se scintilacioni detektor. Jednostruka apsorpciometrija fotona koristi se samo kod snimanja perifernog skeleta, a DPA i kod snimanja centralnog skeleta. Ipak, cena izotopa i vreme poluraspada od 240 dana predstavljali su smetnju za razvoj ove opreme. Tako, radioizotopi i gama zračenje vrlo brzo zamenjeni su generatorima i X-zračenjem [2].

Jednostruka apsorpciomerija X-zraka (*single energy X-ray absorptiometry* – SXA ili SEXA) zasniva se na istim fizičkim principima kao i SPA, osim što koristi X-zrake. Jednostruka apsorpciomerija X-zraka zamenila je SPA u skeniranju apendikularnog skeleta i isključila potrebu za izotopima.

Dvostruka apsorpciometrija X-zraka (*dual energy X-ray absorptiometry* – DXA ili DEXA) predstavlja zlatni standard za merenje koštane mase kod osteoporoze. Prvi komercijalni skener ove vrste upotrebljen je 1978. godine. Ova metoda, u odnosu na DPA, omogućila je bolju analizu apendikularnog i aksijalnog skeleta zahvaljujući brzini pregleda, većem *beam* intenzitetu, boljoj rezoluciji koja je omogućila jasniju identifikaciju granica vertebralnih tela i bolju preciznost. Ovo je jedina osteodenzitometrijska metoda koja daje visoko-kvalitetne snimke i precizna merenja anteroposterione kičme, lateralne kičme, proksimalnog femura, celog tela, podlaktice i pete [3].

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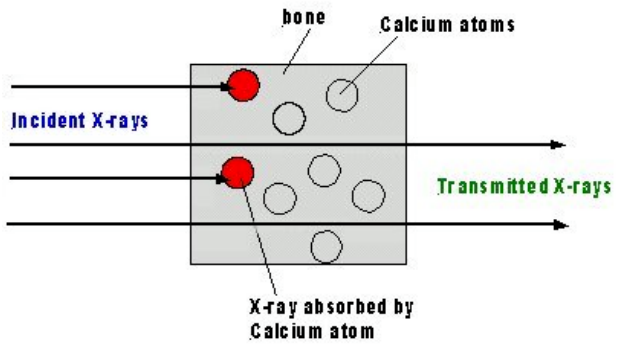
Kvantitativna kompjuterizovana tomografija (*quantitative computed tomography* – QCT) koristi se za merenje BMD aksijalnog skeleta, a periferni QCT (pQCT) se koristi za merenje BMD podlaktice. Koriste se konvencionalni KT skeneri, koji su vrlo skupa radiološka oprema, sa pratećim specijalnim softverom. Prednost QCT je što se može vršiti odvojeno merenje trabekularne od kortikalne kosti. Periferni QCT interesantni su zbog male emisije zračenja. Nedostaci QCT su nešto viša radijaciona doza i visoka cena opreme i pregleda. Najveći nedostatak i problem kod QCT je taj što prisustvo masti u koštanoj srži trabekularne kosti može izazvati greške u merenju BMD i 10–15%. Uopšte, greške kod QCT, u poređenju sa DXA, su veće [4].

Kvantitativni ultrazvuk (*quantitative ultrasound* – QUS) koristi se da se izmere određene karakteristike kosti, obično kalkaneusa, koje su u vezi sa kvalitetom i strukturom kosti, a koje mogu biti korisne za predviđanje stepena rizika od pojave frakture, ali se ne može koristiti za preciznu dijagnozu osteoporoze i praćenje efekta terapije [5, 6]. Promene koje registruje QUS u peti ili drugom delu perifernog skleta ne moraju da odražavaju promene BMD u kičmi ili kuku.

Magnetna rezonacija (MR) nije razvijana za merenje BMD i u kliničkoj praksi koristi se za diferenciranje osteoporotskih promena od tumorskih i inflamacijskih promena kosti, kao i za morfometrijska merenja.

Tehničke karakteristike DXA skenera

Apsorpciometrija X-zraka dvostruke energije predstavlja zlatni standard za merenje koštane mase u određivanju osteoporoze. Princip merenja apsorpcije X-zraka jednostavan je i zasniva se na razlici u merenjima X-zraka koji su apsorbovani atomima kalcijuma u kosti i X-zraka koji su propušteni kroz kost (slika 1).



Sl. 1 – Apsorpcija X-zraka u kosti [7]

Osnovna karakteristika ovakvog DXA skenera je brza promena potencijala rendgenske cevi sa 70 kVp na 140 kVp i to 60 puta u sekundi.

Na DXA skeneru od značaja su i tehnička sredstva kao što su eksterni ili kalibratori na rotacionom disku za tvrdo zračenje i povratnu radijaciju, te K-*edge* filter ili visokonaponski generator koji deli distribuciju X-zraka na dve odvojene komponente. To su „visoke“ i „niske“ energije fotona. Od značaja su i različiti DXA informatički-kompjuterski sistemi. Aktuelne DXA sisteme proizvode *General Electric* (GE) *Medical System, Hologic* i *Norland*. Zavisno od proizvođača skenera, postoje i različita tehnološka i informatička rešenja.

Osnovne razlike i tehnološka rešenja DXA skenera ogledaju se različitim metodama generisanja X-zraka, nivoima energije X-zraka, akviziconim metodama (*fan vs pencil beam*), kalibracijama, detektorima, algoritmima za diferencijaciju koštanih rubova, regionima od interesa, normativima baze podataka.

Proizvodnja dvostruke energije

Hologic koristi visokonaponske generatore koji naizmenično šalju impulse visokog i niskog napona na cev.

General Electric i *Norland* koriste K-*edge* filtere: GE koristi cerijum, a *Norland* samarijum.

Nivoi energije X-zraka

Pikovi dvostruke energije različiti su, pa kod GE iznose 40 i 70 keV, kod *Hologic* 50 i 85 keV, a kod *Norland* 45 i 80 keV.

Osim razlike u snazi opreme DXA skenera, razlika u metodama produkcije dvostruke energije i tipovima detektora nema kliničkog značaja, pa u ovom radu nije potencirana.

Različite metode akvizicije

Pencil beam je metoda kolimisanog zraka koji velikom brzinom prelazi preko objekta snimanja kretanjem sličnim kao kod katodne cevi. Za razliku od katodne cevi kod *pencil beam* metode se laterolateralno zračenje obavlja u oba pravca. *Pencil beam* u stvari je samo jedan zrak i takvo tehničko rešenje bilo je kod prvog KT skenera. Tim jednim zrakom vrši se skeniranje po x-osi u oba pravca i u y-pravcu na XYZ kordinatnom sistemu. Ovakav način snimanja korišten je kod starijih generacija DXA skenera i uslovljavao je duži proces snimanja.

Wide angle fan beam je ograničeno konusno zračenje u formi lepezasto kolimisanog zraka sa pravcem snimanja usmerenim samo ka y-pravcu XYZ koordinatnog sistema. Ovakav način snimanja koristi se kod savremenih DXA skenera i značajno ubrzava proces snimanja. Mana ovog sistema je što na detektorima projektuje veličinu različitu od stvarne veličine objekta od interesa [8].

Narrow angle fan beam je tehnika uskokolimisanog linearnog konusnog zraka koji se pomera kao kod *pencile beam* tehnologije i na taj način se redukuje distorzija koja se formirala usled uvećanja kod *wide angle fan beam* zračenja. Tehničko rešenje dobijeno je pomeranjem stola paralelno sa y-osom XYZ koordinatnog sistema u toku emisije lepezasto kolimisanih X-zraka u x-osi. Na taj način omogućeno je stvaranje realne slike i formiranje algoritama za realnu rekonstruktivnu obradu podataka koštanih parametara kao što su precizna determinacija koštanog mineralnog sadržaja, koštana veličina i geometrija [9].

Kalibratori su instrumenti za kvalitetnu kontrolu i mogu biti eksterni kao što su kod *Norland* i GE ili interni, generisani na rotacionom disku kao kod *Hologic* DXA skenera. Za kalibraciju se koriste različiti modeli fantoma, pa se kod GE koristi aluminijumski fantom, a kod *Hologic* i *Norland* skenera hidroksiapatitni fantom.

Ostale proizvodne razlike DXA skenera vezane su za softverska rešenja i neće biti analizirane u ovom radu.

Komparativna analiza osteodenzitometrijskih metoda

Ova komparativna analiza najvećih i najznačajnijih DXA sistema ima cilj da dâ osnov za male razlike u proceni tačnosti ove opreme. Procena tačnosti DXA skenera izvršena je tako što je vršena analiza na femuru i mekom tkivu kadavera. Kalcijum i pepeo razdvojeni su direktnom hemijskom analizom i na osnovu izvršenih komparacija dobijen je rezultat da je *Lunar* davao 3–5% veće vrednosti, *Norland* 3–6% niže, a *Hologic* 1% niže vrednosti [10]. To govori u prilog tome da je DXA opravdano zlatni standard za merenje osteoporoze, zbog toga što su moguća odstupanja od stvarnih vrednosti kod merenja koštane gustine kod QUS i QCT i do 15%.

Klinička upotreba DXA skenera ranije je bila orjentisana samo na dobijanje informacija o BMD što je omogućavalo precizno dijagnostikovanje osteoporoze. Regioni od interesa kod snimanja DXA skenerom su lumbalna kičma od 1–4. pršljenskog tela, kuk sa regionima vrata femura, Vardovog trougla i trohantera, a odskora i celo telo i podlaktica [11]. Noviji denzitometri sa c-lukom imaju i mogućnost snimanja lateralne projekcije kičme što omogućava još i morfometrijska merenja i 3D-rekonstrukciju i preciznu procenu vertebralnih fraktura [12]. Lateralnim snimanjem, takođe, mogu se izbeći lažni nalazi uzrokovani aortnim kalcifikacijama i degenerativnim promenama aksijalnog skeleta, kao i neprepoznatim frakturama pršljenskih tela [13]. Važno je napomenuti da je efektivna doza X-zračenja kod radiografije lumbalne kičme više od 10 puta veća nego kod lateralnog DXA skeniranja.

Merenje BMD od presudnog je značaja za otkrivanje osteoporoze i meri se na dva načina: kao volumen kosti (g/cm^3) i naziva se volumetrijski denzitet i na poznatoj površini kosti (g/cm^2) i naziva se prostorni denzitet. Osnovni preduslov za mogućnost ovakve vrste merenja je da je radiološka oprema digitalna jer se u formuli za površinu koriste veličine piksela, a u formuli za masu veličine voksela (slika 2).

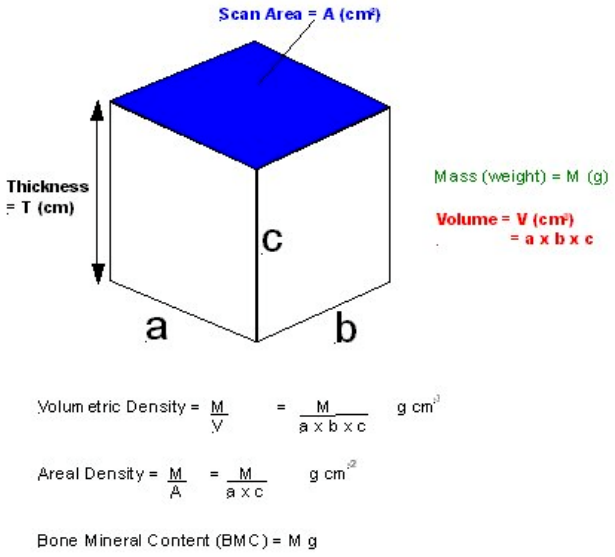
Kao standard za normalnu koštanu gustinu uzet je prosek koštane gustine kod zdrave žene stare 25–30 godina. Ta vrednost u DXA merenjima označava se kao T-skor. Odstupanja BMD od tog proseka DXA skener izražava u standardnim devijacijama (SD). Na osnovu odstupanja BMD u odnosu na T-skor, koje se izražava u SD napravljena je skala po kojoj se stepenuje gubitak koštane mase.

Denzitometrijski kriterijumi DXA za stepenovanje osteoporoze su: normalno – razlika BMD u odnosu na T-skor iznosi do -1 SD; osteopenija – razlika BMD u odnosu na T-skor iznosi od -1 SD do -2,5 SD; osteoporoza – razlika BMD u odnosu na T-skor iznosi više od -2,5 SD; teška osteoporoza – razlika BMD u odnosu na T-skor iznosi više od -2,5 SD uz prisustvo jedne ili više patoloških fraktura.

Alternativni parametar T-skoru je Z-skor. On izražava odnos očekivane (vezane za životno doba) i dobijene vrednosti mineralne koštane gustine i ima veću dijagnostičku vrednost u utvrđivanju stepena senilne osteoporoze, ali ne definiše osteoporozu. Za svako odstupanje Z-skora od -1 SD rizik od nastajanja frakture povećava se kao u slučaju odstupanja T-skora za -2,5 SD kod mlađih osoba.

Radijacijske doze kod dijagnostikovanja osteoporoze

Sve radiološke metode koje se koriste za osteodenzitometriju koriste niske doze X-zračenja, čak i na kičmi i kuku, a naročito na perifernom skeletu. Jedno od pogrešnih mišljenja je da su doze kod QCT mnogo veće nego doze X-zračenja kod DXA. Ranije, u protokolu DXA pregleda lumbalne kičme pre DXA skeniranja bilo je neophodno uraditi radiografiju lumbalne kičme radi procene degenerativnih procesa na kičmi i kalcifikacija na aorti koje bi mogle dati lažni nalaz. U tom svetlu, QCT bio je u prednosti nad DXA sa prethodnom radiografijom lumbalne kičme. Aktuele doze kod svih osteodenzitometrijskih procedura manje su od 10 mrem (1 mrem – 10 μSv). Radi poređenja, radijacija prirodnog okruženja je 300 mrem/godišnje, radiografija kičme je 70 mrem, mamografija je 45 mrem, a put oko sveta transkontinentalnim letom avionom je 6 mrem (tabela 1).



Sl. 2 – Shema i formule za izračunavanje mineralne gustine kosti [7]

Tabela 1

Efektivne doze X-zračenja (*International Commission on Radiological Protection* – ICRP 60) kod čestih radioloških procedura i različitih prirodnih zračenja [14]

Vrsta zračenja	Doza zračenja (mrem)
Godišnja radijacija prirodnog okruženja	300
Lateralna radiografija lumbalne kičme	70
Mamografija	45
Stomatološka radiografija	10
Transkontinentalni let oko sveta	6
QCT sa lokalajzerom	3–10
Radiografija grudnog koša	5
DXA, kuk ili kičma	1–6
Jedna nedelja skijanja	1–2
DXA podlaktice ili pete	< 1

Zaključak

Dijagnoza osteoporoze, generalno, zasniva se na merenju BMD na kičmi i proksimalnom femuru. Dvostruka apsorpciometrija X-zraka je jedina osteodenzitometrijska metoda kojom se vrše precizna merenja svih regiona od interesa i koja daje precizne podatke o koštanoj gustini na osnovu kojih se može postaviti dijagnoza osteoporoze. Novi DXA skeneri omogućili su i precizna morfometrijska merenja. Osim toga, DXA je pokazala odlične rezultate u proceni prognoze, praćenju prirodnog toka bolesti i proceni odgovora na pojedine terapijske režime i zbog toga se i dalje može smatrati zlatnim standardom u dijagnostici osteoporoze.

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K A Z U I S T I K A

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Endovaskularni tretman karotidno-kavernozne fistule tip A primenom platinskih spirala

Endovascular treatment of carotid-cavernous fistula type A with platinium coils

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Apstrakt

Uvod. Karotidno-kavernozne fistule patološke su komunikacije između karotidnih arterija ili njihovih grana i kavernoznog sinusa i najčešće su prouzrokovane traumom. Posttraumatske fistule predstavljaju 70% svih karotidno-kavernoznih fistula i to su najčešće fistule visokog protoka (tip A). Taj tip fistula uvek daje izraženu očnu simptomatologiju. **Prikaz bolesnika.** Prikazan je bolesnik muškog pola, starosti 44 godine, sa ustrelnom povredom glave, kod koga se, kao posledica povrede razvila karotidno-kavernozna fistula. U kliničkoj slici postojali su egzoftalmus desnog oka, hemoza i slabljenje vida na tom oku, glavobolja i diplopija. Digitalnom suptrakcionom angiografijom postavljena je dijagnoza karotidno-kavernozne fistule visokog protoka, koja je bila vaskularizovana delom iz leve karotidne arterije, a delom iz vertebrobazilarnog sliva. Nakon završene dijagnostike, urađena je endovaskularna embolizacija platinskim spiralama transarterijskim putem. Kontrolnom angiografijom potvrđeno je da je fistula zatvorena. **Zaključak.** Embolizacija kompleksne karotidno-kavernozne fistule tip A uspešno je izvršena endovaskularnim putem, upotrebom platinskih spirala.

Ključne reči:
karotidno-kavernozna fistula; povreda glave, penetrantna; dijagnoza; angiografija, digitalna suptrakcija; lečenje; neurohirurške procedure; embolizacija, terapijska.

Abstract

Background. Carotid-cavernous fistulas are abnormal communications between carotid arteries or their branches and the cavernous system caused mostly by trauma. Posttraumatic fistulas represent 70% of all carotid-cavernous fistulas and they are mostly high-flow shunts (type A). This type gives characteristic eye symptoms. **Case report.** This paper presents a 44-year old male patient with carotid-cavernous fistula as a result of penetrating head injury. In clinical presentation the patient had exophthalmos, conjunctival chemosis and weakening of vision on the right eye, headache and diplopia. Digital subtracted angiography showed high-flow carotid-cavernous fistula, which was vascularised from the left carotid artery and from vertebrobasilar artery. Endovascular embolization with platinum coils was performed through the transarterial route (endoarterial approach). Check angiogram confirmed that the fistula was closed and that no new communications developed. **Conclusion.** Embolization of complex carotid-cavernous fistula type A was successfully performed with platinum coils by endovascular approach.

Key words:
carotid-cavernous sinus fistula; head injuries, penetrating; diagnosis; angiography, digital subtraction; therapeutics; neurosurgical procedures; embolization, therapeutic.

Uvod

U kavernoznom sinusu nalazi se kavernozni segment arterije karotis interne (ACI) koja daje sitne grane za hipofizu, duru kavernoznog sinusa i kranijalne nerve u sinusu. Arterija karotis eksterna (ACE) vaskularizuje duru kavernoznog sinusa i pravi anastomoze sa perforatorima ACI. Kavernozni sinus drenira se preko donjeg i gornjeg petroznog sinusa u jugularnu venu i sigmoidni sinus. Anastomotične emisarne vene dreniraju kavernozni sinus u pterigoidni venski pleksus. Površinske srednje cerebralne vene ulivaju se u kavernozni sinus. Gornja i donja oftalmična vena dreniraju orbitu u kavernozni sinus.

Karotidno-kavernozne fistule (KKF) specifičan su tip duralnih arteriovenskih (AV) fistula koji karakteriše abnormalan AV šant u kavernoznom sinusu. Fistula tip A najčešće je traumatskog porekla (fraktura baze lobanje ili penetrantne povrede glave sa direktnom lezijom krvnih sudova kavernoznog segmenta). To su fistule brzog i visokog protoka [1]. Krv iz arterije koja ima visok pritisak, meša se sa krvlju venskog krvotoka kavernoznog sinusa dovodeći do retrogradnog protoka kroz vene koje se dreniraju u kavernozni sinus. Ret-

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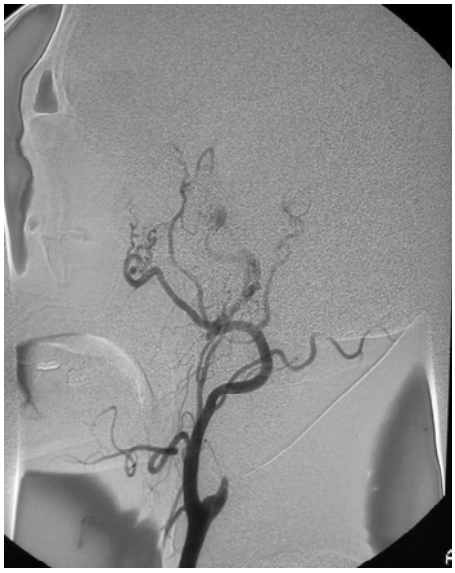
rogradni protok kroz gornju i donju oftalmičnu venu dovodi do dilatacije tih vena sa postupnim ispoljavanjem očne simptomatologije. Fistulu tip A teško je hirurški rešiti zbog anatomske lokalizacije povrede i lošeg opšteg stanja bolesnika.

Prikaz bolesnika

Prikazali smo bolesnika, starog 44 godine, koji je 1996. godine zadobio ustrelnu povredu glave. Ulazna rana bila je temporoparijetalno desno, a projektil se zadržao u visini drugog vratnog pršljena sa desne strane. Primarno je urađena temporalna kraniektomija uz obradu strelnog kanala i stavljen je klips na veću krvareću arteriju. Tada je urađena ligatura ACI iznad račve zbog njene lezije u petroznom segmentu. Neposredno nakon operacije razvio se hidrocefalus zbog čega je plasiran ventrikularni drenažni sistem. U kliničkoj slici, nakon otpusta bolesnika, dominirala je levostrana hemipareza teškog stepena. Tri godine posle povrede javili su se pulsirajući egzoftalmus, hemoza, slabljenje vida, glavobolja i diplopija, uz održavanje levostrane hemipareze. Klinička slika upućivala je na KKF, a digitalnom suptrakcionom angiografijom (DSA) verifikovana je fistula tip A.

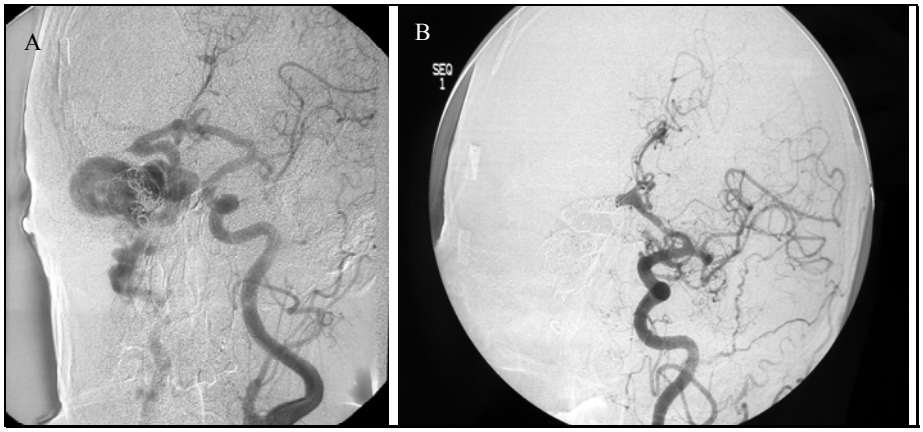
Navedena klinička slika održavala se sve do 2004. godine kada je urađena prva embolizacija fistule i spirale plasirane u dilatirani kavernozni sinus koji je nepotpuno zatvoren. Posle embolizacije došlo je do progresije egzoftalmusa, himoze, slabljenja vida, a javila se i vrtoglavica. Bolesnik je u februaru 2007. godine ponovo primljen u bolnicu radi embolizacije zbog permanentne progresije kliničke slike.

Endovaskularni tretman sproveden je u opštoj anesteziji. Angiografija je izvedena na monoplan aparatu firme General Electric. Vaskularni put obezbeđen je plasiranjem uvodnika lumena 6 Frencha kroz desnu zajedničku femoralnu arteriju, a dijagnostičkim kateterom dijametra 5 Frencha ponovo je urađena pancefalna angiografija. Utvrđena je okluzija desne ACI neposredno iznad račve kao posledica ligature (slika 1).



Sl. 1 – Digitalna supfrakciona angiografija: profilna projekcija desne zajedničke karotidne arterije sa okluzijom arterije karotis interne iznad račve pri čemu grane spoljašnje karotidne arterije ne pune fistulu.

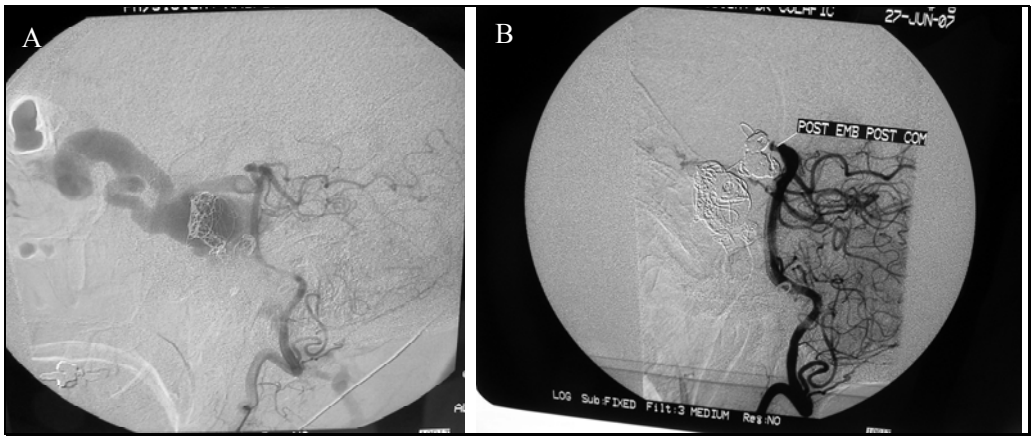
Fistula se punila preko prednje komunikantne arterije iz leve ACI, kao i iz vertebrobazilarnog sliva preko zadnje komunikantne arterije. Kavernozni sinus je bio proširen kao i oftalmične i facijalne vene, zbog retrogradnog protoka krvi (slike 2A, 3A, 4 i 5). Bolesnik je neposredno pre početka terapijske procedure primio 5 000 IJ heparina intravenski u bolusu. U toku procedure u intervalima od po jednog časa aplikovano je po 1 000 IJ heparina. Kateter uvodnik (Terumo 6 French) pozicioniran je u distalni segment leve ACI. Kroz njega je sproveden mikrokateter (Vasko 10, Balt) preko vodič sajle 0,010 (Exspedition, ev3, MTI) u područje fistule,



Sl. 2 – Karotidno-kavernozna fistula tip A (digitalna suptrakciona angiografija, anteriorna projekcija leve arterije karotis interne)

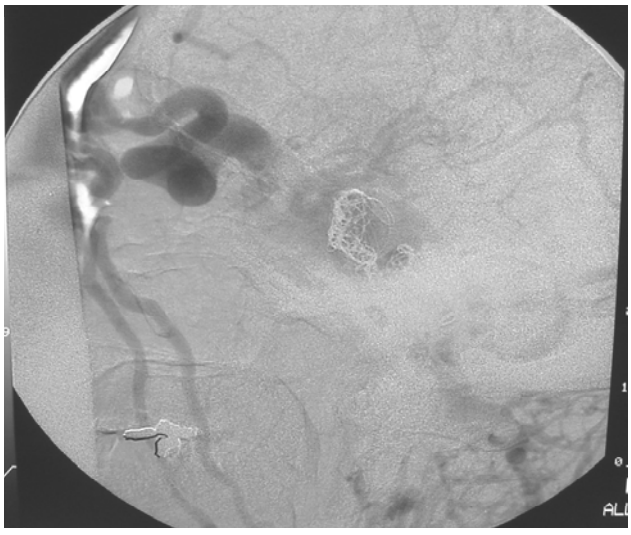
A. Prošireni desni segment A1 puni se preko prednje komunikantne arterije iz leve arterije karotis interne, levi A1 segment takođe je dilatiran. Protok kroz arteriju cerebri mediju slabiji je zbog fenomena krađe krvi uzrokovanog velikim protokom kroz fistulu. Kavernozni sinus je proširen sa enormno dilatiranim oftalmičnim venama.

B. Leva arterija karotis interna posle embolizacije mehanički odvojivim spiralama (Balt) sa potpunim zatvaranjem desnog A1 segmenta, što je dovelo do zatvaranja fistule, uz prekid retrogradnog toka u kavernozni sinus. Vena oftalmika se ne prikazuje, što je znak potpunog zatvaranja fistule.

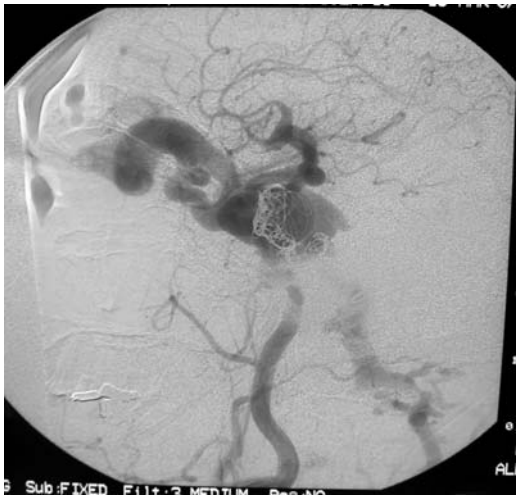


Sl. 3 – Karotidno-kavernozna fistula tip A (digitalna suptrakciona angiografija, profilna projekcija leve vertebralne arterije)
A. Proširena zadnja komunikantna arterija preko koje se puni kavernozni sinus i dilatirane oftalmične vene.
B. Leva vertebralna arterija nakon embolizacije i zatvaranja zadnje komunikantne arterije sa četiri električno odvojive spirale (ev3, MTI), pri čemu je fistula potpuno zatvorena. Ne prikazuju se kavernozni sinus niti oftalmične vene. Uočava se znatno bolja irigacija kroz grane bazilarne arterije zbog toga što nema krađe krvi preko fistule.

korišćenjem *road map* i *real/time* fluoroskopije radi pozicioniranja mikrokatetera pre plasiranja spirala. U nastavku, fistula je zatvorena sa pet mehanički odvojivih spirala (Balt), zatvaranjem A1 segmenta desne arterije cerebri anterior, preko prednje komunikantne arterije (slika 2B). Potom, sprovedena je ista procedura kroz levu vertebralnu arteriju pri čemu je okludirana zadnja komunikantna arterija sa četiri električno odvojive spirale (ev3, MTI) što je dovelo do potpunog zatvaranja fistule (slika 3B). Dva dana nakon intervencije došlo je do povlačenja egzoftalmusa, nestala je diplopija, a vid je bio poboljšan. Istovremeno, glavobolja i vrtoglavica su se povukle. Kontrolnim kliničkim pregledom posle šest meseci konstantovano je da bolesnik nema očnu simptomatologiju, niti vrtoglavicu. Tada je urađena DSA kojom je potvrđeno da je fistula zatvorena.



Sl. 4 – Karotidno-kavernozna fistula tip A (digitalna suptrakciona angiografija, profilna projekcija leve arterije karotis interne, venska faza): proširene facijalne vene.



Sl. 5 – Karotidno-kavernozna fistula tip A (digitalna suptrakciona angiografija, profilna projekcija leve arterije karotis interne, arterijska faza): spirale u zadnjem delu kavernoznog sinusa redukuju protok kroz petrozne sinuse i izrazito proširene oftalmične vene.

Diskusija

Postoji nekoliko podela KKF. U odnosu na etiologiju, KKF mogu biti traumatske, spontane i jatrogene. Prema brzini protoka dele se na fistule niskog i fistule brzog protoka. U odnosu na anatomiju KKF se dele na direktne fistule, koje podrazumevaju direktnu komunikaciju između ACI i kavernoznog sinusa, i indirektne KKF koje su definisane kao fistule između meningealnih grančica i kavernoznog sinusa.

Klinički korišćena klasifikacija KKF je anatomski-angiografska, po Barrow i sar. [2]. Oni opisuju četiri tipa fistula. Tip A su fistule brzog i visokog protoka koji je posledica direktne komunikacije ACI i kavernoznog sinusa usled rascepa zida arterije u kavernoznom sinusu. Najčešće su traumatskog porekla (fraktura baze lobanje ili penetrantna pov-

reda glave) – tip A1. Kod 20% bolesnika fistula je sekundarna, kao posledica rupture aneurizme ACI u kavernoznom sinusu tip A2 [3]. Fistule tip B retke su i predstavljaju duralni šant meningealnih grana ACI sa kavernoznim sinusom. Fistule tip C su duralni šant meningealnih grana ACE sa sinusom. Fistule tip D su kombinacija B i C. Poslednje tri fistule su spontane fistule niskog i sporog protoka. Karotidno-kavernozne fistule čine 12% svih AV fistula. Tip A je češći kod mlađih osoba. Ostali tipovi, B, C i D, češći su kod žena posle 50. godine života, sa odnosom 7:1 [4, 5]. Angiografskim pregledom bolesnika utvrđena je fistula brzog i visokog protoka tip A, koja je posledica ustrelne povrede glave projektilom koji je prouzrokovao frakturu baze lobanje i leziju krvnih sudova kavernoznog segmenta. Venska drenaža kod KKF može biti prednja, preko oftalmičkih vena, ili zadnja preko petroznih sinusa, što uslovljava i kliničku sliku. Drenaža je, najčešće, mešovita. Prednja drenaža daje dramatičnu simptomatologiju. Orbitalne manifestacije su mnogo manje izražene kada se fistula drenira u donji petrozni sinus [6]. Kod prikazanog bolesnika postojala je prednja drenaža, jer je predhodna neuspela embolizacija dovela do redukcije protoka krvi kroz petrozne sinuse. Ovo je preusmerilo krvotok u oftalmične vene, prouzrokujući njihovu enormnu dilataciju, što je dovelo do progresije kliničke slike (slika 5).

Očne komplikacije KKF uključuju oftalmičnu vensku hipertenziju i orbitalnu vensku kongestiju, zatim diplopiju, slabljenje vida i paralizu 3, 4, 5. i 6. kranijalnog nerva. Centralna retinalna venska okluzija, retinopatija i glaukom najčešće su komplikacije KKF. Glavobolja sa šumom u glavi i pulsirajući egzoftalmus često su prve kliničke manifestacije. Terapija kod tipa A može biti hirurška ligatura ACI ispod i iznad fistule i interventne endovaskularne procedure. Hirurškim putem nije bilo moguće rešiti ovu fistulu jer se kavernozni sinus punio iz leve ACI i iz bazilarne arterije. Endovaskularni tretman sa balonom u cilju zatvaranja fistule prvi je primenio Serbinenko [7] 1974. godine. Upotreba platinskih spirala za zatvaranje fistule predstavlja metodu koja se koristi poslednjih petnaest godina [8]. Razvoj intrakranijalnih stentova omogućio je njihovu primenu u kombinaciji sa spiralama za zatvaranje fistule. Postoje dve mogućnosti: 1) plasirati stent u predelu fistule, a onda kroz stent ispuniti i zatvoriti fistulu spiralama i 2) fistulu zatvoriti spiralama, a zatim staviti stent kao podršku da bi se sprečila migracija spirala u lumen arterije [9, 10]. Mogu se koristiti i stent graftovi u cilju zatvaranja rascepa na krvnom sudu, mada je ponekad njihova primena otežana zbog rigidnosti kod tortuoznih krvnih sudova [11–13]. Transarterijski pristup najčešće je korišćeni put za plasiranje spirala, kao što je bilo kod ovog bolesnika, a ponekad se može koristiti kombinacija transarterijskog i transvenoznog pristupa. Venski pristup podrazumeva kateterizaciju donjeg petroznog sinusa ili gornje oftalmične vene [14]. Indikacije za endovaskularni tretman su: progredirajući egzoftalmus, slabljenje vida, paraliza abducensa, teška glavobolja. Ostali tipovi KKF imaju visok stepen spontane regresije. Nekada je dovoljna kratkotrajna kompresija na karotidne arterije da bi se one zatvorile. Dijagnoza KKF postavlja se na osnovu multislajsne kompjuterizovane tomografije (MSCT) angiografije, magnetne rezonancije angiografije, mada DSA još uvek predstavlja zlatni standard u dijagnostici.

Zaključak

Embolizacija kompleksne karotidno-kavernozne fistule tip A koja je bila vaskularizovana iz leve ACI i bazilarne arterije uspešno je izvršena endovaskularnim putem upotrebom platinskih spirala. Prednost ove tehnike je minimalna invazivnost, mogućnost kombinovanja i ponavljanja ukoliko se primenom prve procedure fistula kompletno ne zatvori.

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Atypical proliferating mucinous tumors of gigantic dimensions

Atipični proliferativni mucinozni tumor ogromnih dimenzija

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Abstract

Background. Ovarian tumors of low malignant potential (LMP) are also known as atypically proliferating tumors. Ovarian tumors of LPM account for approximately 15% of all epithelial ovarian cancers. Mean age of occurrence is 40 years and they are 15–20 cm in diameter. **Case report.** A 32-year-old female patient was hospitalized as an urgent case with a large tumor mass that filled the entire abdomen. Cyst was 100 × 70 cm dimensions belonging to the right ovary and filled with 18 liters of content. Right adnexectomy, resection of the second ovary, as well as biopsy of the omentum were performed. Lymphadenectomy of the right iliac and obturator area was also performed. After receiving definitive histopathological results it was decided to perform a radical reoperation. On the 10th postoperative day relaparotomy, total hysterectomy and left adnexectomy were performed. The patient was released on the 6th postoperative day. She used to come to regular examinations up to date. **Conclusion.** This case is a proof that LMP tumors have low malignant potential, they grow slowly and can reach great proportions.

Key words:
ovarian neoplasms; gynecologic surgical procedures; histology; reoperation.

Apstrakt

Uvod. Tumori ovarijuma niskog malignog potencijala (*low malignant potential* – LMP) poznati su i kao atipični proliferativni tumori. Ovi tumori ovarijuma predstavljaju otprilike 15% svih epitelijalnih ovarijalnih karcinoma. Najčešće se javljaju oko 40. godine života i prečnika su oko 15–20 cm. **Prikaz bolesnika.** Bolesnica, stara 32 godine, sa velikom tumorskom masom koja je ispunjavala ceo abdomen, hospitalizovana je kao hitan slučaj. Cista je pripadala desnom ovarijumu, dimenzija 100 × 70 cm sa tečnim sadržajem od 18 litara. Urađena je desna adneksektomija sa resekcijom levog jajnika i biopsijom omentuma. Takođe, urađena je limfadenektomija desnog ilijačnog i opturatornog regiona. Posle dobijanja definitivnog patohistološkog rezultata odlučeno je da se uradi radikalizacija prethodne operacije. Desetog postoperativnog dana izvedena je relaparotomija, totalna histerektomija i leva adneksektomija. Bolesnica je otpuštena šestog postoperativnog dana. Do danas je na redovnim kontrolama. **Zaključak.** Ovaj slučaj dokaz je da atipični proliferativni mucinozni tumori imaju nizak maligni potencijal, da sporo rastu i da mogu dostići velike razmere.

Ključne reči:
jajnik, neoplazme; hirurgija, ginekološka, procedure; histologija; reoperacija.

Introduction

Ovarian tumors of low malignant potential (LMP), also known as atypically proliferating tumors, comprise a group of tumors showing greater epithelial proliferation than seen in benign serous cystadenoma, although they are by definition noninvasive. Ovarian tumors of LMP account for approximately 15% of all epithelial ovarian cancers. Mean age of occurrence is 40 years [1]. Ovarian LMP tumors range bet-ween benign adenoma and ovarian cancer, where the absence of stromal invasion is an absolute criterion for setting of diagnosis [2]. About 5–10% of LMP tumors behave very aggressively as ovarian carcinoma, while the rest behave as benign ovarian tumors [3]. These neoplasms do not differ macroscopically from benign changes. They are multilocular, cystic lesions with a smooth surface of an average diameter of 15–20 cm.

Case report

A 32-year-old female patient was hospitalized at the Institute for Gynecology and Obstetrics of the Clinical Center of Serbia in April 2006 as an urgent case with a large tumor mass that filled the entire abdomen. The abdomen was ex-

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tremely painful, and the skin on the stomach had cracked and become infected due to overstretching (Figure 1).

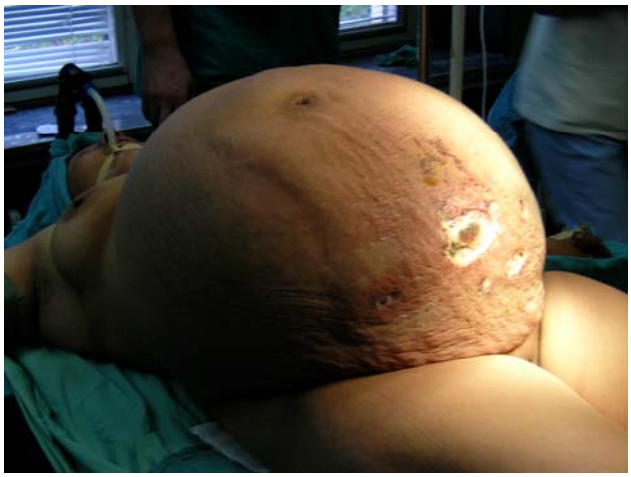
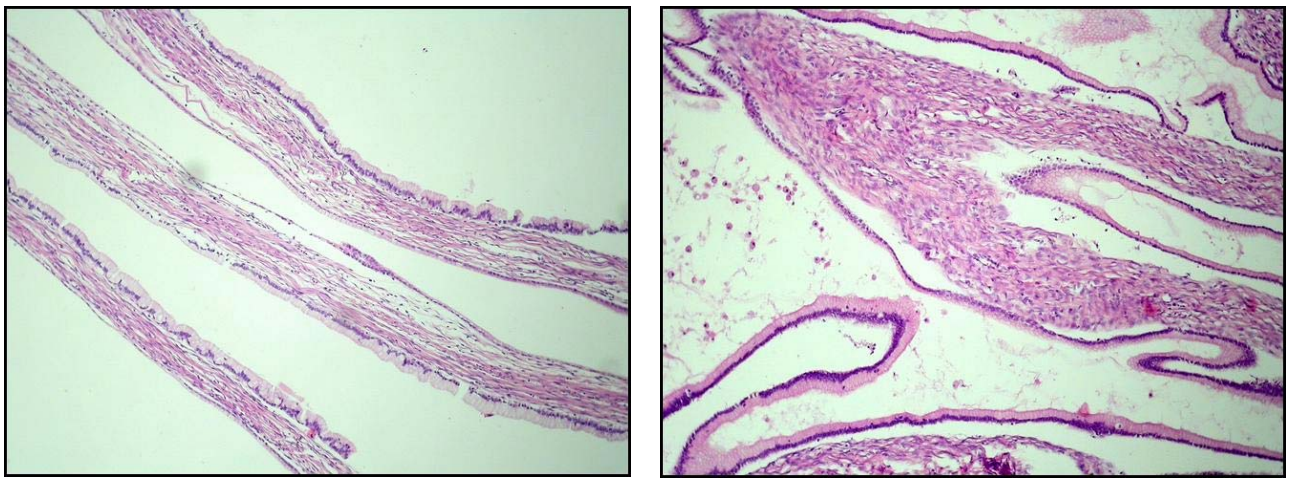


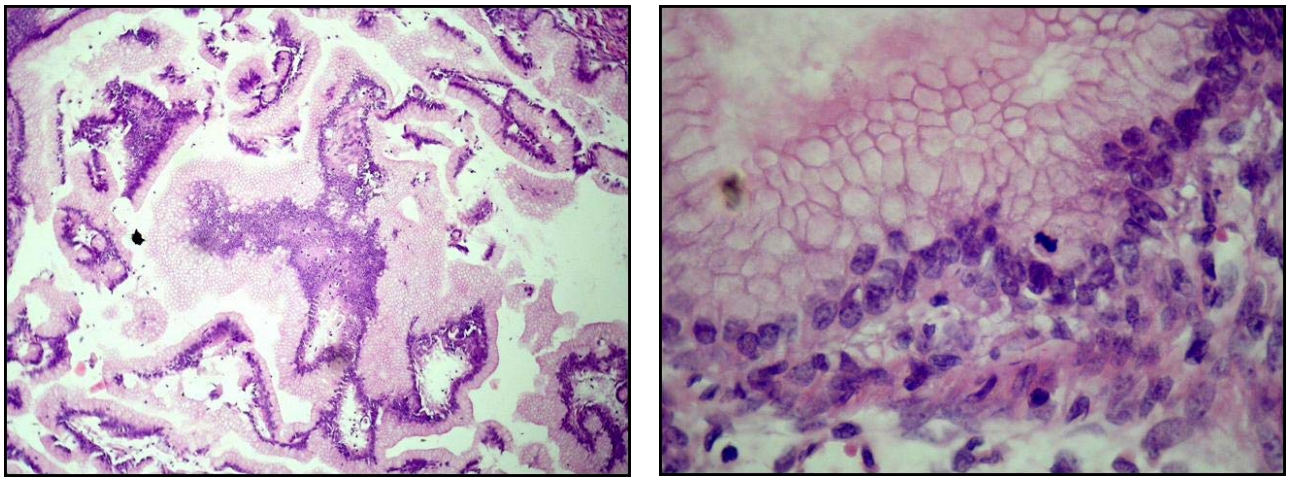
Fig. 1 – Preoperative picture of abdomen and cracked skin

The patient had noticed growth of her stomach over the past two years, but had not reported it earlier to a doctor. Her stool and urine were regular. She gave birth five times, the last time three years ago. Laboratory analyses on admission to the hospital indicated increases values of leukocytes, sedimentation rate, platelets, C-reactive protein and fibrinogen. Tumor markers Ca 125 and Ca 19.9 exhibited slightly higher values. Ca 125 was 53.3 U/ml (referential values < 21 U/ml) while Ca 19.9 was 108 U/ml (referential values 0–33 U/ml). *Staphylococcus aureus* was isolated bacteriologically from the skin changes on the stomach. Antibiotic and anticoagulant therapy was initiated. After the infection was cured the patient was operated. Upon opening the stomach a cyst was located, with dimensions 100 cm × 70 cm of a smooth pearl white colored capsule belonging to the right ovary. The left ovary and uterus exhibited normal characteristics upon macroscopic inspection. During the operation, in order to permit evacuation of the tumor from the stomach, 18 liters of thick, mucous, chocolate colored content was extracted from the cyst and was sent for cytological analysis (Figures 2 and 3). Right adnexectomy was performed, resection of the second ovary, as well as biopsy of the omentum. Lymphadenectomy of the right iliac and obturator area was also performed.



a) b)

Fig. 2 – Benign mucinous cystadenoma (HE × 40)



a) b)

Fig. 3 – Benign mucinous cystadenoma borderline (HE × 40)

The histopathological report described a macroscopic oval cystic formation with a growths mass of 1 330 grams previously opened and emptied with a smooth surface and without growths (Figure 4). The interior surface was predominantly smooth with two polypoid formations of 25 and 50 mm, with honeycomb-like cross-sectional appearance. The cyst walls were lamellar, ranging in thickness from 5 to 10 mm. Along the exterior surface there was an unchanged tube with the length of 110 mm and diameter 5 mm. Microscopic analysis indicated a mucinous cystadenoma with partially present areas containing numerous papilar prominences with pseudostratified epitheloma exhibiting signs of nuclear and cellular irregularities and occasional presence of mitosis. There were no signs of epithel invasion in the ovary stroma, nor there was any penetration of the capsule of the ovary. Two samples of the omentum with a total mass of 100 grams only indicated signs of fibrosis. In the 14 analyzed lymph nodes only reactive changes were noted. A follicular cyst was removed from the left ovary. The definitive histological diagnosis was "Atypically proliferating mucinous tumor of the right ovary (Border line)". The stage of the tumor disease was designated as FIGO Ia classification.

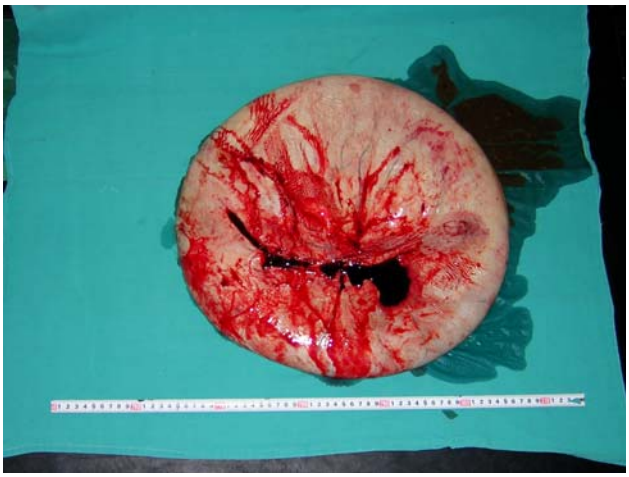


Fig. 4 – Macroscopic oval cystic formation, emptied

After receiving definitive histopathological results it was decided to perform a radical reoperation. On the 10th postoperative day a relaparotomy was performed, total hysterectomy and left adnexectomy. In the histological preparation following radicalization normal microscopic characteristics of the uterus and the second ovary were described. The postoperative period was normal. The scar healed *per primam*. The patient was released on the 6th postoperative day. The patient used to come to regular examinations up to date.

Discussion

Papillary serous low malignant potential tumors are characterized by malignant features and metastatic potential, yet display a benign clinical course [4]. Mucinous LMP tumors are bigger than serous ones with average diameter from 17 to 20 cm and they are rarely bilateral [5]. The patient presented in this paper had tumor localized at only one side ovary. Ovarian LMP tumor was bigger than any described in the literature. We removed the contens of 18 liters (Figure 1). Patients usually refer with pelvic mass and complain of abdominal and pelvic pain, increased abdominal diameter or abnormal bleeding. Ultrasound and computerized tomography could help in diagnosis of ovarian mass. Serum Ca 125 is not always elevated and can be near the normal range even if tumor is present [6]. Only high malignant tumors produce significant increase of serum Ca 125. The patient presented here had only a elevation of serum Ca 125. The patient had abdominal *staphylococcus* skin infection. Primary surgical treatment for patients with LMP tumors and already given births are similar as for invasive ovarian disease and include total abdominal hysterectomy, bilateral salpingoophorectomy and disease staging. We performed radical operation because the patient already had had five deliveries.

Conclusion

This case is a proof that atypical proliferating mucinous tumors have low malignant potential. They grow slowly and can reach great proportions, as in the presented patient.

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Позив на рекламирање у 2009. години

У прилици смо да вам понудимо могућност оглашавања и рекламирања производа и услуга у часопису „**Војносанитетски преглед**“ (ВСП). То је сигурно најбољи вид и најзаступљенији начин упознавања евентуалних корисника са вашим услугама и производима.

Часопис „**Војносанитетски преглед**“, званични орган лекара и фармацеута Војске Србије, научно-стручног је карактера и објављује радове из свих области медицине, стоматологије и фармације. Радове равноправно објављују стручњаци из војних и цивилних установа и из иностранства. Штампа се на српском и енглеском језику. Часопис излази непрекидно од 1944. године до сада. Једини је часопис у земљи који излази месечно (12 бројева), на око 100 страна А4 формата, а повремено се објављују и тематски додаци (суплементи). Путем размене или претплате ВСП се шаље у 23 земље света. Радове објављене у ВСП-у индексирају: *Science Citation Index Expended (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline)*, *Excerpta Medica (EMBASE), EBSCO* (преко ове базе ВСП је *on line* доступан од 2002. године у *pdf* формату) и *Biomedicina Serbica*.

Цене реклама и огласа у часопису „Војносанитетски преглед“ у 2009. години су:

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P R I K A Z K N J I G E

Osnovni koncepti u farmakologiji

Vodič za studente

Treće američko (2006) i prvo izdanje na srpskom jeziku (2008)

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Strana 301, cena 555 dinara

Zemlja sa najrazvijenijom farmaceutskom industrijom u svetu su SAD. To diktira i najsavremeniju farmakoterapiju koju ta industrija širi svojom ofanzivnom farmakoinformativnom delatnošću usmerenom prema svim zdravstvenim radnicima, u prvom redu lekarima, farmaceutima i stomatolozima. Takvo stanje prelama se kroz nastavu i na studente medicinskih, farmaceutskih, stomatoloških i drugih fakulteta koji u svojim studijama imaju i predmet Farmakologiju.

U cilju lakšeg savladavanja gradiva pripremaju se posebni udžbenici koji su za studente medicine u SAD brojni. Njihov sadržaj je ujednačen, ali volumen varira, i to od 400–500, pa preko 700–800, sve do 2 500 stranica i više. Nisu svi, a posebno oni najvoluminozniji, namenjeni samo studentima, već i nastavnicima u pripremi nastave. Međutim, činjenica da oni postoje, uvek vodi izazovu nastavnika da se neki delovi u celosti ugrade i u studentsku nastavu, pa sledstveno tome po istom obimu i u studentska pitanja kod polaganja ispita.

To je i bio razlog da profesorka Janet Stringer sa Medicinskog fakulteta u Hjustonu, napiše kondenzovani udžbenik farmakologije, zadivljujuće jednostavan, pregledan i sveobuhvatan. On može da se koristi na dva načina. Prvo, da se predmet nauči iz jednog od standardnih udžbenika farmakologije koje u praksi preporučuje svaki fakultet na pojedinim univerzitetima, pa da se usvojeno znanje zatim proveri preko ovog udžbenika. Druga mogućnost je da se ovaj udžbenik maksimalno savlada, a gradivo dato u njemu zatim proveri prema uputstvima autorke. Posle toga, da se isto proširi iz gradiva standardnog udžbenika. U tom pogledu izbor je na svakom studentu pojedinačno.

Ovo posebno ističemo stoga što je originalni naslov „*Basic Concepts in Pharmacology. A Student's Survival Guide*“ imao za cilj da ukaže da student koji dobro savlada ovaj udžbenik dobije takav temelj u farmakologiji u svim oblastima farmakoterapije, da ako ga dobro savlada, a u svakom slučaju i dopuni, ima velike šanse i da „preživi“ ispit. Tako pripremljen, udžbenik je u kratkom roku doživeo tri izdanja u SAD. Kao dodatna mogućnost, odraz je standarda za američke studente medicine u ovom domenu, a korišten na jedan od dva napred navedena načina, jednako je pogodan za sve studente fakulteta u našoj zemlji koji u svom programu imaju predmet farmakologija: medicinskih, farmaceutskih, stomatoloških, kao i onih veterinarske medicine i molekularne biologije.

Pored toga, ovaj udžbenik koji predstavlja i temelj farmakologije, mogao bi izuzetno dobro da posluži i lekarima i stomatolozima u ambulantama i domovima zdravlja i farmaceutima u apotekama, kao brz i pouzdan izvor informacija u svim oblastima farmakoterapije, prikazane kroz 46 poglavlja,

uključujući i toksikologiju. U tom smislu, on za njih ima dve pogodnosti: indeks, dat za celovito gradivo, i veoma praktičan pregled lekova po grupama, od kojih su skoro svi registrovani za primenu i u našoj zemlji. Na taj način data je mogućnost širokom krugu korisnika da samo u roku od nekoliko minuta nađu pouzdane podatke o terapijskoj nameni lekova, o mehanizmu dejstva, o međusobnoj efikasnosti, kao i o njihovim najćeščim neželjenim dejstvima.

To su, upravo, i razlozi što se ovaj udžbenik može preporučiti ne samo studentima navedenih fakulteta, već i širokom krugu zdravstvenih radnika u našoj zamlji.

Posebno treba zahvaliti njegovom izdavaču, Data Status iz Beograda, koji je imao razumevanja da u svoj obiman izdavački program uvrsti i jedan ovakav, nadasve praktičan i koristan udžbenik, kao i na veoma popularnoj ceni koja će posebno pogodovati skromnom studentskom budžetu.

Dragana Stanković

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Tabele

Sve tabele štampaju se sa proredom 1,5 na posebnom listu hartije. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) full name of each author;

c) name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

2. ***Abstract and key words***

The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c - t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

3. ***Text***

The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

In the **Introduction** repeat the title of the article, excluding the names of authors. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Methods. Describe your selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Identify the methods, apparatus (manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approvement of the Ethnics Committe for the tests in humans and enimals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as "in press". Information from manuscripts not yet accepted should be cited in the text as "unpublished observations". References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-428.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tables

Type each table double-spaced on a separate sheet. Number tables consecutively in the order of their first citation in the text in the upper right corner **(Table 1)** and supply a brief title for each. Place explanatory matter in footnotes, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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Figures are submitted in triplicate, and for the final version also on diskette/CD. Photos should be sharp, glossy black and white photographic prints, not larger than 203 × 254 mm. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

Legends for illustrations are typed on a separate page, with arabic numerals corresponding to the illustrations. Identify and explain each one clearly in the legend symbols, arrows, numbers, or letters used to identify parts of the illustrations. Explain the internal scale and identify the method of staining in photomicrographs.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

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