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VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine

Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Uprava za vojno zdravstvo MO Srbije

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The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

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This september, 70 years of continuous publication of the *Vojnosanitetski pregled* (Military Medical Review), a scientific journal of physicians and pharmacists of the Serbian Army will be marked. It is one of the leading medical journals in Serbia, indexed in well-known indexing databases including Science Citation Index Expanded (see Editorial, p. 805–8).

U septembru ove godine navršava se 70 godina kontinuiranog izlaženja „Vojnosanitetskog pregleda“ (VSP), naučnog časopisa lekara i farmaceuta Vojske Srbije. VSP je jedan od vodećih medicinskih časopisa u Srbiji, indeksiran u najpoznatijim svetskim bazama naučne publicistike, uključujući i *Science Citation Index Expanded* (SCIe) (vidi Uvodnik, str. 805–8).

EDITORIAL/UVODNIK

Seventy years of the *Vojnosanitetski pregled*

Sedamdeset godina „Vojnosanitetskog pregleda“

Silva Dobrić

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In September this year, 70 years since the beginning of publication of Vojnosanitetski pregled (VSP), scientific and professional journal of physicians and pharmacists of the Serbian Army, will be marked ranking it among the oldest medical journals in Serbia. Five years ago, when the Journal celebrated its 65th birthday, its history was presented in more detail [1], therefore on this occasion only the most significant moments that have marked its 70 years of existence will be given.

The first issue of the VSP was printed in September, 1944, in Bari, Italy, during World War II continuing the tradition of military medical scientific and professional journalism in our region which began by publishing the Vojno-sanitetski glasnik in the period 1930–1941. From the very beginning, to both professional and technical quality of the Journal was paid a great attention, respecting all the international standards and conventions for the scientific periodicals publishing. Even then, the articles published in the VSP, besides summaries in Serbian, had also summaries in English, Russian and French, which ensured them good international visibility. That is the reason, probably, why the VSP in the late 50s and early 60s of the last century was covered by several well-known bibliographic abstract and citation journals and, later, their electronic databases, such as Index Medicus (MEDLINE), Excerpta Medica (EMBASE), Biological Abstracts, International Pharmaceutical Abstracts, Chemical Abstracts. From the 2002, VSP has been included in EBSCO database through which the articles published in it can be obtained in full text.

The frequency of the journal publication changed over the past 70 years. Since the founding of the Journal to 1972 it has been published in 12 issues *per* year, which was an indication of the large influx of manuscripts, since 1973 it has been published in six issues anually, and since 2005 again a monthly publication of the Journal has been established (currently the VSP is only biomedical journal in the country with such a frequency of publication). The disintegration of the former Yugoslavia and the war on its territory at the beginning of the 90s of the last century had negative impact on the overall scientific production in Ser-

U septembru ove godine navršava se 70 godina od početka izlaženja „Vojnosanitetskog pregleda“ (VSP), naučno-stručnog časopisa lekara i farmaceuta Vojske Srbije, što ga svrstava u red najstarijih medicinskih časopisa u Srbiji. Pre pet godina, kada je obeležavan njegov 65. rođendan, detaljnije je prikazan istorijat časopisa [1], tako da će ovom prilikom biti izneti samo najznačajniji momenti koji su obeležili ovih 70 godina postojanja.

Prvi broj VSP-a objavljen je u septembru 1944. godine, u Bariju, Italija, za vreme Drugog svetskog rata, čime je nastavljena tradicija vojnomedicinske naučne i stručne časopisne publicistike na našim prostorima, započeta izlaženjem „Vojno-sanitetskog glasnika“ u periodu 1930–1941. godina. Od samog početka i stručnom i tehničkom kvalitetu časopisa poklanjala se velika pažnja, uz uvažavanje svih međunarodnih standarda i konvencija za izdavanje naučne periodike. Već tada, radovi objavljeni u VSP, pored sažetka na srpskom jeziku, imali su i sažetak na engleskom, ruskom i francuskom jeziku, što im je obezbeđivalo dobru međunarodnu vidljivost. Zbog toga je, verovatno, VSP već krajem 50-ih i početkom 60-ih godina prošlog veka ušao u sistem indeksiranja nekoliko poznatih bibliografskih i bibliografsko-apstraktnih časopisa i, kasnije, njihovih elektronskih baza, kao što su: *Index Medicus* (MEDLINE), *Excerpta Medica* (EMBASE), *Biological Abstracts*, *International Pharmaceutical Abstracts*, *Chemical Abstracts*. Od 2002. godine, VSP je uvršten i u bazu EBSCO preko koje se radovi objavljeni u njemu mogu dobiti u punom tekstu.

Dinamika izlaženja časopisa menjala se tokom proteklih 70 godina. Od osnivanja časopisa do 1972. godine, izlazilo je i 12 brojeva godišnje, što je bio pokazatelj velikog priliva radova, od 1973. godine prelazi se na izdavanje šest brojeva u godini, a od 2005. godine ponovo se uspostavlja mesečno izlaženje časopisa (trenutno je VSP jedini biomedicinski časopis u zemlji sa takvom dinamikom izlaženja). Raspad bivše SFRJ i rat na njenim prostorima početkom 90-ih godina prošlog veka negativno se odrazio na

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bia, and it was not spared either the VSP. However, despite the reduced inflow of manuscripts and difficulties in regular publication, the Journal "survived". In order to provide a large number of papers, in 1995 Editorial Board and Publisher of the VSP decided to award author(s) who in the last year published the most articles in the Journal. This led to a large influx of papers, but most authors were from the Military Medical Academy (MMA) in Belgrade. Since the members of the Editorial Board of the Journal, as well as reviewers were mainly from the MMA, too, there was a possibility of bias and consequently publishing less quality articles. This is the reason why in the early 2000s it was decided that experts outside the military health care institutions be included as members of the Editorial Board of the Journal, as well as reviewers with the provision of at least double anonymous peer-reviews. In addition, it was also decided that, according to the opinion of the reviewers, especially significant papers except in Serbian could also be published in English in order to become more "visible", with a tendency to extend this practice to other papers. These measures, along with maintained regular publication frequency, at the time when publication of other biomedical journals in the country has been late for several months, have resulted in an increasing influx of papers by authors from the so-called civilian medical institutions providing the possibility to publish the Journal monthly again. The practice of monthly publication of the VSP began again in 2005 during which the process of forming the International Editorial Board was finished. In this manner, formal requirements for inclusion of the Journal into international journal society was fulfilled. At the same time, the Journal was enriched with new sections (Letters to the Editor, Comments, Critical Reviews, etc), received a logo and a new, modern look. Also, the influx of papers by authors from abroad has been increased, as well as a growing number of articles published in English (eg. in 2007 every fifth, and in 2008 every fourth article). In addition, the selection criteria for acceptance of papers have got more strict, and previous peer-review procedure have been established during which the authors have been advised how to technically, professionally and in English writhing improve their papers before officially sent to reviewers. A special attention was paid to prevent the occurrence of plagiarism and self-plagiarism adopting a series of measures for their detection and sanctioning. All this contributed to the increase in the quality of papers published in the VSP and increase their visibility on the international level resulting in 2008 by its inclusion into the famous citation databases *Science Citation Index Expanded* (SCIe) of the Institute for Scientific Information (ISI), Philadelphia, USA, now a part of the Thomson Reuters company, and obtaining the first impact factor (IF) in 2010.

Having in mind that the first IF of any journal can be obtained three years after inclusion into ISI databases such as SCIe, as in the case of the VSP meant in 2011, but for 2010, the Editorial Board of the Journal continued working to improve its quality. Increased influx of papers, especially after the publication of news about the inclusion in SCIe database, not only by authors from Serbia, but from all over the world, as well as the involvement of foreign reviewers with consequent rigorous selection of manuscripts received, further contributed to improving quality of articles selected for publication in the VSP. Along with the increase in the number of papers received for publication (between

ukupnu naučnu publicistiku u Srbiji, pa toga nije bio pošteđen ni VSP. Ipak, uprkos smanjenom prilivu radova i poteškoćama u redovnom izlaženju, časopis je opstao. U cilju obezbeđenja većeg broja radova, uredništvo i izdavač VSP-a 1995. godine odlučuju da svake godine nagrade autora kome u protekloj godini bude objavljen veći broj radova na stranicama časopisa. To je dovelo do većeg priliva radova, ali uglavnom od autora iz Vojnomedicinske akademije (VMA) u Beogradu. Kako su i članovi uredništva i izdavačkog saveta časopisa, kao i recenzenti bili, uglavnom, iz VMA, postojala je mogućnost da se zbog „linije nezameranja" u časopisu počnu objavljivati i manje kvalitetni radovi. Ovo je bio razlog što je početkom 2000-ih odlučeno da se Uređivački odbor časopisa, kao i krug recenzenata, prošire i na stručnjake izvan vojnog saniteta uz obezbeđenje najmanje dvostruke anonimne recenzije. Osim toga, bilo je predviđeno da se, na osnovu mišljenja recenzenata, posebno značajni radovi, osim na srpskom, objave i na engleskom jeziku da bi postali „vidljiviji", sa tendencijom da se ta praksa proširi i na ostale radove. Ove mere, uz obnovljenu redovnost izlaženja, u vreme kada su drugi biomedicinski časopisi u zemlji kasnili sa izlaženjem i po nekoliko meseci, imale su za posledicu sve veći priliv radova i od autora iz civilnih zdravstvenih institucija, tako da je ponovo bilo omogućeno izlaženje časopisa svakog meseca. Praksa mesečnog izlaženja VSP-a započeta je 2005. godine, tokom koje je završen i proces formiranja Međunarodnog uređivačkog odbora. Ovim su se stekli i formalni uslovi za ulazak u društvo međunarodnih časopisa. Istovremeno, časopis je obogaćen novim rubrikama (pisma uredništvu, komentari, kritički osvrti i sl), dobio je logo i novi, moderniji izgled, a povećao se i priliv radova autora iz inostranstva, kao i sve veći broj radova objavljenih na engleskom jeziku (npr. u 2007. godini svaki peti, a u 2008. svaki četvrti rad). Osim toga, pooštreni su kriterijumi za prihvatanje radova, a uveden je i postupak predrecenzije, tokom koga je autorima ukazivano na koji način da tehnički, stručno i jezički poboljšaju rad pre nego što se zvanično uputi recenzentima. Posebno se vodilo računa o sprečavanju pojave plagijarizma i autoplagijarizma donošenjem niza mera za njihovo otkrivanje i sankcionisanje. Sve ovo, doprinelo je povećanju kvaliteta radova objavljenih u VSP-u i povećanju njihove vidljivosti na međunarodnom nivou, što je 2008. godine rezultiralo i prijemom VSP-a u sistem praćenja čuvene citatne baze *Science Citation Index Expanded* (SCIe) Instituta za naučne informacije (ISI) iz Filadlefije, sada u sklopu kompanije Thomson Reuters i dobijenjem prvog impakt faktora (IF) u 2010. godini.

Znajući da časopis svoj prvi IF dobije posle tri godine od ulaska u sistem praćenja citatnih baza ISI, što je u slučaju VSP značilo 2011, ali za prethodnu 2010. godinu, uredništvo časopisa nastavilo je da radi na poboljšanju njegovog kvaliteta. Povećan priliv radova, pogotovo posle objavljivanja vesti o uključenju u SCIe, i to ne samo od autora iz Srbije, već iz celog sveta, kao i angažovanje inostranih recenzenata s posledičnom rigoroznijom selekcijom primljenih rukopisa, dodatno su doprineli poboljšanju kvaliteta radova odabranih za objavljivanje u VSP-u. Uporedo

2005 and 2007 225–235 papers, and after 2008 about 350), the interest of readers for articles published in it has also been increased. According to data obtained from the EBSCO database, through which the articles published in the VSP were available in full text, in 2008 when the VSP was included in SCIe database, the number of accesses and downloads of articles published in the VSP was 2,340, and in 2009 it was already five times higher (11,562), with a continuous increase in the coming years (in 2012 the number was 38,376, in 2013 39,436 or slightly more than 100 accesses daily).

In the period after the VSP was included in SCIe database the number of published articles that are commonly cited (Original articles, General reviews, Current topics, Case reports, etc) have also been increased. As an illustration, a total of 145 articles was published in 2005, 160 in 2008, 170 in 2009 and 2010, each, and more than 180 in the years 2011, 2012 and 2013. This increase is the result of attempts that papers successfully passing the peer-review process as soon as possible make available to the scientific and professional community, although in this manner, because of the way of IF calculation (IF for any year is the quotient of the number of citations in that year of articles published in the last two years and the total number of articles published in these two years), its value, despite the increase in the number of citations, can be reduced. This is, at the same time, the best proof that the Editorial Board of the VSP has not wanted by reducing the number of published articles to reduce the denominator in the formula for calculating IF and in a such way to affect its value. Besides, by placing electronic version of the Journal on its web-site (www.vma.mod.gov.rs/vsp), with the possibility of free access to the content of each article, visibility and accessibility of published articles have additionally been increased.

The first IF of the VSP (IF for 2010) was 0.199 classifying it, at that moment, on the 135th position among the 153 most influential journals in the field of General and Internal Medicine. Its value for the 2011 dropped to 0.179, but for 2012 it rose to 0.210. The latest IF of the Journal, for 2013, was released in late July this year, amounting 0.269, which is an increase of 35.7% in relation to the value of the first IF. Since from the inclusion of the VSP in SCIe database a number of articles that have been published in the Journal increased by more than 10%, taking into account the method of calculating IF, it can be concluded that this increase in the IF value is the result of the actual increase in citations of these articles.

Joining a scientific journal in citation system of ISI databases does not mean, by default, a permanent status as one of the most influential journal in the world in a particular scientific field. On the contrary, only then begins the real struggle for "survival", constantly proving that this privileged status is really deserved. Having this in mind, the Editorial Board of the VSP, after obtaining the first IF, continued with activities to improving the Journal quality in all domains [2–5].

In the 2011 VSP entered into the so-called DOI (Digital Object Identifier) system that allows identification of documents in electronic form and establishes a permanent connection to the Internet site where the original document is located. In this manner, articles with DOI number published in an electronic form can be found and cited even before their publishing in printed version

sa porastom broja pristiglih radova za objavljivanje (između 2005. i 2007. godine, 225–235 radova, a posle 2008. oko 350), porasla je i zainteresovanost čitalaca za članke objavljene u njemu. Prema podacima stručnih službi baze EBSCO, preko koje su članci objavljeni u VSP dostupni u punom tekstu, 2008. godine kada je VSP uvršten u bazu SCIe, broj pristupa časopisu i preuzimanja članaka iz njega iznosio je 2 340, da bi već 2009. godine bio pet puta veći (11 562), uz stalni porast u narednim godinama (2012, taj broj je iznosio 38 376, a prošle, 2013, 39 436 ili nešto više od 100 pristupa dnevno).

U periodu posle ulaska VSP u sistem praćenja baze SCIe povećan je i broj objavljenih članaka koji se najčešće citiraju (originalni članci, opšti pregledi, aktuelne teme, prikazi slučajeva i sl). Ilustracije radi, u 2005. godini objavljeno je ukupno 145 članaka, 2008. godine 160, 2009. i 2010. 170, a od 2011. godine broj objavljenih članaka u VSP-u prelazi 180. Ovaj porast objavljenih članaka rezultat je nastojanja da se radovi koji uspešno prođu postupak recenziranja što pre stave na uvid naučnoj i stručnoj javnosti, iako se na ovaj način, zbog načina izračunavanja IF (IF za određenu godinu predstavlja kvocijent broja citata u toj godini za radove objavljene u prethodne dve godine i broja radova objavljenih u te dve godine), njegova vrednost, uprkos porastu broja citata, može smanjiti. Ovo je, ujedno, i najbolji dokaz da uredništvo VSP nije želelo da smanjenjem broja objavljenih radova, a time i imenioca u formuli za izračunavanje IF, utiče na njegovu vrednost. Osim toga, postavljanjem elektronske verzije časopisa na njegovu Internet stranicu (www.vma.mod.gov.rs/vsp), sa mogućnošću slobodnog pristupa sadržaju svakog članka, dodatno se povećala vidljivost i dostupnost objavljenih radova.

Prvi IF VSP (IF za 2010. godinu) iznosio je 0,199 svrstavši ga, u tom momentu, na 135. poziciju između 153 najuticajnija časopisa iz oblasti Opšte i interne medicine. Njegova vrednost za 2011. godinu smanjila se na 0,179, da bi u 2012. godini porasla na 0,210. Najnoviji IF VSP-a za 2013. godinu, objavljen krajem jula ove godine, iznosi 0,269, što je porast od 35,7% u odnosu na vrednost prvog IF. S obzirom da je od ulaska VSP u bazu SCIe broj radova koji se objave na njegovim stranicama porastao za više od 10%, a uzimajući u obzir način izračunavanja IF, može se zaključiti da je ovaj porast vrednosti IF rezultat stvarnog porasta citiranosti radova objavljenih u njemu.

Ulazak časopisa u sistem praćenja citatnih baza ISI ne znači, po automatizmu, i trajno rešenje njegovog statusa kao jednog od najuticajnijih časopisa sveta u određenoj naučnoj oblasti. Naprotiv, tek tada počinje prava borba za opstanak, neprestano dokazivanje da je taj privilegovani status zaista i zaslužen. Imajući ovo u vidu, uredništvo VSP nastavilo je i posle dobijanja IF sa uvođenjem novina čiji je cilj unapređenje časopisa u svim domenima [2–5].

U 2011. godini VSP je ušao u tzv. DOI (Digital Object Identifier) sistem koji omogućava identifikaciju dokumenta u elektronskom obliku i uspostavljanje stalne veze do Internet stranice na kojoj se originalni dokument nalazi. Zahvaljujući DOI broju članak u elektronskom obliku može da bude pronađen i citiran i pre nego što iziđe štampana verzija

of the VSP. Thanks to this, the VSP for more than two years has been publishing 4–6 articles each month in electronic form as On-Line First. Also, since the beginning of 2011 only manuscripts prepared in English have been accepted for publications in the VSP in order to get their content as close as possible to readers from abroad. The exception to this are some articles on the history of medicine which, by quoting the text in Serbian in its original form could lose the authenticity by translating in English. Publication of articles in English have probably contributed to their higher citation as data from the EBSCO database indicate that articles in the VSP published in English have the greatest number of approaches from institutions around the world.

In 2012 the VSP moved to the system of electronic editing (so-called e-UR) which allows easier communication between the Editorial Board of the Journal and the authors on the one side, and the Editorial Board and reviewers on the other, facilitates the monitoring of the status of a paper from the moment of its receipt in the Editorial Office to the final status, and provides checking of plagiarism and self-plagiarism, which has already on several occasions prevented publication of such articles, and, we hope, will reduce such misconduct in future and discourage those authors prone to dishonesty in science. Since July, 2012 we have been using the improved version of the system called ASEESTANT which ofer some additional benefits: the control of references correctness, as well as the selection of appropriate keywords according to the Thesaurus of the U.S. National Library of Medicine from Betesda, which as standardized terms are used in all medical scientific publications. Another novelty is the requirement that the authors in accordance with the recommendations of the International Committee of Medical Journal Editors (International Committee of Medical Journal Editors - ICMJE) [6] provide a signed statement on the contribution to the research and manuscript preparation and the absence of conflict of interests in order to ensure the transparency of information about authorship and funding and, thereby, strengthening ethical principles in scientific publications.

Earlier this year, domestic and international Editorial Board of the Journal was renewed with a few new members, recognized experts in various fields of biomedicine, from home and abroad who, we believe, will significantly contribute to further development of the Journal [7].

In their and my own name, I wish all the best and longevity to our septuagenarian with the desire that its future anniversaries be crowned with even greater successes!

časopisa, što VSP već više od dve godine koristi objavljivanjem svakog meseca po 4–6 članaka u elektronskom obliku, kao *On-Line First*. Takođe, od početka 2011. godine primaju se isključivo radovi na engleskom jeziku da bi se njihov sadržaj što više približio i čitaocima iz inostranstva. Izuzetak od toga su pojedini prilozi iz istorije medicine koji bi, zbog navođenja teksta u izvornom obliku, prevođenjem na engleski izgubili na autentičnosti. Objavljivanje radova na engleskom jeziku verovatno je doprinelo i njihovoj većoj citiranosti na šta nas upućuju i podaci iz baze EBSCO da članci iz VSP-a napisani na engleskom jeziku beleže, već godinama, i najveći broj pristupa i to iz institucija širom sveta.

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Početkom ove godine, obnovljen je domaći i internacionalni Uređivački odbor časopisa sa nekoliko novih članova, priznatih stručnjaka iz različitih oblasti biomedicine, iz zemlje i inostranstva koji će, verujemo, značajno doprineti daljem razvoju VSP-a [7].

U njihovo i svoje ime, želim mnogo sreće i dugovečnosti našem sedamdestogodišnjaku, sa željom da njegove buduće godišnjice budu krunisane još većim uspesima!

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Quantitative and qualitative gait assessments in Parkinson's disease patients

Kvantitativna i kvalitativna procena obrasca hoda kod bolesnika sa Parkinsonovom bolešću

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Abstract

Background/Aim. Postural impairments and gait disorders in Parkinson's disease (PD) affect limits of stability, impaire postural adjustment, and evoke poor responses to perturbation. In the later stage of the disease, some patients can suffer from episodic features such as freezing of gait (FOG). Objective gait assessment and monitoring progress of the disease can give clinicians and therapist important information about changes in gait pattern and potential gait deviations, in order to prevent concomitant falls. The aim of this study was to propose a method for identification of freezing episodes and gait disturbances in patients with PD. A wireless inertial sensor system can be used to provide follow-up of the treatment effects or progress of the disease. **Methods.** The system is simple for mounting a subject, comfortable, simple for installing and recording, reliable and provides high-quality sensor data. A total of 12 patients were recorded and tested. Software calculates various gait parameters that could be estimated. User friendly visual tool provides information about changes in gait characteristics, either in a form of spectrogram or by observing spatiotemporal parameters. Based on these parameters, the algorithm performs classification of strides and identification of FOG types. **Results.** The described stride classification was merged with an algorithm for stride reconstruction resulting in a useful graphical tool that allows clinicians to inspect and analyze subject's movements. **Conclusion.** The described gait assessment system can be used for detection and categorization of gait disturbances by applying rule-based classification based on stride length, stride time, and frequency of the shank segment movements. The method provides an valuable graphical interface which is easy to interpret and provides clinicians and therapists with valuable information regarding the temporal changes in gait.

Key words:
parkinson disease; gait, disorders, neurologic; disease progression; biomedical engineering.

Apstrakt

Uvod/Cilj. Poremećaji hoda i ravnoteže kod bolesnika sa Parkinsonovom bolešću (PD) uključuju i poremećaje stabilnosti, održavanja ravnoteže prilikom hoda i nemogućnost adekvatne reakcije na iznenadne perturbacije. U kasnijim fazama bolesti neki bolesnici razvijaju i epizode motornog bloka, odnosno „frizing" tokom hoda. Objektivno praćenje i merenje karakteristika hoda i promena obrasca hoda tokom progresije bolesti mogu pomoći kliničarima jer ukazuju na promene koje bi dovele do padova i ugrozile bolesnika. Cilj rada bio je razvoj metode koja bi identifikovala ovakve epizode kod bolesnika sa Parkinsonovom bolesti. Razvijeni bežični sistem sa senzorima mogao bi se koristiti za posmatranje efekata terapije ili progresije bolesti. **Metode.** U radu je prikazan sistem za objektivnu procenu obrasca hoda. Korišćenjem bežičnog senzorskog sistema koji koristi akcelerometre, žiroskope i senzore sile, moguće je dobiti procenu parametara hoda, ali i identifikovati „frizing" epizode karakteristične za PD. Uz pomoć ovog sistema snimljeno je 12 bolesnika, te je na osnovu snimljenih signala razvijen novi softverski alat koji omogućava praćenje parametara hoda. **Rezultati.** Na osnovu dužine koraka, trajanja koraka i frekvencije pokreta, razvijen je algoritam za klasifikaciju tipova koraka i uočavanje promena frekvencija pokreta tokom hoda. Prikaz rezultata ovog sistema je dat kroz primer jednog bolesnika. **Zaključak.** Opisani sistem za procenu hoda može biti korišćen za kategorizaciju poremećaja hoda kroz posmatranje promena u dužini i trajanju koraka, kao i frekvencija segmenata noge. Razvijeni metod omogućava iliustrativni prikaz i grafički interfejs koji je jednostavan za interpretaciju i omogućava dobijanje informacija koje kliničarima mogu ukazati na trenutne promene u obrascu hoda.

Ključne reči:
parkinsonova bolest; hod, poremećaji, neurološki; bolest, progresija; biomedicinsko inženjerstvo.

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Introduction

Postural and gait disorders are the most disabling cardinal motor signs found in people with Parkinson's disease (PD). Patients with PD have been reported to have postural impairments including reduction of limits of stability, impaired postural adjustment and poor responses to perturbation [1–3]. Gait disturbances include slow gait speed, shorter step and stride length, and increased variability of stride time (i.e., cycle time) as well as of stride length. In the later stage of disease progression, some patients can suffer from episodic features such as freezing of gait (FOG) [4, 5]. Gait and balance deficits predispose people with PD to falls. In a 20-year follow-up study, it has been reported that 87% of individuals with PD experienced one or more falls and 35% sustained injuries resulting from falls during walking [6]. Falls can lead to physical injuries and psychological traumas such as fear of falling. This results in functional mobility restriction, loss of independence, social isolation, decreased quality of life with increased risk of institutionalization and consequently increased mortality rate. Therefore, the objective gait assessment and monitoring progress of the disease can give clinicians and physical therapist important information about changes in the gait pattern and potential gait deviations, as well as supply data regarding disturbed gait pattern, especially for patients who exhibit FOG episodes and concomitant falls.

We have developed a wireless sensor system for assessment and evaluation of gait patterns for patients with various gait disturbances [7, 8]. The system uses inertial sensors (3D accelerometers and gyroscopes) which record body kinematics and estimation of gait parameters in any environment and for unlimited number of steps during a subject's walk.

This paper presents a new method for identification and classification of freezing episodes for patients with PD. This method, merged with the analysis of standardized temporospatial gait parameters and 3D trajectory reconstruction provides an illustrative clinical tool providing quantitative and qualitative information about a patient's gait pattern and its changes. This assessment was performed in a more detailed and more objective manner than it can be obtained by visual observation or any of existing clinical rating scales.

This system can also be used to provide follow-up of the treatment effects or progress of the disease. In some cases, given medications can stabilize gait by increasing the stride length and/or decreasing stride-to-stride variability [9–11]. The benefit of this treatment can be in monitoring the stride characteristic, length, and timing, before and after treatment application. Therefore, the presented method is an illustrative clinical tool to monitor gait pattern and gait pattern changes.

Methods

Instrumentation – Wireless sensor system for gait analysis

The proposed system, named SENSY was designed and developed within collaboration between the Faculty of Electrical Engineering, University of Belgrade, Serbia and Tecnalia Research Center, San Sebastian, Spain [12]. SENSY hardware comprises six peripheral inertial measurement units (IMUs), one per each leg segment of both legs, and a central PC communication unit connected to a USB port of a remote computer, where signals are monitored and stored (Figure 1). Foot IMUs include connectors for force sensors, which can be attached to the system, either incorporated in shoe insoles or as independent force sensors. Force sensors used in this study were integrated in shoe insoles, with active areas under heel, metatarsals, and toes (Figure 1, left). Each IMU comprises a 3-D analog accelerometer and a 3-D analog gyro sensor. The IMUs are powered by rechargeable batteries.

Fig. 1 – Sensor system mounted on a subject. Left: mounting scheme with inertial sensors placed on leg segments and shoe insole with integrated force sensors; Middle and right: photos of the mounted system, front view and profile.

The developed system is light and compact (30 g per unit), simple for mounting on a subject, comfortable to wear, simple for installing and recording, and presents a reliable system which provides high-quality sensor data. Custom-made acquisition software was developed in LabWindows CVI program (National Instruments, USA) enables real-time monitoring and automated storage of recorded data. Further

signal processing of recorded data is performed in Matlab (MathWorks Inc, USA).

Subjects

A total of 12 patients with idiopathic PD (age of 60 ± 9 years, range 41–71 years) participated in this study. The demographic and clinical data on the study group are shown in Table 1. The recordings were performed at the Clinic for Neurology, Clinical Centre of Serbia, Belgrade, Serbia. The study was performed in accordance to the ethical standards of the Declaration of Helsinki. All the participants gave informed written consent prior to the participation in the study.

Table 1

Patients' demographics, motor and clinical data

Patient	Age (years)	Onset (years)	HY	UPDRS III	NFOG-Q	FOG UPDRS item
#1	59	5	2	45	27	3
#2	57	13	3	65	28	3
#3	64	8	2	35	11	2
#4	66	5	2	43	/	2
#5	70	20	2	42	/	2
#6	57	27	3.5	56	26	2
#7	69	7	3	34	22	2
#8	61	18	2.5	40	17	2
#9	50	5	2.5	24	27	2
#10	41	12	/	/	11	2
#11	71	2	4	56	25	3
#12	60	8	3	/	/	/
Mean	60.4	10.8	2.7	44	21.5	2.27
± SD	± 8.7	± 7.4	± 0.68	± 12.16	± 6.85	± 0.47

HY – Hoehn and Yahr scale, UPDRS – Unified Parkinson's Disease Rating Scale, NFOG-Q – New Freezing of Gait Questionnaire, FOG – Freezing of gait.

The patients were recorded and tested during "off" periods of the disease. At the study entry, the stage of the disease was scored using the Hoehn and Yahr (HY) stage score (this scale is widely used for overall Parkinson's disease severity assessment [13]), the patient disability and motor evaluation of condition by using the Unified Parkinson's Disease Rating Scale (UPDRS) [14], and the global cognitive function using the Mini-Mental State Examination (MMSE) [15]. The inclusion criteria for appropriate data collection were the following: a Hoehn and Yahr disease stage score from 2 to 4, a stable and optimized antiparkinsonian treatment during 4 weeks prior to study entry, and appropriate cognitive functions - MMSE score more than 25.

The patients were assessed by neurologists with Hoehn and Yahr scale, Unified Parkinson's Disease Rating Scale, New Questionnaire (scale for quantitative and qualitative FOG assessment and its influence to activities of daily living [16] (NFOG-Q) and timed "up-and-go" test (TUG) with obstacles [17]. See Table 1 for test details. Based on observation and clinical tests the patients were classified as freezers (PD+FOG).

Experiment setup and recording protocol

The sensor system was mounted on the patients' limbs, as shown in Figure 1. IMUs were placed laterally on leg segments (thigh, shank, and foot of both legs). Shoe insoles with integrated force sensitive resistors (FSR) were used as shown in Figure 1, left panel. The patients were asked to walk along a complex pathway, as illustrated in Figure 2. The pathway was created to provoke freezing episodes in patients. It included start/stop, turns, U-turns, and passing through doorways with different widths. Each trial started from the chair where the patient was sitting. Upon a voice command, the patient had to stand up and start walking straight towards the door 1 to pass the doorway, turn left towards (very narrow) door 2, to make a U-turn, return through door 2, then to go straight along the corridor, where he/she would pass (wide) door 3, several strides later make another U-turn around the obstacle, pass through door 3 again, turn left, pass through door 1, return to the chair, and sit down. This session was repeated four times per subject, with 5-minute breaks between trials. Directions of the U-turn rotations were chosen by the patient. All experiments were recorded with a video camera. Clinicians used videos to identify gait disturbances and FOG episodes (type and duration), and these data were further used for validation of our method.

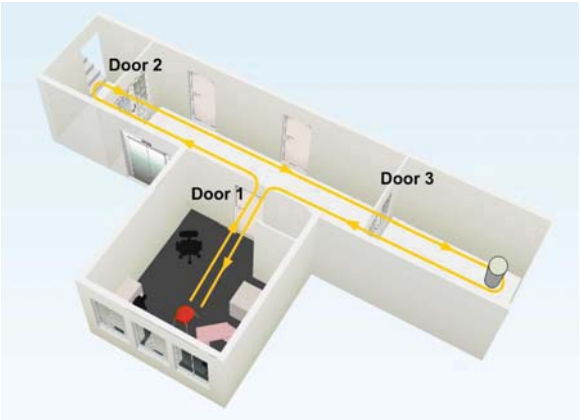

Fig. 2 – Floor plan with the pathway along which the patients were walking (starting from the Lab room, through the doors and hallways, and back).

Data processing

1. Spatiotemporal gait parameters

In order to estimate gait parameters, reliable and accurate estimations of the particular gait events are needed. Heel contact and toe-off delimit swing and stance phases of the gait cycle, so that identification of these events can be used for estimation of various temporal gait parameters. One of the most reliable and easiest methods is by applying thresholds to heel switch or FSR data [18]. The detection of gait events from force sensors is a simple and reliable technique, especially for applications to patients with pathological gait, where it is difficult to adapt rules or thresholds for detection methods for gait events based on IMUs [19]. The estimation of temporal gait parameters is provided by the ground reaction force obtained from force sensors placed under the heel, metatarsal, and toe area of the foot.

FSRs were used to detect gait phases by summing signals from one leg, normalizing to the maximum of the sum, and applying a threshold (THfsr) at 5% of the normalized sum. Intervals where the signal is beyond THfsr are considered to belong to the stance phase, while the other intervals (the normalized sum is below THfsr) are considered to be the swing phase.

Spatial gait parameters (e.g. stride and step length) require the data recorded from accelerometers. Double numerical integration of linear accelerations (recorded by accelerometers) provides information about spatial displacement. For estimation of the stride length, we performed double integration of acceleration signals recorded from foot segments and transformed to the global coordinate system. However, due to the integration drift typical for this type of sensors, a drift-elimination procedure needs to be performed. One successful method for drift elimination is based on a polynomial drift approximation. In this way, we obtained estimation of stride length with only a 4% error [20].

By applying the definitions of gait phases and temporal gait parameters to the detected gait events, SENSY software calculates various gait parameters for each stride within recorded sequence. A list of gait parameters that could be estimated by this system include: stride time, cadence, velocity, stride length, swing time, stance time, swing/stance ratio, single support time, double support time, symmetry etc. A stride parameter that cannot be estimated by this system is the stride width, since that would require information from additional sensors.

2. Reconstruction of gait trajectory

In order to provide 3-D gait analysis and trajectory reconstruction, the software employs transformation matrices and combines human locomotion and biomechanical constraints in order to fuse accelerometer and gyroscope data. Polynomial fitting eliminates the drift [12].

3. Frequency analysis based on spectrogram

PD patients may exhibit changes in movement frequencies of limb segments during gait. Normal walking is typically characterized with frequencies from 0.5 Hz to 3 Hz (vertical shank acceleration), while FOG in patients with alternate leg trembling is typically manifested with a tremor in the range from 3 Hz to 8 Hz. FOG patients can also experience motor blocks without performing movement at all [21–23]. Therefore, it is very practical to display spectrum as a function of time, so that a clinician can follow changes of patient's stride frequency, correlate these alterations to existing obstacles along the path, and estimate duration and intensity of these disorders.

A very intuitive and "user friendly" visual tool for frequency follow-up is a spectrogram, calculated from the temporal signal by using the short-time Fourier transform preformed for each stride independently. The spectrogram used in this application is a graph with the horizontal axis showing time or number of strides, while the vertical axis corresponds to the frequency. A third dimension indicates the amplitude of a particular frequency at a particular time instant, which is coded by the intensity or color of each pixel of the image. We selected the Jet color map in Matlab software, where low amplitudes are represented by cold color tones (starting from navy blue for the lowest amplitude), heading towards warmer colors with amplitude increase, finishing with dark red color for the highest amplitude, as shown in Figure 3 example.

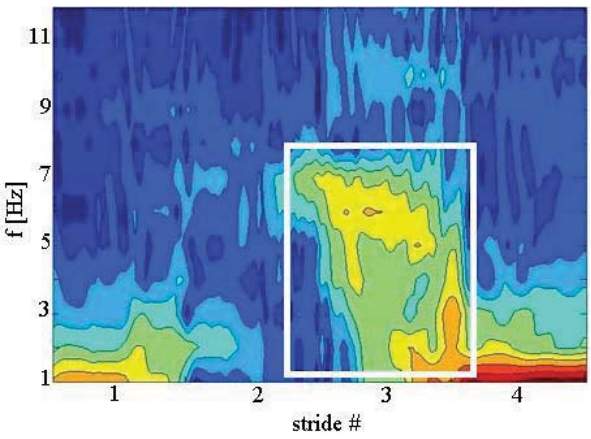

Fig. 3 – Example of spectrogram: FOG episode begins as a motor block (stride #2) and is followed by FOG with tremor episode of the frequencies 5–7 Hz (stride #3), as depicted by the frame.

4. Stride classification and identification of freezing of gait episodes

We illustrated the method with examples of stride-to-stride variability and FOG appearances, both described by the stride length (SL), stride duration (SD), and power spectrum. If we classify each stride based on these three parameters, we can assign each stride to a certain class type according to: normal, short, shuffling, FOG with tremor or FOG with complete motor block. Thresholds for stride length and duration can be calculated from straight-line gait sequence (suggested length not less than 5 m). The thresholds are then defined from mean ± SD of the two observed parameters (Figure 4), while for shuffling strides (typical for FOG) the threshold for stride length is set to 20 cm. If the patient's gait

pattern is severely disturbed even for the straight-line gait sequence, the clinician can apply thresholds obtained from the available data or can manually adjust the threshold according to his/her assessment.

Figure 4 shows an example of the threshold setup.

Classification of strides and FOG types were made accordingly to the rules presented in Tables 2 and 3.

Results

The described stride classification was merged with an algorithm for stride reconstruction [12], resulting in a useful graphical tool that allows clinicians to inspect and analyze a subject's movement. Along the reconstructed trajectory gait disturbances are highlighted for detailed gait analysis.

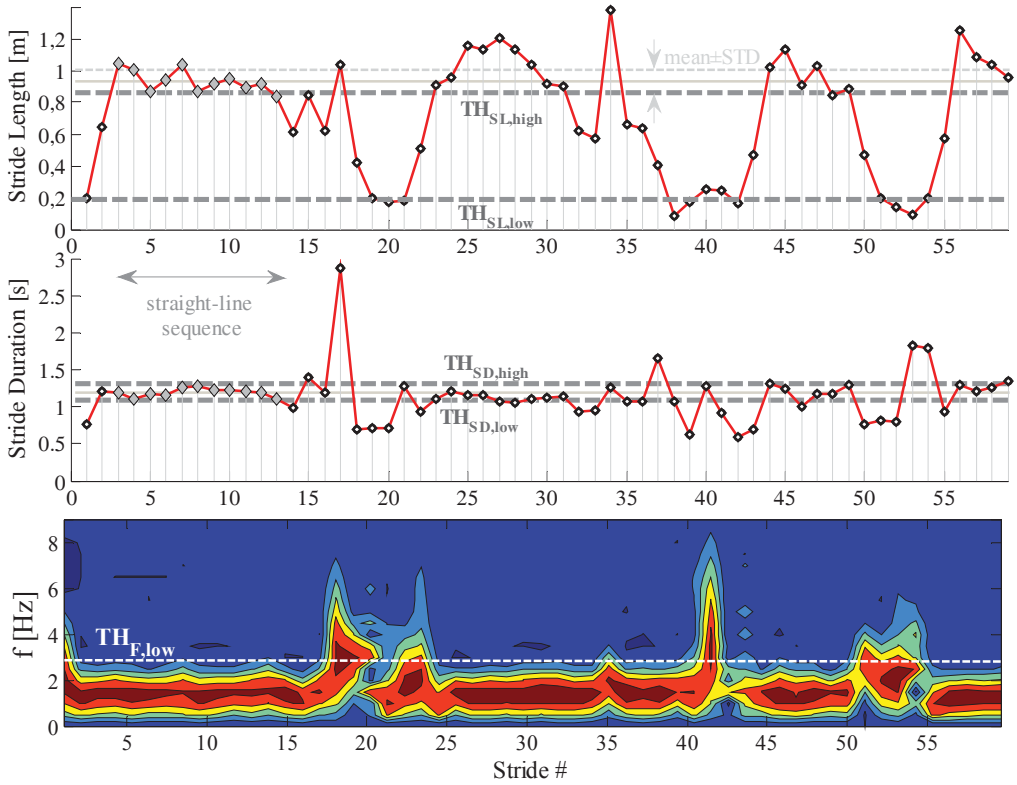

Fig. 4 – An example of stride-to-stride variability of a Parkinson's disease (PD) patient's gait shown by the stride length (top), stride duration (middle), and spectrogram (bottom). Thresholds are shown with thick dashed horizontal lines ($TH_{SL,high}$, $TH_{SL,low}$, $TH_{SD,high}$, $TH_{SD,low}$, $TH_{F,low}$). Mean values and standard deviations of stride length and duration are calculated from initial strides while the patient was walking along a straight path (grey markers). Spectral analysis is shown as a spectrogram, with the white dashed line (threshold value) separating frequencies during "normal" gait and gait with trembling/tremor.

Table 2

Typical values for gait parameters with lower and higher thresholds (TH) applied for classification

Gait parameter	Normal (typical)	TH_{low}	TH_{high}
Stride length (SL) [m]	0.7–1.3	0.2	0.5
Stride duration (SD) [s]	0.8–1.2	0.5	2
Dominant frequency band (F_1 –F_2) [Hz]	0.5–3	3	8

Table 3

Rule-based stride classification and identification of freezing of gait (FOG) episodes.

Stride or FOG type	Rule (Condition)
Normal	Default
Short stride	$TH_{SL,low} < SL < TH_{SL,high}$
Shuffling stride	$SL < TH_{SL,low}$
FOG motor block	$Freq < TH_{F,high}$ & $ST > TH_{ST,high}$
FOG & trembling	$Freq > TH_{F,low}$ & $ST > TH_{ST,high}$

TH – threshold; SL – stride length; ST – stride time.

This graphical tool plots the trajectory in 3D as a stick diagram (Figure 5), where one "leg" represents each stride, and the gait disturbances are color coded according to the following scheme: black = normal, blue = short, green = shuffling, red = FOG with trembling, and pink = FOG with motor block. The algorithm identifies and marks freezing episodes by different colors and indicates gait sequences which should be observed and analyzed more carefully.

As an example of recorded gait shown in Figure 4, such generated plot is presented in Figure 5. The following is a

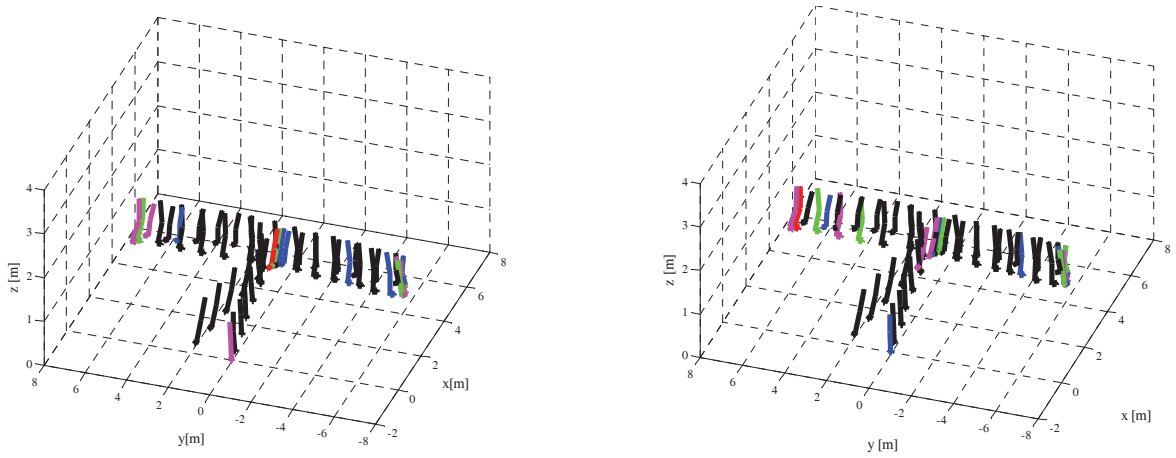

Fig. 5 – Illustration of 3D gait trajectory for the left and the right leg (left and right panel) with identified and classified freezing of gate (FOG) episodes shown by color coding. Stride types are color coded according to following: black – normal, blue – short, green – shuffling, red – FOG with tremor, pink – FOG with motor block. Left leg and right leg comparison shows asymmetry of the existing gait disturbances.

detailed description of Figure 5: the changes in the gait pattern were present at the beginning of the sequence (start hesitation), which is shown at (0, 0, 0) coordinate in Figure 5. The patient had the hesitation start at the first step. Further on, he experienced gait disturbances with FOG after passing through door 2 and during turning. He had a complete motor block on the left leg while he was trying to initiate/continue walking with the right leg (manifested by FOG with tremor and shuffling). Thereafter, he successfully proceeded through the hallway towards door 3 and through door 3, but once again he experienced problems during the U-turn. While turning around the obstacle, he had short shuffling strides combined with a complete motor block. Further, he started walking towards door 1, which manifested as a regular gait until he reached the door, where he dramatically decreased the stride length and eventually exhibited FOG with leg trembling. After this episode, he passed through door 1, walked forward and returned to the chair with his natural gait pattern.

This method for identification and classification of FOG episodes has been evaluated by experienced clinicians who compared the system/method outputs with video recordings of the same episodes. Comparison showed an excellent matching between visual observations and sensor detection of FOG episodes (both sensitivity and specificity test showed more than 95% success). Furthermore, sensors were able to quantify particular episodes and provide more detailed information also about the strides which preceded them.

Estimated gait parameters for gait sequence presented in Figures 4 and 5 are shown in Table 4. The parameters are shown in terms of their mean values, standard deviations and coefficient of variations (calculated according to $CV = \frac{STD}{mean} \cdot 100\ \%$), for the left and right leg separately.

Table 4

Gait parameters for the observed gait sequence

Gait parameters	mean L	SD L	mean R	SD R	CV L [%]	CV R [%]
Step count	107.00	/	/	/	/	/
Ambulation time [s]	68.12	/	/	/	/	/
Cadence [steps/min]	94.25	/	/	/	/	/
Stride counts	51.00	/	57.00	/	/	/
Distance [m]	37.79	/	36.21	/	/	/
Velocity [m/s]	0.65	0.31	0.66	0.32	47.55	49.46
Stride time [s]	1.31	0.98	1.20	0.50	74.57	42.07
Swing time [s]	0.34	0.13	0.33	0.14	37.41	42.56
Stance time [s]	0.97	0.98	0.86	0.54	100.94	62.69
Cycle time Dif [s]	-0.12	1.10	0.00	0.00	0.00	0.00
Single supp time [s]	0.36	0.13	0.31	0.16	36.88	49.82
Double supp time [s]	0.61	0.95	0.55	0.61	156.54	110.56
Swing cycle [%]	28.85	10.26	29.33	10.98	35.58	37.45
Stance cycle [%]	71.15	10.26	70.67	10.98	14.43	15.54
Single supp cycle [%]	30.54	10.29	27.27	11.92	33.70	43.70
Double supp cycle [%]	40.61	18.84	43.40	21.75	46.39	50.11
Stride length [m]	0.74	0.34	0.72	0.33	45.52	46.28

L/R – left or right leg; SD – standard deviation; CV – coefficient of variation.

Discussion

The described gait assessment system can be used to detect and categorize gait disturbances by applying the rule-based classification according to stride length, stride time,

and frequency of the shank segment movements. The spectrogram used in this application is easy to interpret and provides clinicians and therapists with valuable information regarding the temporal changes in the frequencies of lower limb's movement. This method discriminates among normal walking, appearance of FOG with leg trembling, and appearance of motor blocks during walking. This could point towards valuable information about walking unsteadiness and possible falls.

The SENSY system with the proposed method provides full information about FOG episodes in PD patients, as well as changes in gait patterns which could precede these episodes. However, the decisions about the proper and accurate classification of gait disturbances (such as short strides *vs* normal strides, or short strides *vs* shuffling) are sensitive to the established threshold values. Therefore, the visual tool (shown in Figure 5) should be considered in combination with the stride-to-stride variability data (Figure 4) with shown thresholds. These thresholds can be also adapted and changed manually by a referred clinician, if needed.

Unlike the clinical rating scales commonly used for FOG assessment (presented in the last two columns of Table 1), this assessment provides information about the types of stride or FOG episodes, its duration and intensity. Another advantage of the system, compared to clinical scales is that there are no issues with subjectivity which is based on the observer's experience and individual bias, or subjectivity of the patient responding the questionnaire.

Compared to other ambulatory systems for FOG detection [8, 21, 23], the described system provides more detailed information combining data from all three leg segments of both legs, and classify strides according to five previously defined states (normal or abnormal), while other systems typically classify gait patterns according to normal *vs* FOG, where some differentiate FOG to FOG with motor block or FOG with trembling.

When it comes to patients with gait disturbances that are present in PD, the gait analysis performed in a clinical environment frequently does not actually capture patient's real state of locomotion, i.e., the gait pattern which is prevalent. Being aware that their gait is being observed and recorded, patients often (consciously or subconsciously) change their gait pattern, trying to walk "better", faster, or the opposite, to emphasize their movement disorders and problems they have. Therefore, having a gait assessment system which could be used in home environment, as a holter monitor, would provide clear and objective image about patient's state in a quiet environment, as well as frequency and duration of experienced gait disturbances and episodes. This could be arranged by simple hardware adaptation, allowing the sensor units to store data internally, instead of sending the data to a remote PC.

Conclusion

For patients with PD, the objective spatiotemporal measure of gait disturbances provides a very important tool not only to follow the progress of the disease, but also to investigate how the patients respond to treatment (medication) and to monitor effects of prescribed therapies on gait characteristics. Also, since the proposed system could be used as a holter monitor, the required time for patients to stay in hospital could be significantly shortened. A patient can be examined during the day, sensor system attached to their limbs and returned home with a standalone holter unit to record gait while at home environment. In this way, clinicians can get real picture about patient's gait disturbances (types and timings) while at home, during their usual activities without being at the hospital. Furthermore, having illustrative graphic clinical tool to monitor gait pattern and gait pattern changes enables even inexperienced clinicians to note the changes in gait pattern, and signal the concomitant walking problems in monitored patients to clinicians or to patient's caregivers.

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Glycaemic control and prevalence of hypoglycaemic events in children and adolescents with type 1 diabetes mellitus treated with insulin analogues

Glikemijska kontrola i prevalencija hipoglikemija kod dece i adolescenata sa dijabetesom melitusom tipa 1 lečenih insulinskim analozima

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Abstract

Background/Aim. An ideal insulin regimen for children and adolescents with type 1 diabetes mellitus (T1DM) should be physiological, flexibile and predictable, protecting against hypoglycaemia. The aim of this study was to evaluate the influence of insulin analogues on glycaemic control and the occurance of hypoglycaemic episodes in children and adolescents with T1DM. **Methods.** The study group consisted of 151 children and adolescents (90 boys, 61 girls) treated with human insulins for at least 12 months before introducing insulin analogues. All the patients were divided into two groups: the group I consisted of 72 (47.7%) patients treated with three injections of regular human insulin before meals and long-acting analogue (RHI/LA), and the group II of 79 (52.3%) patients treated with a combination of rapid-acting and long-acting analogue (RA/LA). The levels of glycated hemoglobin (HbA1c) and the number of hypoglycaemic episodes were assessed at the beginning of therapy with insulin analogues, and after 6 and 12 months. **Results.** The mean HbA1c was significantly lower in the group I (RHI/LA) after 6 months (9.15% *vs* 8.20%, $p < 0.001$) and after 12 months (9.15% *vs* 8.13%, $p < 0.001$) as well as in the group II (RA/LA) after 6 months (9.40% *vs* 8.24%, $p < 0.001$) and after 12 months of insulin analogues treatment (9.40% *vs* 8.38%, $p < 0.001$). The frequency of severe hypoglycaemia was significantly lower in both groups after 6 months (in the group I from 61.1% to 4.2% and in the group II from 54.4% to 1.3%, $p < 0.001$), and after 12 months (in the group I from 61.1% to 1.4% and in the group II from 54.4% to 1.3%, $p < 0.001$). **Conclusion.** Significantly better HbA1c values and lower risk of severe hypoglycaemia were established in children and adolescents with T1DM treated with insulin analogues.

Key words:
diabetes melitus, type 1; child; adolescent; hypoglycemia; insulin; treatment outcome.

Apstrakt

Uvod/Cilj. Idealan insulinski režim za decu i adolescente sa dijabetesom melitusom tipa 1 (DMT1) trebalo bi da bude fiziološki, fleksibilan i predvidljiv, kao i da štiti od hipoglikemija. Cilj ove studije bio je procena uticaja insulinskih analoga na stepen glikemijske kontrole i učestalost hipoglikemijskih epizoda kod dece i adolescenata sa DMT1. **Metode.** Ciljna grupa obuhvatila je 151 dete i adolescenta (90 dečaka, 61 devojčica) koji su dobijali humane insuline bar 12 meseci pre uvođenja insulinskih analoga. Bolesnici su bili podeljeni u dve grupe: u prvoj je bilo 72 (47,7%) dece lečene sa tri injekcije regularnog humanog insulina pre obroka i dugodelujućim analogom insulina (RHI/DA), a u drugoj grupi 79 (52,3%) dece lečene kombinacijom brzodelujućeg i dugodelujućeg analoga insulina (BA/DA). Nivoi HbA1c i broj hipoglikemijskih epizoda registrovani su na početku terapije insulinskim analozima, i posle 6 i 12 meseci. **Rezultati.** Srednja vrednost glikoziranog hemoglobina (HbA1c) bila je značajno niža u prvoj grupi (RHI/DA) posle 6 meseci (9,15% *vs* 8,20%, $p < 0,001$) i posle 12 meseci (9,15% *vs* 8,13%, $p < 0,001$), kao i u drugoj grupi (BA/DA) posle 6 meseci (9,40% *vs* 8,24%, $p < 0,001$) i posle 12 meseci lečenja insulinskim analozima (9,40% *vs* 8,38%, $p < 0,001$). Učestalost teških hipoglikemija bila je značajno niža u obe grupe posle 6 meseci (u prvoj grupi sa 61,1% na 4,2% i u drugoj sa 54,4% na 1,3%, $p < 0,001$) i posle 12 meseci (u prvoj grupi sa 61,1% na 1,4% i u drugoj sa 54,4% na 1,3%, $p < 0,001$). **Zaključak.** Kod dece i adolescenata sa DMT1 lečenih insulinskim analozima utvrđen je značajno niži nivo HbA1c i manji rizik od teških hipoglikemija.

Ključne reči:
dijabetes melitus, tip 1; deca; adolescenti; hipoglikemija; insulin; lečenje, ishod.

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Introduction

Ideally, insulin regimen for children and adolescents with type 1 diabetes mellitus (T1DM) should be physiological, flexibile, and predictable, in order to protect against hypoglycaemia [1, 2]. This goal is particularly difficult to achieve in the paediatric patients, due to their susceptibility to hypoglycaemia, fluctuating insulin requirements caused by exercise, illness, variable carbohydrate intake, psychosocial and physiologic issues related to age, puberty, and weight gain [3, 4]. The Diabetes Control and Complications Trial (DCCT) and other landmark studies have shown that intensive insulin therapy is associated with an increased risk of hypoglycaemia [3].

Compared to regular human insulin (RHI), the new rapid-acting insulin analogues (RA, insulin aspart and lispro) more closely resemble postprandial endogenous insulin secretion by their faster onset and shorter duration of action which reduces the risk of hypoglycaemia between meals and during the first part of night as well as a need for snacks between meals [5, 6].

Long-acting insulin analogues (LA, insulin detemir and glargine), have been developed with the aim of providing a constant, flat and reproducible supply of basal insulin. Their action starts within 1 to 2 hours and diminishes within 16 to 24 hours, with no pronounced peaks, which lowers the risk of diurnal and nocturnal hypoglycaemia [4, 7, 8].

The aim of this retrospective study was to evaluate the influence of rapid-acting and long-acting insulin analogues on metabolic control and frequency of hypoglycaemic events in children and adolescents with T1DM.

Methods

The study group consisted of 151 children and adolescents (90 boys, 61 girls) treated with human insulins (total daily dose ≤ 1.5 U/kg) (Table 1). The primary inclusion criterion was intensive treatment with human insulins for at least 12 months. The second inclusion criterion was introducing insulin analogues due to unsatisfactory metabolic control. All the patients were divided into two groups: the group I consisted of 72 (47.7%) children treated with three injections of regular human insulin before meals and long-acting analogue (insulin detemir or glargine) at bedtime (RHI/LA) and the group II of 79 (52.3%) children treated with rapid-acting analogue (insulin aspart) as premeal insulin and long-acting analogue at bedtime (RA/LA). The mean age of patients at the beginning of treatment with insulin analogues was 13.0 ± 2.2 years in the group I and 13.5 ± 2.4 years in the group II. A follow-up period for all the subjects was 12 months, excluding 8 patients who were lost for follow-up after 6 months because of transfer to adult endocrinologist.

In this observational, retrospective study data were collected from medical records. The levels of glycated hemoglobin (HbA1c) and the number of hypoglycaemic episodes were assessed at the beginning, and 6 and 12 months after introducing insulin analogues. Hypoglycaemic episodes were classified as minor (child could help itself) and major – severe (requiring assistance to treat).

All data were analysed using the statistical package SPSS (version 17.0). Data were reported as absolute numbers and percentages, or as means and standard deviations (SDs). Student's *t*-test was used to assess the statistical significance of differences between different insulin regimens and between the groups. Pearson's χ^2-test was used for comparison of categorical variables. Changes in the means of frequency and severity of hypoglycemic episodes were assessed using Friedman and Wilcoxon signed ranks tests. *p*-values of less than 0.05 were considered as statistically significant.

Results

The mean HbA1c was significantly lower in both groups after 6 and 12 months. In the group I (RHI/LA) HbA1c was lower after 6 months (9.15% *vs* 8.20%) and after 12 months (9.15% *vs* 8.13%) as well as in the group II (RA/LA) after 6 months (9.40% *vs* 8.24%) and after 12 months of insulin analogues treatment (9.40% *vs* 8.38%) as shown in Table 2. There were no significant statistical differences in HbA1c between the groups at the beginning and 6 and 12 months after introducing insulin analogues.

The frequency of hypoglycaemic episodes was significantly lower in both groups 6 months after introducing insulin analogues (100% to 87.5% in the group I, and 100% to 89.9% in the group II), and 12 months after introducing analogues (100% to 83.3% in the group I, and 100% to 75.9% in the group II). The frequency of minor hypoglycaemic events was higher in both groups (Table 3) after 6 months (in the group I from 38.9% to 83.3%, and in the group II from 45.6% to 88.6%) and after 12 months (in the group I from 38.9% to 76.4%, and in the group II from 45.6% to 69.9%) while the frequency of severe hypoglycaemic events was significantly lower in both groups after 6

Table 1

Baseline characteristics of the 151 children and adolescents with type 1 diabetes mellitus

Patients characteristics	Regular human insulin/Long-acting analogue (RHI/LA)	Rapid-acting analogue/Long-acting analogue (RA/LA)
All children, n (%)	72 (47.7)	79 (52.3)
Boys, n (%)	47 (65.3)	43 (54.4)
Girls, n (%)	25 (34.7)	36 (45.6)
Age of introducing analogues (years), $\bar{x} \pm SD$	13.0 ± 2.2	13.5 ± 2.4
HbA1c (%), $\bar{x} \pm SD$	9.15 ± 2.24	9.40 ± 1.67

Table 2

Glycated hemoglobin (HbA1c) before introducing insulin analogues and after 6 and 12 months

Insulin therapy	HbA1c (%), $\bar{x} \pm SD$			Difference	95% CI	*p*-value
	before	after 6 months	after 12 months			
RHI/LA	9.15 ± 2.25	8.20 ± 1.71		0.96	(0.6–1.2)	< 0.001
			8.13 ± 1.63	1.01	(0.6–1.3)	< 0.001
RA/LA	9.40 ± 1.67	8.24 ± 1.47		1.16	(0.9–1.4)	< 0.001
			8.38 ± 1.66	1.04	(0.7–1.4)	< 0.001

RHI/LA – Combination of regular human insulin (RHI) and long-acting analogue (LA); RA/LA – Combination of rapid-acting analogue (RA) and long-acting analogue (LA).

Table 3

The frequency of hypoglycaemic events 6 and 12 months after introducing insulin analogues

Insulin therapy		Hypoglycaemic events, n (%)			
		without	minor	severe	*p* value
RHI/LA	Before	0	28 (38.9)	44 (61.1)	
	After 6 months	9 (12.5)	60 (83.3)	3 (4.2)	< 0.0001
	After 12 months	12 (16.7)	55 (76.4)	1 (1.4)	< 0.0001
RA/LA	Before	0	36 (45.6)	43(54.4)	
	After 6 months	8 (10.1)	70 (88.6)	1 (1.3)	< 0.0001
	After 12 months	19 (24.1)	55 (69.9)	1 (1.3)	< 0.0001
Total	Before	0	64 (42.4)	87 (57.6)	
	After 6 months	17 (11.3)	130 (86.1)	4 (2.6)	< 0.0001
	After 12 months	31 (20.5)	110 (72.8)	2 (1.3)	< 0.0001

RHI/LA – Combination of regular human insulin (RHI) and long-acting analogue (LA); RA/LA – Combination of rapid-acting analogue (RA) and long-acting analogue (LA).

months (in the first group decreased from 61.1% to 4.2%, and in the group II from 54.4% to 1.3%) and after 12 months (in the group I from 61.1% to 1.4%, and in the group II from 54.4% to 1.3%). There were no statistically significant differences in frequency of hypoglycaemic episodes between the groups at the beginning, and 6 and 12 months after introducing insulin analogues.

Discussion

It is widely accepted that the traditional insulins used in basal-bolus therapy, regular human and neutral protamine hagedorn (NPH) insulin, do not accurately reproduce the physiological insulin profile. Insulin analogues have demonstrated certain clinical improvements over regular human insulin, and NPH insulin [9–11]. Data indicate that the combination of rapid-acting and long-acting analogues leads to overall improved glycaemic control in T1DM [5, 11, 12].

The risk of hypoglycaemia is the most feared adverse event among diabetes mellitus patients and medical staff in relation to insulin treatment [13, 14]. Severe hypoglycaemia may lead to long-term cognitive impairment in children below 6 years of age and similar effects may also apply for older children [15, 16]. Treatment with insulin analogues is associated with lower risk of hypoglycaemia, especially severe ones, in children and adolescents with T1DM. It is likely that a combination of rapid-acting and long-acting insulin analogues produces a more physiological insulin secretion and thereby reduces the risk of severe hypoglycaemia [12].

In this retrospective study all the patients were already on basal-bolus therapy recieving three injections of regular human insulin before meals and NPH insulin at bedtime. Introducing long-acting insulin at bedtime or the combination of mealtime rapid-acting and bedtime long-acting insulin analogue resulted in improved glycaemic control with lower risk of severe hypoglycaemia. The patients in both groups experienced a decrease in HbA1c levels after introducing insulin analogues with a small, but statistically significant difference of 0.96% in the group I and 1.16% in the group II after 6 months, and 1.01% and 1.04% after 12 months. The mean HbA1c levels were still significantly lower 12 months after introducing insulin analogues in both groups. The frequency of severe hypoglycaemia was significantly lower in both groups 6 and 12 months after introducing insulin analogues, but there were no statistically significant differences between the groups. There were more patients with minor hypoglycaemia, but those were ones that had severe hypoglycaemic events before introducing insulin analogues.

In the large-scale multicentre trial, Hermansen et al. [5] showed that combination of insulin analogues, insulin detemir and insulin aspart, in addition to a significant improvement in HbA1c, provides a lower risk of hypoglycaemia than NPH and regular human insulin treatment. A meta-analytsis of the Cochrane Metabolic and Endocrine Disorders Group reviewed 42 randomized controlled trials that compared the effect of intensified therapy regimens with rapid-acting insulins to regular insulin in adults. The analyses demonstrated a small, but statistically significant decrease in HbA1c using rapid-acting insulin analogues [6, 17]. They mimic the normal mealtime insulin response more closely than injection of regular human insulin and thereby improve postprandial glycaemic control [5, 10].

There are limited data regarding the use of rapid-acting and long-acting insulin analogues in children and adolescents

compared to adults with T1DM. None showed a significant decrease in HbA1c levels, and only one demonstrated lower rates of hypoglycaemic episodes. Only few studies showed a significant decrease in morning fasting blood glucose levels and in the frequency of severe diurnal and nocturnal hypoglycaemic episodes [18]. Chase et al. [19] demonstrated a decrease of HbA1c in addition to a significant decrease in severe hypoglycaemia. In the first large-scale multicentre study Robertson et al. [2] showed the efficancy and safety of insulin detemir in children and adolescents with T1DM. The lower risk of severe hypoglycaemia with insulin detemir was achieved in children without compromising glycaemic control. In all age groups the quality of life seemed to improve with the insulin analogues, which was attributed to less fear of hypoglycemia and more flexibility in lifestyle and food intake [4, 6, 17, 19].

Conclusion

This study demonstrated that insulin analogues used in basal-bolus therapy, either only long-acting analogues with premeal regular human insulin or the combination of rapid-acting and long-acting analogues, provide significantly better HbA1c values and lower risk of severe hypoglycaemic events in children and adolescents with T1DM.

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Limb apraxia in multiple sclerosis

Apraksija udova kod multiple skleroze

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Abstract

Background/Aim. There are almost no studies on apraxia in people with multiple sclerosis. Although the white matter is damaged in MS, it is not the only location in which the pathological changes are present. Demyelinated lesions in the cortex have recently been recognized as important components of multiple sclerosis pathology. The aim of this study was to determine whether apraxia is present among people with MS, and the importance of demographic characteristics and impairment of functional systems at conceptualization and execution of movements. **Methods.** The experimental group consisted of 30 patients, mean age 51.34 ± 7.70 years. The patients in the experimental group were diagnosed with MS according to the McDonald criteria. The control group consisted of 30 healthy subjects, mean age 50.30 ± 10.47 years. For research purposes, we used the following instruments: Questionnaire for Collecting Demographic Data, Kurtzke Functional Systems Scores, Waterloo-Sunnybrook Apraxia Battery (WatAB). Execution of motion tasks that are a part of the WatAB were incorporated in the System for the Observation and Analysis of Motor Behavior. **Results.** Our study showed that limb apraxia was common in people with MS. Apraxia was present during pantomime in 26.70% of the patients, and during the imitation of movements in 44.80% of the patients. Gender, age, education level, duration of disease and a form of MS did not determine the quality of conceptualization and execution of movements. The time elapsed from the last exacerbation was a determinant of quality of executed movements. Impairments of functional systems predicted impairments of movement execution. The expanded disability scale score correlated with the severity of apraxia. **Conclusion.** Our study confirm the presence of apraxia in MS. It is necessary to carry out further studies using functional magnetic resonance imaging, as well as the conduct longitudinal studies to determine the precise structure of motor behavior in people with MS.

Key words:
apraxias; diagnosis; multiple sclerosis; questionnaires; severity of illness index.

Apstrakt

Uvod/Cilj. Istraživanja apraksije kod osoba sa multiplom sklerozom (MS) gotovo da nema. Iako je oštećenje bele mase prisutno, ono ne predstvlja jedinu lokaciju u CNS-u na kojoj su prisutne patološke promene kod MS. U skorije vreme smatra se da je kora velikog mozga veoma važna lokacija na kojoj dolazi do patoloških promena kod osoba koje boluju od MS. Cilj ovog istraživanja bio je da se utvrdi da li među osobama sa MS ima onih kod kojih je prisutna apraksija, kao i značaj demografskih karakteristika i oštećenja funkcionalnih sistema za konceptualizaciju i izvođenje pokreta. **Metode.** Eksperimentalnu grupu činilo je 30 učesnika starosti 51,34 ± 7,70 godina. Učesnicima eksperimentalne grupe dijagnostikovana je MS prema Mekdonaldovom dijagnostičkom kriterijumu. Kontrolnu grupu sačinjavalo je 30 zdravih osoba starosti 50,30 ± 10,47 godina. Za potrebe istraživanja korišćeni su podaci dobijeni Upitnikom za prikupljanje demografskih podataka i osnovnih podataka o bolesti, Kurzke-ovim skorovima funkcionalnih sistema i Adaptiranom Vaterlo baterijom za procenu apraksije (ova baterija korišćena je u kombinaciji sa Sistemom za opservaciju i analizu motornog ponašanja). **Rezultati.** Naše istraživanje je pokazalo da apraksija udova predstavlja čestu pojavu od osoba sa MS. Apraksija na zadacima izvođenja pantomime bila je prisutna kod 26,70% bolesnika, a na zadacima izvođenja imitacije pokreta kod 44,80% bolesnika. Pol, godine života, stepen obrazovanja, dužina trajanja bolesti i oblik bolesti nisu determinisali kvalitet konceptualizacije i izvođenja pokreta kod osoba sa MS. Vreme proteklo od poslednje egzacerbacije predstavljalo je determinantu kvaliteta izvedenih pokreta. Oštećenja funkcionalnih sistema kod osoba sa MS predviđala su pristustvo oštećenja izvršenja pokreta. Proširena skala funkcionalne onesposobljenosti bila je u korelaciji sa težinom apraksije. **Zaključak.** Naše istraživanje otkrilo je prisustvo apraksije kod MS. Potrebno je izvršiti dalja istaživanja uz korišćenje funkcionalne magnetne rezonance, kao i sprovođenje longitudinalnih studija kako bi se preciznije utvrdila struktura motornog ponašanja kod osoba sa MS.

Ključne reči:
apraksija; dijagnoza; multipla skleroza; upitnici; bolest, indeks težine.

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Introduction

Apraxia is defined as a disorder of learned movements, which is not caused by muscle and/or neurological factors (e.g. weakness, akinesis, aphasia, cognitive resources decline, vision problems, etc.) [1–3]. Neuropsychology was mostly engaged in studying apraxia especially in persons with brain injury, Alzheimer's disease, Parkinson's disease, corticobasal degeneration, and other. The most investigated relationship was the one between the localized impairment and apraxia occurrence.

Multiple sclerosis (MS) is usually regarded as a disease of the white matter [4]. Lesions of the white matter that include demyelination and neuronal damage are very visible on magnetic resonance imaging (MRI) and macroscopically during autopsy [5, 6]. Detecting white matter lesions with MRI is an essential signal for the presence of MS. Cortex has recently been recognized as an important location in which pathological changes occur in patients suffering from MS [7, 8]. MS is a chronic inflammatory disease of the central nervous system characterized by multifocal demyelination and axonal damage, which affects both white and gray matter of the cerebral cortex, deep gray matter nuclei and the spinal cord. The appearance of apraxia is expected due to lesions or degeneration of certain areas of cortex and/or by damaging the parietofrontal pathways [9].

There are almost no scholarly papers on apraxia in persons with MS. The only research that addresses the relationship of MS and apraxia is the one conducted by Kamm et al. [9].

For that reasons the aim of this study was to determine the frequency of aproxia in patients with MS, and its relation to demographic characteristics, severity of illness, type of illness, disease duration, and time elapsed since the last exacerbation.

Methods

This study included participants of both sexes, 18–65 years of age, divided into control and experimental groups of similar size. The sample was unrepresentative and convinient, depending on the avalilability of the participants.

The experimental group included patients with MS diagnosed by the McDonalds diagnostic criteria [10]. One of the criteria for inclusion in the sample was the score achieved on the Expanded Disability Status Scale (EDSS) that was greater than or equal to 1. All the patients of the experimental group were the members of the Multiple Sclerosis Society of Serbia. Also, the inclusive criteria for the experimental group meant that the subject was able to independently read and understand the information from the form which confirmed the consent to participate in the research. All the patients of the experimental group read, understood and signed a form confirming the consent for participation in the research. The patients of the experimental group did not have the history of nor are currently subjected to alcohol and/or psychoactive substance use, in the last two years pregnant women, persons with the history of neurological damage which cannot be treated as a result of MS, persons with dementia, persons suffering from psychiatric disorders, persons with significant motor disorders (such as tremor, bradykinesia, dyskinesia), persons with peripheral conditions (e.g., arthritis) that may compromise motor function, individuals with developmental disabilities, persons that cannot understand the assessment procedure due to some cognitive deficit.

The control group formation criterion was to be healthy, and as for the experimental group, to read, understand and sign the form, confirming their consent to participate in research. Exclusive criteria for the control group were the same as for the experimental one.

The control group was introduced to demographic characteristics of the experimental group.

The System for the Observation and Analysis of Motor Behavior (SOAMB) consists of hardware and software components. Required central processing unit (CPU) and memory of the system were provided by the computer Dell Inspiron PP29L. An additional monitor for displaying tasks was used, 20-inch diagonal display with 1,280 × 1,024 resolution and the image refresh rate of 75 Hz. For the audio material reproduction loudspeakers Genius were used. For the purpose of recording movements a web camera Logitech Webcam C905 was used, which could record in high definition, 1,600 × 1,200 resolution, 30 frames per sec.

The computer program for acquisition, analysis and partial data processing was created in Java programming language. The program allows creation of the profile for each participant. The task creation which participants should perform, using sound, pictures or video files, was enabled.

In this study all the tasks of WatAB were developed in video format and uploaded into the program. In this way we avoided the possibility for the examiner to issue tasks at different times and in different manners. For example, the possibility that some of the examiner perform movement imitation tasks in different ways for different participants in the study was avoided, providing a greater degree of objectivity in the use of WatAB [11].

The research was conducted in the facilities of the Society of MS Serbia, on the territory of Belgrade and Aranđelovac.

For data analysis and processing, software packages Microsoft Excel and SPSS were used. From the statistical techniques we used descriptive, correlation, regression, and discriminate techniques. The results are presented in tables and figures.

Results

The demographic characteristics of the control and the experimental group are presented in Table 1. Health status characteristics in the patients with MS are presented in Table 2.

The results of the participants of the control and experimental groups in the WatAB

We compared the results of both groups in WatAB. Table 3 shows the comparative values of the results of the participants on the scales and subscales. For the group comparison, we used the *t*-test for independent samples. There was a

Table 1

Demographic characteristics of the study participlants

Characteristics	Control group	Multiple sclerose group
Age (years), $\bar{x}$ ± SD	50.30 ± 10.47	51.34 ± 7.70
Sex, n (%)		
male	50 (15)	46.7 (14)
female	50 (15)	53.3 (16)
The highest education completed (years), n (%)		
0	3.30 (1)	0 (0)
8	20.00 (6)	3.30 (1)
12	46.7 (14)	70.10 (21)
15+	30.00 (9)	26.60 (8)

Table 2

Characteristics of the patients with multiple sclerosis (MS)

Type of MS	Distribution	Age (year)	Age at time of diagnosis (year)	Last remission period (years)	EDSS (points)
	n (%)	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
RRMS	10 (33.3)	49.41 ± 7.61	32.20 ± 10.28	5.14 ± 3.34	4.20 ± 2.26
PPMS	10 (33.3)	53.48 ± 7.38	35.50 ± 9.58	8.00 ± 0.23	4.00 ± 1.93
SPMS	2 (6.7)	58.92 ± 0.11	34.50 ± 7.78	3.00 ± 0.12	5.50 ± 3.53
N/A*	8 (26.6)	48.96 ± 8.19	35.42 ± 6.24	2.85 ± 5.43	4.94 ± 2.81
Total	30 (100)	/	/	/	/
Average	/	51.34 ± 7.70	34.28 ± 8.74	4.49 ± 3.91	4.42 ± 2.30

***New available date is not present in documentation; EDSS – Expanded Disability Status Scale; RRMS – relapsed-remitting MS; PPMS – primary progressive MS; SPMS – secondary progressive MS.**

statistically significant difference between the control and the experimental group on the conceptual scale, $p < 0.05$. The difference between the mean values of group characteristics was large [eta squared (n2) = 0.10)]. There was a statistically significant difference in the recognition subscale ($p < 0.05$, n2 = 0.10).

Also, a statistically significant difference between the two groups on productive scale and almost all of its subscales was found.

Figure 1 shows deviations of those with MS, compared to a typical population. Deviations are represented by Z scores.

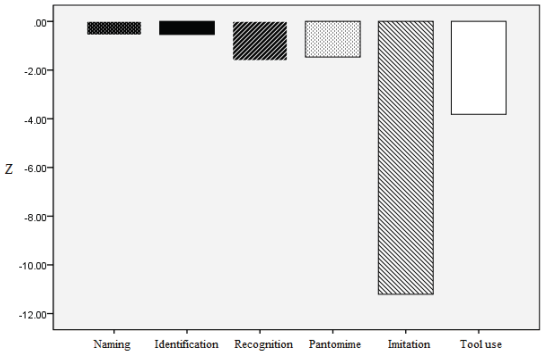

Fig. 1 – Z-scores at the Adapted Waterloo-Sunnybrook Apraxia Battery.

The presence of apraxia in the patients with MS

In order to determine the number of the study patients with from MS and the presence of apraxia we used the Roy`s research group approach. We analyzed the subscale of pantomime, imitation as well as the whole production scale. The patietns with a deviation up to 1 SD were classified as those with no present apraxia. Those with discrepancy in scores greater than 1 SD, and less than 2 SD were classified as borderline conditions, while those with a variation in perform-

Table 3

The scores of the control and the multiple sclerose (MS) group on the Adapted Waterloo-Sunnybrook Apraxia Battery

Types of tasks	Control group ($\bar{x}$ ± SD)	MS group ($\bar{x}$ ± SD)
Conceptual scale*	97.17 ± 4.43	92.61 ± 9.81
naming	97.04 ± 4.99	94.25 ± 8.20
identification	97.83 ± 5.27	95.29 ± 8.27
recognition*	95.97 ± 5.92	85.60 ± 21.91
Production scale*	97.16 ± 2.90	84.84 ± 23.34
pantomime*	93.98 ± 6.08	85.07 ± 15.83
concurrent imitation*	99.20 ± 1.08	83.83 ± 27.60
delayed imitation*	98.09 ± 2.54	78.86 ± 33.84
Tool use*	98.39 ± 3.29	85.83 ± 24.34

***$p < 0.05$ (statistically significant difference).**

ance in these subscales and scales greater than 2 SD belonged to the group with the presence of apraxia. In this way, the obtained results showed that the performance on the subscale pantomime among those with apraxia can be classified in 8 (26.70%) participants. At the subscale movement imitation in the group with MS and the presence of apraxia, was classified in 13 (44.80%) participants. On the overall production scale apraxia was present in 12 (42.90%) of the patients. The overall results of this procedure are shown in Table 4.

tional impairment of the system and the time from the last exacerbation. Comparisons were performed by analysis of variance.

There was no statistically significant difference in the results of these subscales in relation to the form of the disease (the group 1: relapsing-remitting, the group 2: primary progressive, the group 3: secondary progressive).

There was no statistically significant difference in the results, nor in the duration of MS (the group 1: up to 12.33; the group 2: from 12.34 to 18.83, and the group 3: over 18.84).

Table 4

The presence of apraxia in the patients with multiple sclerosis

The presence of apraxia	Pantomime	Imitation	Production scale
	n (%)	n (%)	n (%)
Yes	8 (26.70)	13 (44.80)	12 (42.90)
Borderline	6 (20.00)	4 (13.80)	2 (7.10)
No	16 (53.30)	12 (41.40)	14 (50.00)

The WatAB results concerning gender, age and the level of education

The results of the patients in the subscales of the WatAB in relation to gender were compared by *t*-test for independent samples, while the analysis of variance was used to compare these results in relation to age and the level of education. The results were compared on the subscales of naming, identification, recognition, pantomime, imitation and use of tools/objects.

There was no statistically significant difference in relation to the results of these subscales regarding gender (Figure 2), as well as age (group 1: < 45.42 years; the group 2: from 45.53 to 55.00 years; and the group 3: more than 55.01 years) and the degree of education (group 1: 0 years of education; the group 2: 8 years of education; the group 3: 12 years of education; group 4: 15+ years of education)].

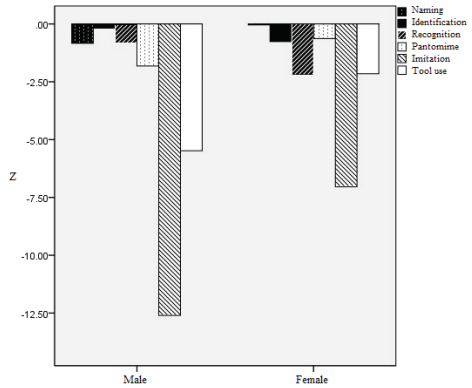

Fig. 2 – Z-scores at the Adapted Waterloo-Sunnybrook Apraxia Battery in relation to gender.

The WatAB results concerning type, duration, degree of functional impairment of the system and the time elapsed from the last exacerbation

The results on the subscales of naming, identification, recognition, pantomime, imitation and use of tools/objects were compared in relation to type, duration, degree of functional impairment of the system and the time from the last exacerbation. Comparisons were performed by analysis of variance.

The results on subscales identification, recognition, pantomime, imitation and use of tools/objects in relation to the time from the last exacerbations were compared by the *t*-test of independent samples. The group 1 consisted of participants with the last exacerbation 4 years ago or less, while the group 2 consisted of participants with the last exacerbation 4 years or more before. We found only one statistically significant difference between the group 1 (= -1.57 ± 2.03) and the group 2 (= -0.65 ± 0.39) in the pantomime subscale, t (11) = -2.62, p = 0.024 (two-sided). The difference between the mean values o f the characteristics of the groups (mean difference = -2.22, 95% Cl: -4.09 to -0.35) was very large (n = 0.38).

In order to additionally explain the obtained results, we used the Pearson's correlation to establish whether there was a relationship between the time from the last exacerbation and the results of the subscales WatAB. The assumptions of the normality, linearity and homogeneity were satisfied. There was a correlation between achievements on the subscale of recognition and the time elapsed from the last exacerbation (r = 0.59, p < 0.05), as well as correlations between scores on the subscale of pantomime and the time from the last exacerbation (r = 0.58, p < 0.05). Shorter time elapsed from the last exacerbation was accompanied by lower Z scores on the subscales of recognition and pantomime people with MS.

The possibility for the degree of functional systems impairments to predict the results in the subscales pantomime, imitation and use of tools of the WatAB

The possibility of the degree of damage measured by FSS to predict results in the WatAB subscales was estimated in a sample of patients with MS. We used the standard multiple regression, for each of WatAB subscales.

For predicting the results on the subscale of pantomime, a preliminary analysis showed that the assumptions of normality, linearity, multicollinearity and homogeneity of variance were not violated. The model as a whole explained 50.1% of the total variance, F (7, 22) = 5.15, p < 0.05, so it can be said that scores on the FSS significantly predict the results

on the subscale of pantomime in the WatAB. The regression coefficients and standard errors are shown in Table 5.

Table 5

Regression coefficients and standard errors (SE) in predicting the quality the performance of pantomime, imitation and using tools

Variable	Pantomime			Imitation			Using tools		
	B	SE_B	β	B	SE_B	β	B	SE_B	β
Constant	2.01	0.86		17.37	6.28		5.89	2.60	
Pyramidal	-0.15	0.27	-0.08	-3.01	1.97	-0.22	-1.42	0.82	-0.28
Cerebellar	-0.16	0.31	-0.09	-1.56	2.30	-0.11	-0.81	0.95	-0.15
Brainstem	-0.32	0.30	-0.16	-3.52	2.19	-0.24	-2.18	0.91	-0.39*
Sensory	0.18	0.43	0.06	2.45	3.14	0.11	-0.96	1.30	-0.12
Visual	-0.34	0.29	-0.19	-1.11	2.12	-0.08	-0.27	0.88	-0.05
Cerebral or Mental	-1.01	0.37	-0.38*	-7.75	2.71	-0.38*	-0.54	1.12	-0.07
Bowel and Bladder	-1.13	0.35	-0.54*	-6.86	2.58	-0.43*	-1.21	1.07	-0.20

***$p < 0.05$; B – non-standardized regression coefficient, β – standardized coefficient.**

For predicting the results in the motion imitation subscale preliminary analysis showed that the assumptions of normality, linearity, multicollinearity and homogeneity of variance were not violated. The model as a whole explained 54.8% of the total variance, $F(7, 21) = 5.85$, $p < 0.05$, so it can be said that scores on the FSS significantly predict the results on the subscale of motion imitation on WatAB. The regression coefficients and standard errors are shown in Table 5.

For predicting results in the use of tools subscale preliminary analysis showed that the assumptions of normality, linearity, multicollinearity and homogeneity of variance were not violated. The model as a whole explained 46.8% of the total variance, $F(7, 20) = 4.39$, $p < 0.05$, so it can be said that scores on the FSS significantly predict the results on the subscale of using tools in WatAB. The regression coefficients and standard errors are shown in Table 5.

The results in WatAB associated with EDSS scores

We used the Pearson's correlation to establish whether there was any relationship between the EDSS and achievements of the WatAB subscales. The assumptions of the normality, linearity and homogeneity were satisfied. There were strong correlations between the results on the subscales of pantomime ($r = -0.72$, $p < 0.01$), imitation ($r = -0.76$, $p < 0.01$), tool use ($r = -0.75$, $p < 0.01$) and the EDSS. A higher EDSS was accompanied by lower Z scores on the subscales of pantomime, imitation and tool use in people with MS.

Discussion

The obtained results indicate that limb apraxia very often occurs in population with MS. In this population apraxia is present more often in imitation than in pantomime tasks. The obtained results consist of the results of a few other studies in terms of the general presence of apraxia in persons with MS. According to Staff et al. [12] the percentage of apraxia presence in the population of persons with MS is much lower – only 13%. On the other hand, Kamm et al. [9] have found that limb apraxia is present in 26.3% of participants with MS.

Praxic abilities of women are better than in men throughout their entire development [13] and healthy women perform voluntary movement with fewer errors than men [14]. In patients with brain lesions praxis system is much more dependent on the anterior region of the left hemisphere in women than in men [15–17], which may suggest that more focused representation allows greater precision of voluntary movements control in women [15]. Women have a better ability to implement complex motion and pre-planning movement than men during the execution and control [18]. Pozzilli et al. [19] in their study using MRI show that there are differences in lesion characteristics between men and women with MS. The authors conclude that lesions in men are less inflammatory, but more destructive than in lesions in women. Therefore, we considered the possibility that the general tendency for women to have less apraxic errors in the execution of movements than men does not have to be present in persons with MS. We showed the parallel display of the results of men and women on the WatAB subscales. It is obvious that women in the subscales of the pantomime, imitation and use of tools have minor deviations from the control group in comparison to men. However, the difference between men and women did not reach statistical significance.

The participants in the study did not have different results in the WatAB in relation to age and disease duration. We believe that this result is quite expected considering the nature of progression of the disease, which is indicated by the existence of its subtypes according to the mode of progression (relapsing-remitting, primary progressive, secondary progressive, etc). This means that the disease can quickly progress to some younger people than in older or *vice versa*, and that there is no rule or correlation between age and disease progression. Kamm et al. [9] designed a linear regression model, for which the result on the EDSS is the best single predictor, while among other predictors are the duration of MS and the age of the participants. Participants in their study had lower EDSS compared to participants in our study. That could be the reason for results diversity. Also, the authors, did not consider the individual effects of these variables within the model. Dimeck et al. [20] conducted a study in which the sample consisted of healthy participants who performed concurrent and delayed imitation of movement. A statistically significant difference between older and younger participants had been obtained. Changes in conditions of imitation also gave a statistically significant differ-

ence in achievements. If the age and duration of disease play a role in the results of participants with MS, for more accurate determination of their impact a longitudinal study on a number of people, with occasional reevaluation of praxic abilities should be carried out. Otherwise, the influence of age and disease duration on the results of the tasks of apraxia assessment is not visible due heterogeneous illness progression of the participants in the study.

It was expected that this claim would be supported by the results of the participants regarding MS type. However, there was no statistically significant difference between the groups, while conflicting results have been obtained by Kamm et al. [9]. Participants in their study with the relapsing-remitting form of MS had significantly higher scores on the praxis assessment compared to the participants with primary progressive and secondary progressive forms of MS. There were no statistically significant differences between the groups of participants with primary progressive and secondary progressive type of MS. It remains to be further explored.

The time from the last exacerbation has great effect only on the results on the subscale of pantomime. These results contribute to the statement that the time after exacerbations in people with MS leads to recovery of the function, and that this recovery implies the features of conceptualization and execution of movement. We will refrain from further interpretation of the obtained results because they are limited in the sense that they represent only the condition in one timely moment for each individual, that is, they do not have a longitudinal character. Stamenova et al. [21] investigated the long-term recovery of limb apraxia after brain injury. Participants in the study (with acute and chronic conditions) on all tasks showed signs of recovery except on the tasks of identifying actions. Faster recovery showed acute and subacute patients on the tasks of pantomime. The study of Stamenova et al. [21] has similarity to the results from our study.

The degree of functional system impairments in patients with MS predicted the success of the performance of pantomime imitation and use of tools. Patients with apraxia often do not have problems in using real tools. Our study confirms that significant predictors of the success in performing pantomime and imitation differ from significant predictors of the success in the use of tools. When performing pantomime and imitation, requirements for use of cognitive functions could be increased. In the case of pantomime performance it is necessary to create visual representations of the tools used or representations of social situations in which persons use some form of representative nontransitive pantomime. In case of movement imitation it is essential to receive information about the action performed by someone else, to decode information about what is seen, to form ideas about the movement that is to be executed and to properly activate effectors for movement performance. On the other hand, when using the real tools, through the sensory system the information about the tool (shape, weight, etc.) is obtained. In this sense, action performance with real tools requires a higher degree of activation of other systems in the brain (especially learned actions), as well as greater load of the musculoskeletal system.

The role of the system for bladder and bowel control in the prediction of apraxia may seem surprising at first glance, but it should be emphasized that from the neurological point of view some elements of this system overlap with other functional elements of other systems. The bladder and bowel control is partly influenced by our own will. Fowler et al. [22] suggest that clinical observation studies and observation studies using MRI suggest that the frontal lobe plays an important role in determining the appropriate moment for micturition. Some studies [23, 24] show that the right inferior frontal gyrus, which is a part of the prefrontal cortex was active when the bladder was full in patients. It is believed that the prefrontal cortex is involved in planning complex cognitive behaviors, personality characteristics reflects, plays a role in the expression of appropriate social behavior as well as the functions of attention and response selection [25]. This system damage in our research emerges as a predictor of quality of pantomime and imitation together with impairment of mental function.

Finally, higher EDSS were associated with poorer performance on tasks of pantomime, imitation and tool use. EDSS and apraxia connection exists solely to the production of movement tasks, but not the conceptualization of movement (identification, recognition and naming). EDSS is based on FSS and other motor skills, which is why this correlation is understandable. Unlike Kamm et al. [9], who showed that more MS patients having apraxia have higher EDSS, our research in this regard did not address the frequency of apraxia in relation to EDSS. Our research confirms that if EDDS is higher, there will be present a more severe form of apraxia as shown by the results in all the production subscales.

Conclusion

Limb apraxia is frequent in persons with multiple sclerosis, and its occurrence is different when it comes to performing pantomime and movement imitation. Praxic performance depends on the time elapsed from the last exacerbation and the Expended Disability Scale score. Functional systems impairments in patients with MS may predict the quality of movement execution. A connection between sex, age, type of disease and apraxia in people with multiple sclerosis should be the subject of further research.

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Histological characteristics and markers of proliferation and differentiation in rat brain with experimental glioma

Histološke karakteristike i markeri proliferacije i diferencijacije u mozgu pacova sa eksperimentalnim gliomom

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Abstract

Background/Aim. The cell line C6 is a continuous cell line of rat glioma and, as a transplantable line, is frequently used for induction into *in vivo* model of primary brain tumor. It is believed that, pursuant to its histological traits and biological behavior, this experimental tumor corresponds to human anaplastic astrocytoma of grade II/III, which is characterized by proliferative and invasive potency, and marked cell differentiation. The aim of this study was to determine macroscopic analysis of rat brain with implanted tumor during tumorigenesis, histological features of tumor cells of induced brain tumor and markers of proliferation (proliferation cell nuclear antigen – PCNA, cytokeratin – CK 19) and differentiation (glial fibrillary acidic protein –GFAP) in rat brain with implanted tumor. **Methods.** To determine histological structure of the brain with implanted C6 cells, we used brain sections stained for hematoxylin-eosin or kresyl violet, whereas other sections were immunohistochemically stained for GFAP, CK 19 and PCNA. **Results.** A statistically significant difference in weights of the left and right brain hemispheres with implanted tumors during tumorigenesis in as soon as 7 days from the day of inducing tumors was revealed. The tumor was of cellular type, with distinct pleomorphism of cells and frequent hyperchromasia of the nucleus. Immunohistochemical staining for PCNA revealed a significant number of positive cells on the days 7, 14 and 21 day following the implantation of C6 cells. CK 19 positive cells were present in both brain hemispheres, and numerous GFAP positive astrocytes were found around the puncture lesion. **Conclusions.** Within the experimental conditions of the present research, C6 glioma did not demonstrate any relevant deviations concerning development, clinical symptomatology and macroscopic anatomy relative to those already described in the literature.

Key words:
glioma; rats; disease models, animal; immunohistochemistry.

Apstrakt

Uvod/Cilj. Ćelijska linija C6 predstavlja kontinuiranu ćelijsku liniju glioma pacova i kao transplantabilna linija, često se koristi za indukciju *in vivo* modela primarnog moždanog tumora. Smatra se da po histološkim osobinama ili biološkom ponašanju ovaj eksperimentalni tumor odgovara humanom anaplastičnom astrocitomu gradusa II/III, koji karakteriše proliferativni i invazivni potencijal, kao i izražena ćelijska diferencijacija. Cilj ovog rada bio je da se odrede: makroskopska analiza mozgova pacova sa implantiranim tumorom u toku tumorogeneze, histološke karakteristike tumorskih ćelija indukovanog moždanog tumora i markeri proliferacije (proliferativni ćelijski nuklearni antigen – PCNA, citokeratin – CK 19) i diferencijacije (glialni fibrilatni kiseli protein – GFKP) u mozgu pacova sa implantiranim tumorom. **Metode.** Za određivanje histološke građe mozga sa implantiranim C6 ćelijama korišćeni su isečci mozga obojeni hematoksilin-eozinom ili krezil violet bojom, dok su drugi isečci imunohistohemijski obojeni na GFKP, citokeratin 19 i PCNA. **Rezultati.** Utvrđeno je da postoji statistički značajna razlika u težini između leve i desne hemisfere mozgova sa implantiranim tumorima u toku tumorogeneze već 7 dana od indukcije tumora. Tumor je bio celularnog tipa, sa izraženim pleomorfizmom ćelija i čestom hiperhromazijom nukleusa. Imunohistohemijsko bojenje na PCNA pokazalo je veliki broj pozitivnih ćelija posle 7, 14, i 21 dan od implantacije C6 ćelija. Ćelije pozitivne na CK 19 bile su prisutne u obe hemisfere mozga, a brojni GFAP pozitivni astrociti nađeni su oko mesta ubodne lezije. **Zaključak.** U eksperimentalnim uslovima ove studije gliom C6 nije pokazao značajnija odstupanja u smislu razvoja, kliničke simptomatologije i makroskopske anatomije od one koja je već opisana u literaturi.

Ključne reči:
gliom; pacovi; bolest, modeli na životinjama; imunohistohemija.

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Introduction

One of the most frequently used tumor cell lines *in vitro* in neurobiology are C6 cells. The C6 cell line is a continuous cell line of rat glioma, which has been originally induced in Wistar rats by intravenous application of N-methyl nitrosourea [1]. Given that it is also a transplantable line, it is frequently used for *in vivo* induction of primary brain tumor model [2]. It is believed that, pursuant to its histological traits and biological behavior, this experimental tumor corresponds to human anaplastic astrocytoma of grade II/III [3].

Clarification of biochemical pathways for the progression of cell cycle made it possible to identify the PCNA antigen (proliferating cell nuclear antigen), which made a practical tool as a tumor proliferation marker. The PCNA, a member of the cyclin family, is a nuclear protein which attaches to DNA delta polimerase and is necessary for replication of DNA [4]. Its presence is related to late G1 and S phases of cell cycle. Anti-PCNA antibodies are commercially available, and used to determine the proliferative potential of CNS tumor [5]. This antigen proved to be a more reliable and accurate marker of tumor cell proliferation relative to the mitotic index.

Cytokeratin 19 (CK 19) is an acidic protein that makes a part of epithelial cell structure. It contributes to the cell resistance, transduction of signals and regulation of cell migration and invasion [6]. As an intermediate filament excreted by epithelial cells, it is used as a marker for the differentiation of epithelial cells.

Glial fibrillary acidic protein (GFAP) is the key constituent of intermediate filaments with normal, reactive and neoplastic astrocytes. It is considered that the expression of GFAP in astrocytoma is in correlation with cell differentiation, and in inverse relation with proliferative potential [7]. The exception is protoplasmic astrocytoma, which displays either a minimal GFAP immunoreactivity or none at all [8]. Immunohistochemical detection of GFAP is vital in neuropathological research of astrocytoma [9].

The experience so far tells us that when compared with similar experimental models C6 cells grow more homogeneous intracerebrally and imitate the growth of human glioma to a greater extent [10], which was the basis for decision to monitor the said markers in the experimental rat glioma.

The aim of the research was to determine: macroscopic analysis of brains having implanted tumor during the tumorigenesis; histological traits of tumor cells of an induced brain tumor (animal model); PCNA, CK 19 and GFAP in rat brain having implanted tumor.

Methods

C6 cells were used to induce experimental brain tumor. C6 cells were maintained in culture on nutrient medium, Dulbecco Modified Eagle Medium (DMEM), with added 10% v/v inactivated fetal calf serum (FCS) and mixture of antibiotics (penicilin-100 U/mL, streptomicin-100 μg/mL and amphotericin B-25 μg/mL). Flasks with cells were kept in humid environment with present 5% CO_2 at 37°C. In order to prepare cells for cerebral implantation, they were submitted to trypsinization, centrifugation, rinsing and resuspending in phosphate buffered saline (PBS).

Cerebral implantation of C6 cells was performed on Wistar male rats weighing on average 242 grams. They were firstly anesthetized by intraperitoneal application of sodium thiopental (50 mg/kg) and thereafter fixed in place on a wooden platform in the proper position. Next, the skin of head was depilated and disinfected. A scalpel was used for skin incision and subcutaneous tissue along the midline, in anteroposterior direction, for 1.5–2 cm. After removing the periosteum, craniotomy was performed using a dental drill, in the right-hand frontopariental region of the skull, 4 mm to the right and 2–3 mm above the coronary satura. Using the Hamilton syringe, suspension of C6 cells (4×10^6 cells/10 nL PBS) was injected into the right brain hemisphere to the depth of 5 mm. Thereafter, the incision was shut by suture, and animals were placed into individual cages to recover. It took 21–25 days to develop the tumor in animals.

The second group of animals underwent the identical procedure, except that instead of C6 cells suspension, they were injected the same volume of media for making suspension (the group of ostensibly operated animals). Both groups of animals were nourished pursuant to standard hygienic and dietary regime up to the beginning of experiment.

After a light ether anesthesia, the animals were sacrificed by means of decapitation; the brains were carefully taken out and examined for macroscopic presence of tumor. The hemispheres were divided, weighed separately, and subjected to further procedure.

After sacrifying animals with implanted C6 cells, their brains were taken for histological analysis. The brain tissue was fixed in 4% paraformaldehyde (in phosphate puffer, pH 7,4) for 2–3 days at 4°C, and thereafter was performed cryoprotection in 30% Sukrozi and PBS for 2–3 days at 4°C. Then the tissue was sliced by cryotome (Reichart) at the temperature of -25°C. The section thick 14 nm were mounted on the previously jelly-coated microscopic plates and stored at -20°C. The sections were stained for hematoxylin-eosin or kresyl violet in order to visualize the overall histological structure of brain implanted with C6 cells, whereas other ones were immunohistochemically stained for GFAP, CK 19 and PCNA.

The ABC (avidin-biotin-peroxidase rena complex) method [11] was used to determine immunoreactivity as follows: rinsing the sections in PBS; blocking the endogen peroxidase by incubation of sections in 0.3% hydrogen peroxide in methanol; incubation with 20% normal goat serum to block the non-specified associating of secondary antibodies; incubation with solution of primary antibodies (polyclonal anti-GFAP ICN Pharmaceuticals 1:500; monoclonal anti-CK 19 ICN Pharmaceuticals 1:100; monoclonal anti-PCNA Boehringer Manheim 1:100) in PBS with 1% BSA (bovine serum albumin), 1h; incubation with appropriate secondary antibody conjugated by biotin, 1h; incubation with avidin-biotin-peroxidase complex, 30 min; and stained reaction - diaminobenzidine (DAB)-H_2O_2,5 min.

In between all incubations the rinsing in PBS was performed. For methodological control sections incubated with-

out primary antibodies were used. All reactions took place at room temperature.

The DMEM nutrient medium, FCS, ready-made mixtures of antibiotics and antimycotics, solution for trypsinization, all other standard chemicals for maintaining cell cultures, and plastic disposable containers, were given by ICN Pharmaceuticals, Costa Mesa, USA. The C6 rat glioma cells were given by Prof. Dr. Stukalov (Institute for Molecular Genetics, Moscow, Russia).

Male Wistar rats were bred in the Vivarium of the Center for Biomedical Research, ICN Galenika Institute.

Results

Macroscopic analysis of tumor

The presence of tumor was often accompanied by edema, although the tumor itself was not always macroscopically visible. Macroscopic tumors were only visible in 20% of cases, as a bump in the right hemisphere with a 2 mm diameter. Measuring of the right and left hemispheres of brains with implanted tumors during the tumorigenesis revealed a statistically significant difference in weight between the left and the right hemispheres in as soon as 7 days after the tumor incubation event (Table 1).

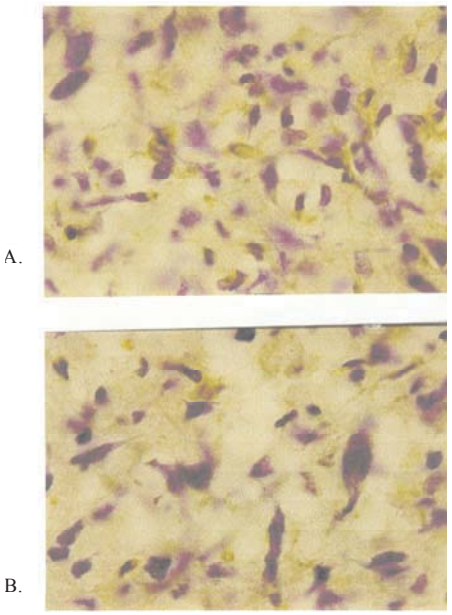

Fig. 1 – Immunohistochemical staining for proliferating cell nuclear antigen (PCNA), ABC method and contrast staining for krezyl-violet.
A) PCNA positive nuclei of C6 cells in the right brain hemisphere 14 days after the implantation (× 1,000). B) The number of PCNA – positive C6 cells in the left hemisphere is substantially lower (× 1,000).

Table 1

Weights of the right and left hemispheres of rat brains with C6 tumor

Days after implanting C6 cells	Brain weights (mg)	
	right hemisphere	left hemisphere
7	702.2 ± 71.4*	603.7 ± 20.2
14	620.0 ± 36.6	622.6 ± 82.5
21	592.6 ± 33.7	598.0 ± 20.2

The results are given as an average value ± standard deviation; * $p < 0.01$.

Microscopic structure of C6 rat glioma

Histological preparations stained by hematoxylin-eosin had a clear place of puncture lesion in the right brain hemisphere, however the tumor mass itself could rarely be noticed, due the tendency of C6 cells' to infiltrative growth. The tumor was of cellular type, with a marked pleomorphism of cells and a frequent hyperchromasia of the nucleus.

Immunohistochemical staining for PCNA revealed numerous PCNA positive cells in the right brain hemisphere migrating along the white matter, and also appearing in the left hemisphere (Figure 1).

The proliferation rate index (number of positive C6 cells relative to all implanted cells) could not be determined, so that the monitoring of this index over time was not possible. However, a significant number of positive cells were noticed on the days 7, 14, and 21 after the implementation of C6 cells, meaning that these cells are still proliferating beyond the day 21.

Immunohistochemical staining for cytokeratin 19 revealed numerous CK 19 positive cells in both hemispheres of the brain (Figure 2).

The preliminary results showed that all C6 cells *in vitro* were CK 19 positive. A significantly higher number of cells were found in the right hemisphere, where C6 cells had been

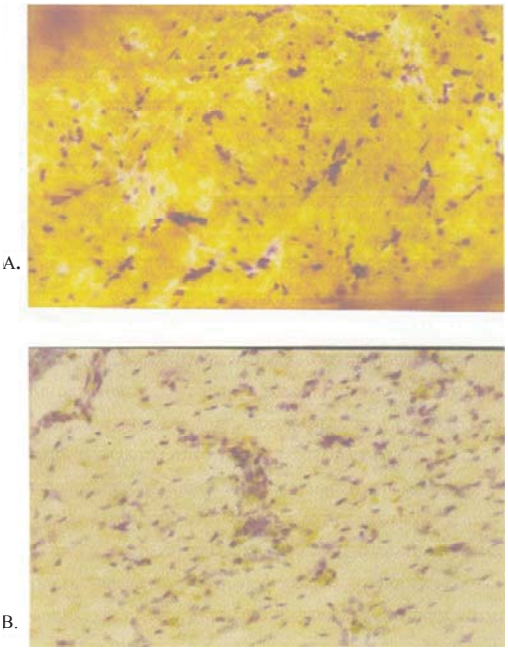

Fig. 2 – Immunohistochemical staining for cytokeratin 19, ABC method and contrast staining for krezyl-violet.
A) Numerous cytokeratin 19–positive cells in the right brain hemisphere 7 days after C6 cells implantation (× 400). B) Individual cytokeratin 19–positive cells in the counter lateral hemisphere indicate that C6 cells infiltrated the entire brain (× 400).

implanted, than in the left hemisphere, where they arrived by means of migration. In the control brain sections of normal rats, CK 19 positive cells were found only in the capillary endothelium.

Immunohistochemical reaction with anti-GFAP antibodies revealed numerous GFAP positive astrocytes that were found close to the puncture lesion spot. These astrocytes showed traits of reactive glia, cells swelling, increase in the number and length of extensions (Figure 3).

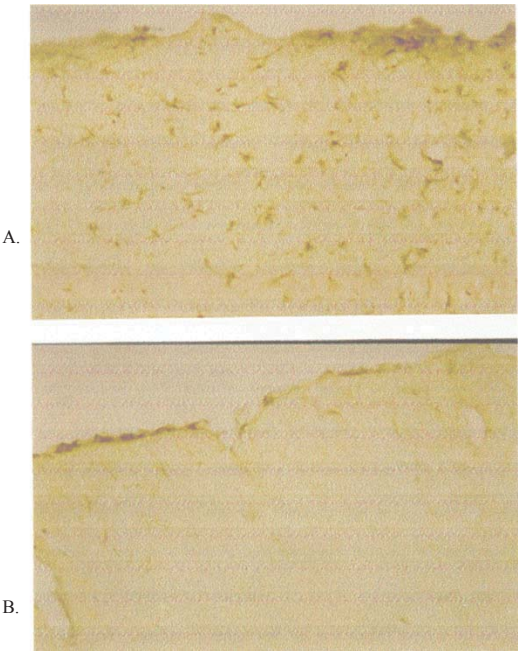

Fig. 3 – Immunohistochemical staining for glial fibrillary acidis protein (GFAP), ABC method.
A) Numerous reactive GFAP–positive astrocytes close to the point of C6 cells implantation (× 400). B) Counter lateral hemisphere – only a small number of normal astrocytes were noticed (× 400).

Implanted C6 cells were not GFAP positive. *In vitro*, C6 cells also did not contain GFAP marker of mature glial phenotype.

Discussion

This study examined experimental brain tumors macroscopically, histologically and immunohistochemically for the presence of proliferation (PCNA) and differentiations (GFAP and CK 19) cell markers.

The study used animal model of primary brain tumor. C6 rat glioma cells are commonly used as glioma cells model for *in vitro* and *in vivo* researches related to tumor cells biology [12]. The C6 glioma cell line has originally been induced on Wistar rats bred by random mating, by means of exposing them to N,N′-nitro-methylurea [2]; once injected into rat brain [13], it proved by its attributes to be morphologically similar to human malignant glioma. A glioma, a tumor on central nervous system that arise from glial cells, primarily occurs in the brain, and comprise more than 70% of all brain tumors. They are histologically malignant [14] and their typical hallmark is cell proliferation [15].

It is considered that C6 glioma is analogous to human glioma of II/III malignancy degree. This study used animals in which tumor had been developing for 21–25 days, unlike other authors who used this model, however with tumor development lasting for 10–11 days [16], 14 days [17], or 11–21 days [18] following the cerebral implantation of C6 cells.

While measuring the left and the right hemispheres of brains with implanted tumor, a difference was observed in weights of the left and the right hemispheres on the day 7 of tumorigenesis, indicating that glioma cells display invasive ability during active division of glioma cells.

As a marker of cell proliferation, PCNA was monitored immunohistochemically in rat brain having implanted tumor. It was observed that numerous PCNA positive cells were located not only close to the spot of C6 cells implementation, but also in the opposite hemisphere, which supports the migration of C6 cells and their infiltration throughout the brain.

The C6 cells are known to migrate away from the site of implantation and infiltrate the adjacent regions of the brain [10]. Quantification of immunohistochemically stained cells was not possible, given the huge number of implanted cells (4 million), so that monitoring of the increase in number of cells during tumorigenesis was not possible. However, as soon as 7 days after implementation of C6 cells, a large number of PCNA positive cells could be observed in both brain hemispheres, which indicating their intensive proliferation.

Immunohistochemical staining for CK 19, an intermediate filament found in C6 cells *in vitro,* also showed numerous positive cells not only at the place of C6 cells implantation, but also in the opposite hemisphere. The control sections of normal brain showed no CK 19 positive glial cells or neurons.

Previous analysis of histological preparations to GFAP revealed a similar morphology of C6 rat glioma and human glioblastoma, formation of glial edge at the glioma periphery, consisting of GFAP-positive reactive astrocytes. Astrogliosis was monitored until death of animal (28th day) [19], (30th day) [20]. Reactive astrocytes with multiple processes encircled not only the primary focus of glioma but also any other place of tumor invasion into the nerve tissue [19, 20].

In addition, the injection of radio-tagged monoclonal antibodies on GFAP model of C6 glioma on rats has indicated their accumulation in tumors. Concentration of antibodies was considerably higher in the tumor-affected hemisphere when compared with the unaffected hemisphere [21].

In this study, the immunohistochemical determination of GFAP revealed that tumor cells do not contain this marker of mature glial phenotype. The C6 cells in culture are also characterized by the absence of GFAP [1], and they also retain this property after implantation and inducing of brain tumor *in vivo*. Having that said, around the area of puncture lesion inflicted during implantation of C6 cells were discernable numerous reactive astrocytes, which were particularly GFAP positive.

Within the experimental conditions of this study, C6 glioma did not reveal any noteworthy deviations in terms of development, clinical symptomatology and macroscopic anatomy other than those already described in literature [2, 22].

Conclusion

Macroscopic analysis of C6 rat glioma revealed invasive ability of glioma cells displayed during active division of glioma cells. Histological preparations of rat brain with C6 glioma clearly displayed puncture lesion in the right brain hemisphere, but the tumor mass could rarely be noticed. Microscopic analysis of C6 rat glioma showed the tumor to be of cellular type, with distinct polymorphism of cells and frequent hyperchromasia of nucleus. Cell proliferation marker PCNA was identified immunohistochemically in C6 cells in both hemispheres of the brain, indicating the proliferative and invasive potential of cells. C6 cells positive to CK 19 were found in both hemispheres of the brain, thus further supporting migration of these cells. Numerous GFAP positive astrocytes were found around the place of puncture lesion. Implanted cells were not GFAP positive.

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Correlation of clinical and neurophysiological findings with health-related quality of life in patients with diabetic polyneuropathy

Korelacija kliničkih i neurofizioloških nalaza sa kvalitetom života bolesnika sa dijabetesnom polineuropatijom

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Abstract

Background/Aim. Diabetic polyneuropathy is defined as the presence of clinical or subclinical symptoms and/or signs of peripheral nerve damage in patients with diabetes mellitus in the absence of the other causes of peripheral neuropathy. The aim of this study was to assess health-related quality of life (HRQoL) in patients with diabetic polyneuropathy and its correlation with clinical and neurophysiological findings. **Methods.** This study comprised 60 patients with distal, symmetric, sensorimotor diabetic polyneuropathy and type 2 diabetes mellitus. For evaluation of clinical findings the following scales were used: Medical Research Council strenth score (MRC sum score), Inflammatory Neuropathy Cause and Treatment (INCAT) disability scale (arm disability and leg disability scales), INCAT sensory sum score, Hamilton depression and anxiety rating scales. Nerve conduction study (NCS) was performed on the motor part of the median and peroneal nerves, the sensory part of the median nerve and sural nerve. All the patients completed the Serbian version of the SF-36 questionnaire as a measure of HRQoL. **Results.** Our results showed mild to moderate QoL impairment in the patients with diabetic polyneuropathy with no difference in physical and mental composite scores ($p > 0.05$). The age of the patients, mean MRC sum score, arm disability scale score, leg disability scale score and mean INCAT sensory sum score correlated with scores in the SF-36 questionnaire ($p < 0.01$). The patients with higher scores of anxiety and depression had significantly worse health perception for all QoL domains, for both composite scores and for the total SF-36 score ($p < 0.01$). Both motor and sensory NCS parameters of the median nerve showed significant correlations with QoL scores ($p < 0.05$). **Conclusion.** Our results showed mild to moderate QoL impairment in the patients with diabetic polyneuropathy. HRQoL significantly correlated with the age of the patients, muscle strength, disability, sensory complaints, depressiveness and anxiety of the patients. Electrophysiological examination of median nerve significantly correlated with QoL in the patients with diabetic polyneuropathy.

Key words:
diabetic neuropathies; neurophysiology; quality of life; questionnaires.

Apstrakt

Uvod/Cilj. Dijabetesna polineuropatija definiše se kao prisustvo kliničkih ili supkliničkih simptoma i/ili znakova oštećenja perifernih živaca kod bolesnika sa dijabetesom melitusom u nedostatku drugih uzroka periferne neuropatije. Cilj rada bio je da se proceni kvalitet života povezan sa zdravljem (HRQoL) kod bolesnika sa dijabetesnom polineuropatijom i njegova povezanost sa kliničkim i neurofiziološkim nalazom. **Metode.** U istraživanju je učestvovalo 60 bolesnika sa distalnom simetričnom senzorimotornom dijabetesnom polineuropatijom u sklopu dijabetesa melitusa tipa 2. Za objektivnu procenu kliničkog nalaza korišćene su sledeće skale: *Medical Research Council strenth score* (MRC skor), *Inflammatory Neuropathy Cause and Treatment* (INCAT) skala invalidnosti (za ruke i noge), INCAT senzorna skala, Hamiltonova skala depresivnosti i anksioznosti. Elektroneurografija (ENG) sprovedena je na motornom delu *n. medianus*-a i *n. peroneus*-a i na senzornom delu *n. medianus*-a i na *n. suralis*-u. Svi bolesnici popunili su srpsku verziju upitnika SF-36. **Rezultati.** Registrovano je blago do umereno sniženje kvaliteta života kod bolesnika sa dijabetesnom polineuropatijom, i to bez razlika u fizičkom i mentalnom domenu ($p > 0,05$). Starost bolesnika, MRC skor, INCAT skala invalidnosti i INCAT senzorna skala bile su u korelaciji sa rezultatima na SF-36 upitniku ($p < 0,01$). Bolesnici sa višim skorom an-

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ksioznosti i depresivnosti imali su značajno lošiju percepciju zdravlja za sve domene, za oba kompozitna skora i ukupni SF-36 skor ($p < 0,01$). Motorni i senzorni ENG parametri za *n. medianus* značajno su korelisali sa HRQoL ($p < 0,05$). **Zaključak.** Naši rezultati pokazuju blago do umereno sniženje kvaliteta života kod bolesnika sa dijabetesnom polineuropatijom. Kvalitet života kod ovih bolesnika je u značajnoj vezi sa starošću bolesnika, njihovom mišićnom snagom, stepenom invalidnosti, senzornim smetnjama, depresivnošću i anksioznošću. Elektrofiziološki nalaz za *n. medianus* značajno je povezan sa kvalitetom života kod bolesnika sa dijabetesnom polineuropatijom.

Ključne reči:
dijabetesne neuropatije; neurofiziologija; kvalitet života; upitnici.

Introduction

Diabetic polyneuropathy is defined as the presence of clinical or subclinical symptoms and/or signs of peripheral nerve damage in patients with diabetes mellitus in the absence of the other causes of peripheral neuropathy [1]. It is one of the most common and most important complications of diabetes, and one of the most frequent polyneuropathies in developed countries [2, 3]. The most frequent form of diabetic polyneuropathy is distal, symmetric, sensorimotor, predominantly sensory polyneuropathy and it is encountered in 30–50% of patients with diabetes [4, 5]. It is mainly of axonal type with secondary demyelination, but with the progress of the disease it can become sensorimotor axonal-demyelinating [6, 7].

Having in mind a high prevalence of diabetes and high treatment expenses, diabetic polyneuropathy has also significant socioeconomic impact [3]. Quality of life (QoL) is worse in patients with diabetes who have complications including diabetic polyneuropathy [8–10]. Venkataraman et al. [11] even found that peripheral neuropathy, among all complications, was associated with the greatest reduction in quality of life. Diabetic patients with neuropathy also have significantly worse trajectory of QoL outcomes over time compared to patients without neuropathy [12]. Previous studies usually compared QoL in diabetic patients with and without complications, but neither of them evaluated influence of clinical and electrophysiological parameters on QoL in a cohort of patients with peripheral diabetic neuropathy.

The aim of this study was to assess QoL in patients with diabetic polyneuropathy and its correlation with clinical and neurophysiological findings.

Methods

This study comprised 60 patients with distal, symmetric, sensorimotor diabetic polyneuropathy as a complication of diabetes mellitus type 2. All the patients were diagnosed and treated at the Neurology Clinic, Clinical Center in Banja Luka, Bosnia and Herzegovina. The other etiology of polyneuropathy was excluded by additional investigations, including urea, creatinine, vitamin B12 and thyroid hormones serum levels, immunological and virusological analysis, electrophoresis of serum and urine proteins with immunofixation, tumor markers. Patients with any other severe disease or macrovascular and microvascular diabetic complications except polyneuropathy, those with cognitive failure, alcohol or drug abuse were excluded from the study. Prior to the enrolment into the study informed consent was obtained from all the patients. The study was approved by the Ethics Committee of the Neurology Clinic in Banja Luka.

A general questionnaire was used to assess demographic characteristics of the investigated patients, including gender, age at onset and duration of diabetic neuropathy, as well as current age.

For evaluation of clinical findings the following scales were used: Medical Research Council strenth score (MRC sum score), Inflammatory Neuropathy Cause and Treatment (INCAT) disability scale (arm disability and leg disability scales), and INCAT sensory sum score. The MRC sum score was calculated by summation of MRC scores for 8 muscle groups bilaterally and the overall score ranges from 0 (total paralysis) to 80 (normal strength) [13]. The arm disability scale quantifies daily activities of the arms, with the span ranging from 0 (no signs of disability) to 5 (most severe disability) [14], while the leg disability scale quantifies walking ability with the results ranging from 0 (walking is not affected) to 5 (wheelchair bound, unable to stand or walk even with help) [14]. The INCAT sensory sum score includes examination of pain and vibration sensibility in arms and legs, as well as two-point discrimination sensibility in arms. The score has values ranging from 0 (normal sensation) to 20 (most severe sensory deficit) [15, 16]. Muscle stretch reflexes of biceps and triceps, as well as patellar and Achilles reflexes were also examined. Every reflex was marked as 1 (absent), 2 (lower) or 3 (normal), so the reflex score had values ranging from 8 (generalized areflexia) to 24 (normal reflexes) [17].

For evaluation of depression, the 21-item Hamilton depression rating scale (Ham-D) was used where a score less than 8 signifies the absence of depression [18]. For evaluation of anxiety, the Hamilton anxiety rating scale (Ham-A) was used where a score less than 18 marks the absence of anxiety [19].

Electroneurography examination was performed by the single examiner on the Oxford Synergy equipment. Temperature of the examined limb was maintained above 31°C. Nerve conduction study (NCS) was performed using surface stimulation and registration electrodes on the standard positions for the examined nerves (motor part of median and peroneal nerves, sensory part of the median nerve and the sural nerve). The following parameters were assessed: motor conduction velocity (MCV), amplitude of the compound muscle action potentials (CMAP) and minimal F wave latency for motor nerves, sensory conduction velocity (SCV) and amplitude of the sensory nerve action potentials (SNAP) for sensory nerves. Polyneuropathy was defined as sensory, motor or sensorimotor according to the type of predomi-

nantly affected nerves. According to the pathophysiological mechanism of nerve damage, polyneuropathy was marked as axonal, demyelinating or axonal-demyelinating according to the criteria published by Tankisi et al. [20].

All the patients completed the Serbian version of the SF-36 questionnaire as a measure of HRQoL [21]. The SF-36 is a generic instrument that measures eight general health concepts: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). Two main scores are available to summarize these scales: physical composite score (PCS) and mental composite score (MCS), as well as total SF-36 score. All these scores fall within a 0–100 scale, with higher scores reflecting better HRQoL.

Statistical analysis included descriptive statistics, Man Whitney U-test, Student's *t*-test, ANOVA and Spearman correlation analysis. Significant testing was two-sided, with alpha sets at 0.05 for a statistical significance and 0.01 for a high statistical significance.

Results

The results of the SF-36 questionnaire are shown in Figure 1. The best subscore was found for PF and the worse for GH. There was no difference in PCS and MCS ($p > 0.05$).

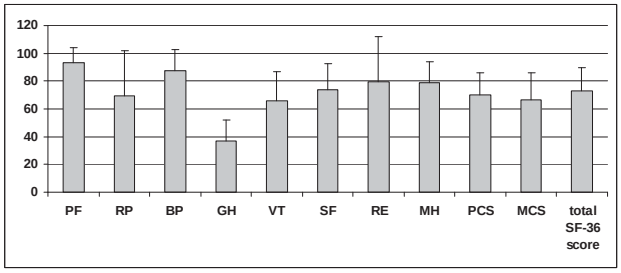

Fig. 1 – Results of the SF-36 questionnaire in the patients with diabetic polyneuropathy in all domains (n = 60). The results are shown as $\bar{x}$ ± standard deviation.

PF – physical functioning; RP – role physical; BP – bodily pain; GH –general health; VT – vitality; SF – social functioning; RE – role emotional; MH – mental health; PCS – physical composite score; MCS – mental composite score.

Demographic data and therapeutic approach in the analyzed patients are shown in Table 1. There was no difference in QoL regarding gender of the patients ($p > 0.05$). Those older at the onset of the disease had significantly lower RP, BP and VT subscores, as well as PCS and the total SF-36 score ($p < 0.05$). There was no correlation between duration of diabetic neuropathy and QoL measured by the SF-36 ($p > 0.05$). The age of the patients significantly correlated with RP, BP, VT, PCS, the total SF-36 score ($p < 0.01$), as well as with GH and MCS ($p < 0.05$).

Table 1

Sociodemographic characteristics and therapeutic approach in the analyzed patients with diabetic polyneuropathy

Demographic characteristics (n = 60)	Values
Sex (%)	
male	50.0
female	50.0
Age at onset, years (mean years ± SD)	44.3 ± 12.0
Current age, years (mean years ± SD)	56.5 ± 9.9
Duration of disease (mean years ± SD)	12.2 ± 6.0
Type of therapy (%)	
only oral hypoglycemics	33.3
insulin	66.7
Hemoglobin A1c (%)	7.3 ± 2.3

The mean MRC sum score of the analyzed patients with diabetic polyneuropathy was 78.2 ± 4.5. The arm disability scale score was 0.13 ± 0.39, leg disability scale score 0.23 ± 0.46 and mean INCAT sensory sum score 2.35 ± 2.53. All these scores correlated with better scores on each domain of SF-36 questionnaire ($p < 0.01$) (Table 2). Mean muscle stretch reflexes score was 17.7 ± 4.7 and it correlated with VT, SF, RE, MH, PCS, MCS and total SF-36 score ($p < 0.01$), as well as with PF, RP and GH ($p < 0.05$).

The average Ham-D score was 4.58 ± 3.76, while average Ham-A was 2.71 ± 2.29. The patients with higher scores of anxiety and depression had significantly worse health perception for all QoL domains, for both composite scores and for the total SF-36 score ($p < 0.01$) (Table 2).

Table 2

Correlation between clinical factors and quality of life (QoL) in the patients with diabetic polyneuropathy (n = 60)

SF-36 domains	MRC sum score	INCAT Arm disability scale	INCAT Leg disability scale	INCAT Sensory sum score	Muscle stretch reflexes score	Ham-D score	Ham-A score
PF	0.609; 0.001	-0.470; 0.001	-0.601; 0.001	-0.508; 0.001	0.294; 0.023	-0.642; 0.001	-0.593; 0.001
RP	0.529; 0.001	-0.513; 0.001	-0.518; 0.001	-0.399; 0.002	0.327; 0.011	-0.468; 0.001	-0.413; 0.001
BP	0.331; 0.010	-0.326; 0.011	-0.393; 0.002	-0.350; 0.006	0.240; 0.065	-0.483; 0.001	-0.479; 0.001
GH	0.386; 0.002	-0.412; 0.001	-0.452; 0.001	-0.414; 0.001	0.299; 0.020	-0.777; 0.001	-0.735; 0.001
VT	0.461; 0.001	-0.417; 0.001	-0.516; 0.001	-0.477; 0.001	0.418; 0.001	-0.824; 0.001	-0.781; 0.001
SF	0.395; 0.002	-0.439; 0.001	-0.501; 0.001	-0.422; 0.001	0.362; 0.005	-0.843; 0.001	-0.793; 0.001
RE	0.360; 0.005	-0.366; 0.004	-0.343; 0.007	-0.385; 0.002	0.402; 0.001	-0.819; 0.001	-0.782; 0.001
MH	0.344; 0.007	-0.414; 0.001	-0.414; 0.001	-0.368; 0.004	0.372; 0.003	-0.804; 0.001	-0.793; 0.001
PCS	0.521; 0.001	-0.460; 0.001	-0.561; 0.001	-0.497; 0.001	0.399; 0.002	-0.752; 0.001	-0.700; 0.001
MCS	0.394; 0.002	-0.413; 0.001	-0.450; 0.001	-0.457; 0.001	0.370; 0.004	-0.902; 0.001	-0.854; 0.001
Total SF-36	0.462; 0.001	-0.442; 0.001	-0.502; 0.001	-0.479; 0.001	0.380; 0.003	-0.862; 0.001	-0.807; 0.001

The results are shown as Spearman's rho; MRC – Medical Research Council strenth score; INCAT – Inflammatory Neuropathy Cause and Treatment; HAM – Hamilton depression (D) or anxiety (A) rating scale; PF – physical functioning; RP – role physical; BP – bodily pain; GH –general health; VT – vitality; SF – social functioning; RE – role emotional; MH – mental health; PCS – physical composite score; MCS – mental composite score.

The results of NCS in our patients with diabetic polyneuropathy are presented in Table 3. Regarding NCS parameters of the motor peroneal nerve, only MCV was in correlation with PF domain (rho = 0.336, $p < 0.009$). Regarding sural nerve, only SNAP amplitude correlated with VT domain (rho = 0.30, $p = 0.013$). Both motor and sensory NCS parameters of median nerve showed significant correlations with QoL scores (Table 4).

Table 3

Nerve conduction study findings in the patients with diabetic polyneuropathy (n = 60)

Investigated nerve conduction parameters	Value ($\bar{x} \pm SD$)
Median nerve (motor part)	
MCV (m/s)	49.77 ± 4.08
CMAP amplitude (mV)	9.18 ± 1.75
F wave latency (m/s)	30.72 ± 2.31
Peroneal nerve	
MCV (m/s)	39.49 ± 4.00
CMAP amplitude (mV)	4.23 ± 1.84
F wave latency (m/s)	56.92 ± 5.62
Median nerve (sensory part)	
SCV (m/s)	46.44 ± 4.71
SNAP amplitude (μV)	9.52 ± 6.54
Sural nerve	
SCV (m/s)	35.89 ± 5.57
SNAP amplitude (μV)	2.80 ± 1.41

MCV– motor conduction velocity; CMAP – compound muscle action potentials; SCV – sensory conduction velocity; SNAP – sensory nerve action potentials.

QoL, especially in patients with diabetic complications including polyneuropathy [9–12]. Physical and mental domains were similarly affected in our patients which is in accordance with few previous studies [22, 23]. This fact signifies the importance of QoL measure in diabetic polyneuropathy since physicians are usually focused on physical symptoms and have neglect for patients' subjective complaints. In line with this observation, we registered the lowest scores for GH and VT domains which is in accordance with other studies [23–25], and it further speaks in favor of the importance of the mental health impairment in patients with diabetic polyneuropathy.

In our study, the patients of older age at onset of the disease and at the moment of investigation had worse health-related QoL, particularly for physical domains. On the other hand, duration of disease did not affect any subscore of QoL. Similar results were published by Lloyd et al [22]. Both findings mean that QoL was in association with normal ageing process but not with duration of diabetic neuropathy itself. On the other hand, there are also publications that showed worse PCS in patients with longer duration of disease [23], but we failed to find this correlation.

According to our results, clinical parameters of severity of diabetic polyneuropathy were in a significant correlation with SF-36 scores. Although muscle strength was pretty good in our patients and disability of arms and legs was minor, these parameters significantly affected all aspects of QoL. Similarly,

Table 4

Correlation between nerve conduction study of the median nerve and quality of life in the patients with diabetic polyneuropathy (n = 60)

SF-36 domains	Motor part of median nerve			Sensory part of median nerve	
	MCV	CMAP amplitude	F wave latency	SCV	SNAP amplitude
PF	0.285; 0.028	0.332; 0.009	-0.501; 0.001	0.560; 0.001	0.318; 0.013
RP	0.048; 0.714	0.221; 0.090	-0.364; 0.004	0.172; 0.189	0.332; 0.010
BP	0.112; 0.395	0.271; 0.037	-0.283; 0.029	0.212; 0.104	0.349; 0.006
GH	0.114; 0.386	0.323; 0.012	-0.236; 0.069	0.181; 0.166	0.423; 0.001
VT	0.154; 0.240	0.286; 0.027	-0.231; 0.076	0.274; 0.034	0.427; 0.001
SF	0.207; 0.113	0.296; 0.021	-0.350; 0.006	0.325; 0.011	0.470; 0.001
RE	0.247; 0.058	0.365; 0.004	-0.129; 0.325	0.319; 0.013	0.300; 0.020
MH	0.133; 0.312	0.268; 0.039	-0.147; 0.264	0.213; 0.102	0.364; 0.004
PCS	0.141; 0.282	0.325; 0.011	-0.383; 0.003	0.289; 0.021	0.438; 0.001
MCS	0.199; 0.128	0.341; 0.008	-0.228; 0.080	0.299; 0.020	0.420; 0.001
Total SF-36	0.185; 0.156	0.353; 0.006	-0.320; 0.013	0.321; 0.012	0.444; 0.001

The results are shown as Spearman's rho; *p*. MCV – motor conduction velocity; CMAP – compound muscle action potentials; SCV – sensory conduction velocity; SNAP – sensory nerve action potentials. PF – physical functioning; RP – role physical; BP – bodily pain; GH –general health; VT – vitality; SF – social functioning; RE – role emotional; MH – mental health; PCS – physical composite score; MCS – mental composite score.

Sensorimotor polyneuropathy was diagnosed in 51 (85%) of the patients and sensory polyneuropathy in 9 (15%). Forty four (73.3%) patients had axonal and 16 (26.7%) patients had axonal-demyelinating polyneuropathy. The patients with sensorimotor polyneuropathy had similar SF-36 scores as patients with pure sensory polyneuropathy ($p > 0.05$). Also, the patients with axonal-demyelinating polyneuropathy had similar QoL like those with pure axonal polyneuropathy ($p > 0.05$).

Discussion

Our results show mild to moderate QoL impairment in the patients with diabetic polyneuropathy. Previous studies showed that diabetes had mild to moderate influence on better QoL was associated with higher values of the MRC sum score and lower INCAT disability scores in patients with immune mediated polyneuropathies [15, 26]. Sensory symptoms were more common than motor in our patients with diabetic neuropathy and their influence on all the aspects of QoL was obvious. According to literature data, patients with immune mediated neuropathies who had higher values of INCAT sensory composite score had worse results for PF, BP and PCS [26, 27]. Thus, sensory complaints may have significant impact on QoL in patients with neuropathies and they should not be neglected by physicians. Finally, we found that even examination of muscle stretch reflexes may be suggestive of worse QoL but this factor was the least important clinical parameter for estimation of QoL in diabetic polyneuropathy.

Mean Ham-D and Ham-A scores were pretty low in our study which is in accordance with previous finding that depressiveness and anxiety are present in only about one third of patients with diabetes [9, 28, 29]. Besides this, our patients with higher scores of anxiety and depression had significantly worse perception of health-related QoL. Similar results were published previously for diabetic patients [30]. Psychological and pharmacological treatment of depression and anxiety may lead to better blood sugar control, better functional status and better perception of QoL in patients with diabetic neuropathy [31].

Established correlations between electrophysiological parameters and QoL are very important since NCS is an objective measure of diabetic polyneuropathy more sensitive than clinical examination [32]. Lower QoL was previously found even in subclinical forms of polyneuropathy diagnosed only by electromyography but not by clinical examination [33]. Certain inconsistent correlations between NCS parameters of peroneal and sural nerves with QoL were found in our and a study by Padua et al. [23]. On the other hand, according to our data NCS findings on the median nerve were more important to estimate QoL in patients with diabetic polyneuropathy. The most significant correlations were observed between CMAP amplitude and QoL, as well as between SNAP amplitude and QoL. This means that axonal damage of the median nerve may be suggestive of worse QoL. This may be explained that nerves of upper extremities are affected later in the course of the disease and that median nerve impairment may lead to severe arm disability including using of tool, writing, driving, clothing etc.

Previous studies reported lower QoL scores in patients with sensorimotor compared to those with sensory polyneuropathy and in axonal-demyelinating compared to axonal type of polyneuropathy [32]. This correlation seems logical and may be explained by natural progression of disease. However, we failed to find these correlations which can be explained with small sample analyzed.

Conclusion

Our results show mild to moderate quality of life (QoL) impairment in the patients with diabetic polyneuropathy. Besides, the age of the patients, muscle strength and disability, sensory complaints also had great influence on QoL. The patients with higher level of anxiety and depressiveness had significantly worse perception of health-related QoL. The most prominent finding in our study is that electrophysiological examination of the median nerve significantly correlates with QoL in the patients with diabetic polyneuropathy.

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The prevalence of pseudoexfoliation syndrome and possible systemic associations in patients scheduled for cataract surgery at the Military Medical Academy in Belgrade

Prevalencija pseudoeksfolijativnog sindroma i moguća udruženost sa sistemskim oboljenjima kod bolesnika predviđenih za hirurgiju katarakte u Vojnomedicinskoj akademiji u Beogradu

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Abstract

Background/Aim. Pseudoexfoliation syndrome (PEX) is an age-related systemic degenerative disorder characterized by the production and progressive accumulation of extracellular fibrillar eosinophilic material in the anterior segment of the eye. The aim of the study was to evaluate several clinical aspects of PEX, such as frequency of PEX and pseudoexfoliation glaucoma (PEXG), intraocular pressure (IOP), the type of lens opacity, and the possible relationship of PEX and systemic diseases. **Methods**. All 674 cataract patients had a comprehensive eye examination, including slitlamp biomicroscopy before and after mydriasis, IOP measurement, and fundus examination. The patients were classified into two groups: the PEX and the non-PEX group. **Results**. The overall prevalence of PEX syndrome was found to be 17.5% (118 patients). The mean age of PEX patients (79.7 ± 6.1 years) was significantly higher when compared with those without PEX (73.5 ± 9.1 years) ($p = 0.000$). The prevalence of PEX syndrome was found to increase with age, from 7.3% in the 7th decade of life to 27% in patients older than 80 years ($p < 0.001$). The most common cataract type in the PEX patients was mature cataract observed in 40.7% of patients. The rest of the patients had mixed (30.5%), nuclear (25.4%), cortical (1.7%) and hypermature cataract (1.7%). Among the PEX patients 44 (37.2%) had glaucoma. Intraocular pressure was significantly higher in eyes with pseudoexfoliations than in eyes without it (17.8 ± 3.2 mmHg and 15.8 ± 2.8 mmHg, respectively; $p = 0.001$). Moreover, the prevalence of coronary heart disease was found to be higher in PEX patients. **Conclusion**. PEX syndrome is a common problem among Serbian patients scheduled for cataract surgery. It represents one of the major glaucoma risk factors and probably associated with ischemic heart disease, intraoperative and postoperative problems in cataract surgery.

Key words:
exfoliation syndrome; cataract; glaucoma; prevalence; coronary artery disease, intraoperative complications; postoperative complications.

Apstrakt

Uvod/Cilj. Pseudoeksfolijativni sindrom (*pseudoexfoliation syndrome* – PEX) je starosni sistemski degenerativni poremećaj koji karakteriše produkcija i progresivna akumulacija vanćelijskog vlaknastog eozinofilnog materijala na spoljašnjim segmentima oka. Cilj ove studije bio je da ispita određene kliničke aspekte PEX, kao što su: učestalost PEX, pseudoeksfolijativni glaukom (PEXG), intraokularni pritisak (IOP), tipovi katarakte i moguća vezu PEX-a sa sistemskim bolestima. **Metode**. Svi bolesnici (n = 674) obuhvaćeni studijom detaljno su oftalmološki pregledani, uključujući pregled na biomikroskopu sa procepnom lampom, pre i posle midrijaze, merenje IOP i pregled očnog dna. Bolesnici su bili podeljeni u dve grupe: sa PEX-om i bez PEX-a. **Rezultati**. Ukupna prevalencija PEX-a bila je 17,5% (118 bolesnika). Prosečna starost bolesnika sa PEX-a bila je (79,7 ± 6,1 godina), što je statistički značajno više u odnosu na bolesnike bez PEX-a (73,5 ± 9,1 godina). Nađeno je da prevalencija PEX-a raste sa starenjem, od

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7,3% u sedmoj deceniji života do 27% kod starijih od 80 godina ($p < 0,001$). Učestalost pojedinih tipova katarakte kod bolesnika sa PEX-om bila je: maturna kod 40,7%, mešovita kod 30,5%, nuklearna kod 25,4%, kortikalna kod 1,7% i hipermaturna kod 1,7% bolesnika. Od 118 bolesnika sa PEX, 44 (37,2%) imalo je glaukom. Vrednosti IOP kod bolesnika sa PEX-om (bez glaukoma) bile su 17,8 ± 3,2 mmHg, što je statistički značajno više ($p = 0,000$) u odnosu na 15,8 ± 2,8 mmHg kod bolesnika bez PEX (bez glaukoma). Prevalencija koronarne bolesti bila je statistički značajno viša kod bolesnika sa PEX. **Zaključak**. PEX je čest kod bolesnika sa kataraktom u našoj populaciji. On predstavlja jedan od glavnih rizika od pojave glaukoma, udružen je sa ishemijskom bolešću srca, kao i intraoperativnim i postoperativnim problemima u hirurgiji katarakte.

Ključne reči:
eksfolijativni sindrom; katarakta; glaukom; prevalenca; koronarna bolest; intraoperativne komplikacije; postoperativne komplikacije.

Introduction

Pseudoexfoliation syndrome (PEX) is an age-related systemic degenerative disorder characterized by production and progressive accumulation of extracellular fibrillar eosinophilic material in the anterior segment of the eye. This material may be found in many ocular tissues including ciliary processes, zonules, anterior lens surface, pupillary margin, corneal endothelium, trabecular meshwork, and conjunctiva. Ocular PEX has been associated with the development of open- and closed-angle glaucoma and cataract with zonular instability [1–3]. It has already been reported that PEX syndrome is a major risk factor in modern extracapsular cataract surgery and phacoemulsification. The risk of intraoperative problems (such as a poorly dilating pupil) zonular weakness with or without lens instability, capsular break and vitreous loss) and postoperative complications (including fibrinoid reaction, posterior synechias, cell deposits and capsule contraction-phimosis) is higher in eyes with this syndrome [4–5]. Currently, PEX is regarded as a systemic disorder, since pseudoexfoliation material has also been identified in the skin and connective tissue portions of various visceral organs. In this regard, previous studies have shown a relationship between PEX and various systemic disorders, such as hypertension, coronary heart disease, stroke, abdominal aortic aneurysm, Alzheimer's disease, asymptomatic myocardial dysfunction, diabetes, and sensorineural hearing loss [6–16]. It is estimated that 60–70 million people worldwide are affected by PEX. The prevalence of PEX increases with age, but shows significant variations between geographical regions. In cataract surgery patients, the PEX prevalence ranges from 0.4% in Chinese to 39.3% in Ethiopian population [17–19]. To our knowledge, the prevalence of PEX in Serbian patients scheduled for cataract surgery has not yet been investigated. Therefore, the aim of our study was to evaluate several clinical aspects of PEX, such as frequency of PEX and pseudoexfoliation glaucoma (PEXG), intraocular pressure (IOP), the type of lens opacity, and the relationship of PEX and systemic diseases.

Methods

This retrospective study included 674 consecutive patients scheduled for cataract surgery (phacoemulsification) with intraocular lens implantation, in the Eye Clinic of Military Medical Academy (MMA) in Belgrade from January to Octobre 2011. The MMA as a 1200-bed tertiary care facility admits around 30,000 patients and performs about 30,000 surgical procedures (more than 4,000 in the Eye Clinic) and more than half a million specialist examinations each year.

All the patients were examined independently by two investigators, with the same instruments, in a dark examination room. A diagnosis of PEX was made after mydriasis with 1% tropicamid and 10% phenylephrine hydrochloride. Slitlamp examination was performed before and 30–40 min after the dilation of the pupils. Secondary cataracts related to trauma, uveitis and steroid use, congenital cataracts, and patients younger than 50 years of age were excluded from the study. Data were collected on the basis of an interview with the patient and from the records of patient files. The data included age, gender, history and duration of any eye and systemic disease, and use of ocular or systemic medications. The study included only the patients who had detailed internal diseases specialist examination in the last six months.

The patients had a comprehensive eye examination including visual acuity testing, refractive work-up, slit-lamp biomicroscopy before and after mydriasis, IOP measurement with Goldmann applanation tonometry, and fundus examination. Clinical diagnosis of PEX was made on the basis of the presence of typical fibrogranular pseudoexfoliation material on the anterior capsule surface and the pupillary margin (Figure 1–3). Pupil size was measured before and after dilation with a specially designed ruler under the same lighting conditions. In pseudophakic and aphakic fellow eyes pseudoexfoliation material was sought in locations other than the

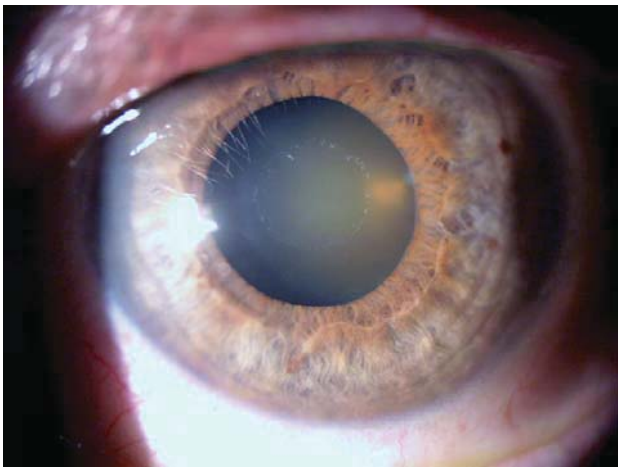

Fig. 1 – A 74-year-old woman with pseudoexfoliation of the anterior lens surface and poorly dilatated pupil.

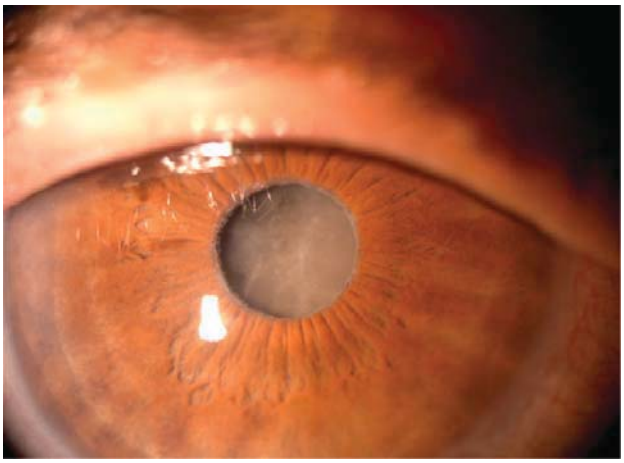

Fig. 2 – A 76-year-old woman with pseudoexfoliation on the pupillary margin and hard mature cataract.

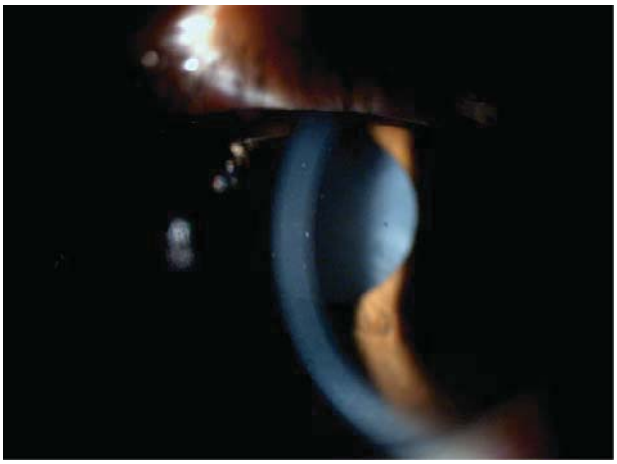

Fig. 3 – A 80-year-old man with pseudoexfoliation on the corneal endothelium.

lens. Other diagnostic features included endothelial pigmentation, loss of pupillary ruff, moth-eaten iris transillumination, Sampaoelesi line, and pigment deposition in the trabecular meshwork. Type of cataract was classified as nuclear, cortical, subcapsular, mixed-nonmature, mature, and hypermature based on slit-lamp biomicroscopy. Gonioscopy was performed only in those patients who had previously established glaucoma or patients who were suspected to have glaucoma based on raised IOP and suspicious optic nerve head findings. The diagnosis of glaucoma was made if the patient had a history of glaucoma surgery or was using glaucoma medication, when IOP was >21 mmHg with glaucomatous optic disc cupping, and visual field defects. Optic nerveheads were considered glaucomatous on biomicroscopical examination with the Volk (90D) lens in the presence of focal or generalized narrowing or disappearance of the neuroretinal rim with an enlarged amount of cupping or pallor. Ocular hypertension (OHT) was defined as IOP > 21 mmHg without optic nervehead changes or visual field defects. Study protocol was in adherence to the tenets of the Declaration of Helsinki.

χ^2 test was used to compare the qualitative variables and Student's *t*-test to compare the means of the continuous quantitative variables. *p*-values under 0.05 were taken to indicate a statistical significance.

Results

The mean age of the patients (318 or 47.2% male, 356 or 52.8% female) was 74.6 ± 8.9 years (range 50–92 years). The mean age of women was 74.7 ± 7.9 years and of men was 74.6 ± 9.8 years ($p > 0.05$). The overall prevalence of PEX syndrome was found to be 17.5% (118 patients). No statistically significant difference in PEX prevalence was found between men (45.8%) and women (54.2%), but a statistically significant difference was observed between the age groups ($p \leq 0.05$). The prevalence of PEX syndrome was found to increase with age, from 7.3% in the 7th decade of life to 27% in patients older than 80 years ($p < 0.001$). The most common cataract type in all the groups was nuclear 36.2%, followed by mixed and mature in 23.1% of the patients, posterior subcapsular in 11.3%, cortical in 5.9%, and hypermature in 0.3% of the patients. In the PEX patients the most comon cataract type was mature 40.7% (Figure 2) mixed 30.5%, nuclear 25.4%, cortical 1.7% and hypermature also 1.7% (Table 1). There was a high statistical diference between the type of cataract in the PEX and non-PEX patients ($p < 0.001$). The mean age of PEX patients (79.7 ± 6.1 years) was significantly higher when compared with all the subjects without PEX (73,5 ± 9,1 years) ($p = 0.000$). PEX was unilateral in 35.4% of the subjects and bilateral in 74.6%. The mean age of the bilateral PEX patients (81.5 ± 10.3 years) was significantly higler when compared with the unilateral PEX patients (74.2 ± 7.5 years). No subjects within 50–59 years age group had PEX at all, whereas 89.9% of the subjects with PEX were older than 70 (Table 2). The most common type of glaucoma was PEXG (6.5%), primary open angle glaucoma (POAG) (5.0%), angle closer glaucoma (2.4%), normal tension glaucoma (1.2%) and ocular hypertension (0.6%). Among the PEX patients 44 of 118 (37.2%) had glaucoma. In eyes scheduled for cataract surgery, IOP

Table 1

The estimated predominant type of cataract in patients with and without pseudoexfoliation syndrome (PEX) scheduled for cataract surgery

Type of cataract	PEX +*	PEX-**	Total	*p* value
Nuclear	30 (25.4)	214 (38.5)	244 (36.2)	> 0.05
Posterior subcapsular	0 (0)	76 (13.7)	76 (11.3)	< 0.05
Cortical	2 (1.7)	38 (6.8)	40 (5.9)	> 0.05
Mixed	36 (30.5)	120 (21.6)	156 (23.1)	> 0.05
Mature	48 (40.7)	108 (19.4)	156 (23.1)	< 0.001
Hypermature	2 (100)	0 (0)	2 (0.3)	< 0.05

Note: The results are expressed as n (%); *Patients with PEX; **Patients without PEX.

Table 2

Prevalence of pseudoexfoliation syndrome (PEX) according to ages, sex, and mean intraocular preasure (IOP)

Parameters	PEX +*	PEX-**	Total	*p* value
Mean age (years)	79.7 ± 6.1	73.5 ± 9.1	76.6 ± 8.9	< 0.001
Mean IOP (mmHg)	17.8 ± 3.2	15.8 ± 2.8		< 0.001
Sex				
male	54 (45.8)	264 (47.5)	318 (47.2)	> 0.05
female	64 (54.2)	292 (52.5)	356 (52.8)	> 0.05
Age groups (years)				
50–59	0 (0)	56 (10.1)	56 (8.3)	
60–69	8 (6.8)	102 (18.3)	110 (16.3)	
70–79	48 (40.7)	248 (44.6)	256 (43.9)	< 0.001
80–89	58 (49.2)	150 (27.0)	208 (30.9)	
> 90	4 (3.4)	0 (0.0)	4 (0.6)	

Note: The results are expressed as means ± SD or n (%); *Patients with PEX; **Patients without PEX.

was higher in eyes with PEX (but without glaucoma) than in those without PEX (17.8 ± 3.2 and 15.8 ± 2.8 mmHg, respectively; $p = 0.001$) (Table 2). The patients with PEX glaucoma had higher IOP compared with POAG patients, but there was no statistical significance (PEX 24.8 and POAG 23.2 mmHg, respectively; $p = 0.340$). The operative eyes with and without PEX did differ in pupil diameter before (3.7 ± 0.5 and 3.8 ± 0.4 mm, respectively; $p = 0.001$), and also after mydriasis (6.4 ± 0,7 and 7.5 ± 0.5 mm, respectively; $p = 0.000$). There was no stistically significant diference in the prevalence of arterial hypertension between the patients with and without PEX ($p = 0.641$). There was a stistically significant difference in the prevalence of ischemic heart disease between the patients with and without PEX ($p < 0.001$). Furthermore, in the patients with diabetes mellitus the prevalence of PEX was statistically significantly lower compared with the patients without PEX ($p < 0.030$). The group of patients with cerebrovascular disease included the patients who had sustaind one or more transient ischemic attacks and the patients with the history of stroke. There was no statistically significant difference in the prevalence of cerebrovascular disease between the patients with and those without PEX ($p = 0.239$) (Table 3).

Ethiopia [19], 42% in Sweden [20] and 16.4% in Turkey [8, 24]. This variationes depend on the examiner or other factors such as patients selection, the ethnic composition of the population, the clinical criteria for diagnosis, and the thoroughness of examination.

In the present study PEX syndrome was found in 17.5% of patients and there was an increase in the prevalence of PEX according to the age group, from 7.3% in the 60–69 years group up to 27.9% in the 80–89 years age group. Our results are comparable with earlier studies which have also shown that PEX is strongly age-dependent disorder, which seldom occurs before the age of 50 [8, 9, 18]. In the current study, patients with PEX were significantly older (79.7 ± 6.1 years) than patients without PEX (73.5 ± 9.1 years) ($p \leq 0.001$). This is in agreement with the results of previous reports, which found that the mean age of cataract patients with PEX was higher than those without PEX [3, 8, 10, 21]. In addition, we did not find any patient under 60 years of age with PEX, and we found only four patients with PEX in the 60–69 age group. Previous studies regarding the difference between the frequency and clinical significance of PEX in men and women are not consistent. Although Hietanen et al. [21] reported that PEX is more frequent in women, recent studies

Table 3

Associated systemic diseases in patietns with psudoexfoliation syndrome (PEX)

Systemic diseases	PEX group	Non PEX group	*p* value
Ischemic heart disease*	68 (57.6)	192 (34.5)	< 0.001
Hypertension	88 (74.6)	398 (71.6)	> 0.05
Diabetes mellitus	12 (10.2)	126 (22.7)	< 0.05
Cerebrovascular diseases	20 (17.0)	52 (8.4)	> 0.05
Heart arrhythmias	24 (20.4)	64 (10.6)	> 0.05

Note: The results are expressed as n (%); *angine pectoris and acute myocardial infarct.

Discussion

To our knowledge this is the first report on PEX among Serbian patients scheduled for cataract surgery. The reported frequency of PEX syndrome among candidates for cataract surgery varies extensively in different geographic regions even from place to place in the same country: 0.3% in Poland, 3% in France, 9% in North American Indians, 16% in Russia, 18% in Norway [20] 25.2% in Finland [21], 28% in Greece [9, 22], 28.7% in Spain [23], 35.4% in Estonia [18], 39.3% in including a larger number of PEX patients have reported opposite results [22, 23, 25]. Morover, Sekeroglu et al. [8] found no difference according to gender. Similarly, our results despite a higher number of women with PEX (54.2%) compared with men (45.8%), did not show a statistically significant difference between this two groups.

The most common cataract type in our PEX patients was mature cataract (40.7%) and nuclear cataract in non-PEX group (31.8%). In contrast, previous studies reported nuclear cataract as the most common type in PEX patients.

For instance, in the study by Kaljurand et Puska [18] nuclear sclerosis was predominated in eyes with PEX compared to those without PEX (57.6% and 36.9%, respectively). Reasons for this discrepancy might be explained by higher mean age of PEX patients in our study (79.7 ± 6.1 years) compared with previous studies (63.7 ± 10.5 in Ethiopia, 74.3 ± 7.0 in Turky and 77.1 ± 9.3 in Poland). Furthermore, the mean age of patints and consistently the most common cataract type in our study could be influenced by waiting lists for cataract surgery wich are among one and two years. The poor economic situation in Serbia could also partly explain higher percentage of mature cataracts in our patients.

The association between PEX and glaucoma is well established. In the eyes with PEX but without glaucoma, the mean IOP was higher than in the eyes without PEX and glaucoma. The reported prevalence of glaucoma in the PEX eyes has been found to vary in different populations: 7% in the United States [26], 7.5% in India [25], 13% in Iran [20], 13.3% in Ethiopia [19], 27.8% in Estonia [18], 28.8% in Crete [22], 30% in Norway [20], 32.1% in Turkey [8], 39.5% in Greece [9], 30% in Finland [21] and 37.2 % in our study. Our results are consistent with these findings. Moreover, PEX is the most common identifiable cause of open-angle glaucoma in the world [27]. The prevalence of PEX among patients with glaucoma has been reported as 1.4% in the United States [26], 26.7% in India [25], 30.5% in Turkey [8], 50% in Finland [21], 54.5% in Estonia [18], 60% in Norway [27], 75% in Sweden [10]. Consistent with previous reports, our results showed that PEXG represented 41.5% of all cases of glaucoma. Furthermore, in the PEX patients without glaucoma, the mean IOP was higher than in the non-PEX group wich is in agrement with previous studies [8–10, 18, 21]. On the basis of these findings, it could be assumed that PEX represents one of major risk factors for glaucoma development.

PEX is a systemic disorder of the extracellular matrix and exfoliation material has been found to be deposited in many organs of the body, including the heart, liver, lung, kidneys, and meninges [2, 4]. To date there are numerous studies regarding the association of ocular PEX syndrome and different systemic diseases. However, the results of the previous studies are not consistent. As elastin is a major component of the extracellular matrix of arterioles, PEX might be associated with vascular diseases [28]. Preliminary reports have suggested an association between PEX and transient ischemic attacks, stroke, heart disease, and cerebrovascular disease. However, our study did not find a stistically significant diference in the prevalence of arterial hypertension, cerebrovascular diseases, cardiac arrhythmias between patients with and without PEX [8–13]. Nevertheless, the prevalence of ischemic heart disease was statistically significantly higher in patients with PEX than in those without PEX. This finding might be explained by the fact that both PEX and ischemic heart disease occur more commonly in older patients. Earlier studies concerning the relationship between diabetes mellitus and PEX have shown that the incidence of PEX was significantly lower in diabetic compared to non-diabetic patients of similar age [29]. Consistently with these findings, our results showed the prevalence of diabetes mellitus in our PEX group was significantly lower than in non-PEX group. Other studies did not find a statisticaly significant difference of diabetes mellitus prevalence in PEX and non-PEX patients [8,12,30]. A possible explanation for differences in the prevalence of PEX in diabetic patients can be explained with geographical differences and environmental contributing factors in causing PEX.

One of the limitations of our study was its retrospective design. Furthermore the correlation of PEX syndrome and systemic disease in this study was evaluated only on the basis of information collected from medical records. In addition, a relatively small number of patients with PEX precludes generalized conclusions on the basis of the obtained results. Thus, further prospective studies are needed to assess the optimal prevalence of PEX syndrome and its association with systemic diseases.

Conclusion

On the basis of our results we could assume that PEX syndrome is a common problem among Serbian patients with age-related cataract. Moreover, it was shown that PEX syndrome is significantly associated with hypermature and mature cataract, reduced pupil dilatation, higher mean IOP, IOP of > 21 mmHg, glaucoma, and ischemic heart disease. Thus, in order to prevent potential ocular and systemic complications, patients who are candidates for cataract surgery should be thoroughly screened for PEX. However, many questions about PEX syndrome still remain obscure. Therefore, the true prevalence of PEX among the general population in Serbia, and its association with cataract, glaucoma and systemic dieases should be further studied through population-based surveys.

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Factors associated with positive outcome of avulsion injuries in children

Faktori koji utiču na pozitivan ishod avulzija zuba kod dece

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Abstract

Introduction/Aim. Avulsions are severe dental injuries with high impact on patients' quality of life due to prolonged treatment and possible severe complications. The aim of the study was to analyze the epidemiological factors related to the occurrence, treatment and outcome of avulsions in Serbian children. **Methods.** This research included 2,194 patients aged 1–18 years with traumatic dental injuries. The history, demographic, clinical and radiographic data of the patients were observed from dental medical records. **Results.** A total of 266 avulsions were observed in 207 patients. The frequency of avulsions was 12% in primary dentition and 5% in permanent dentition. A statistically significant relationship between place, cause of avulsion and outcome was observed. Replantation of permanent teeth was performed in 46.7% with a mean time 6.9 h. Almost a half of permanently avulsed teeth (48.7%) were not retrieved from the accident site and 11.3% of replanted teeth were transported in adequate media. The observed incidence of complications was 31.9%. **Conclusions.** Replantation was not performed in more than a half of all eligible teeth due to either loss or inadequate/delayed transport, which emphasizes the need for preventive strategies and health education in population.

Key words:
tooth avulsion; child preschool; child; adolescent; risk factors; treatment outcome.

Apstrakt

Uvod/Cilj. Avulzije spadaju među najteže povrede zuba kod dece i utiču na njihov psihosocijalni razvoj zbog dugotrajne terapije i mogućih teških komplikacija. Cilj istraživanja bio je analiza epidemioloških faktora povezanih sa nastankom, terapijom i ishodom avulzija kod dece u Srbiji. **Metode.** Ovo istraživanje obuhvatilo je 2 194 ispitanika uzrasta 1–18 godina sa povredama zuba. Podaci su dobijeni iz kartona povreda i stomatoloških kartona, a obuhvatali su: anamnestičke podatke, demografske podatke, podatke iz kliničkog i radiološkog pregleda. **Rezultati.** Zabeleženo je ukupno 266 avulzija kod 207 ispitanika. Učestalost avulzija u mlečnoj denticiji iznosila je 12%, a u stalnoj 5%. Uočena je statistički značajna povezanost između mesta, uzroka povrede i ishoda avulzije. Replantacija stalnih zuba je izvršena kod 46,7% ispitanika sa prosečnim vremenom 6,9 h. Skoro polovina svih avulziranih stalnih zuba (48,7%) nije donešena sa mesta povrede, dok je svega 11,3% replantiranih zuba donešeno u adekvatnom medijumu. Učestalost komplikacija iznosila je 31,9%. **Zaključak.** Replantacija nije vršena kod više od polovine raspoloživih avulziranih zuba zbog neadekvatnog transporta ili zakasnelog dolaska kod stomatologa, što ukazuje na hitnu potrebu za izradom preventivnih strategija i zdravstveno-vaspitnim radom unutar populacije.

Ključne reči:
zub, avulzija; deca, predškolska; deca; adolescenti; faktori rizika; lečenje, ishod.

Introduction

Avulsions are severe dental injuries with a complete traumatic displacement of injured tooth from its socket. Impact leads to injury of pulp tissue in apical region, periodontal injury and accompanied injury of surrounding tissues such as supporting bone and cement [1]. Contamination during injury, extra-alveolar time, environment during transport, and initial treatment procedures influence outcome and frequency of posttraumatic complications after replantation [2, 3].

The frequency of avulsions is estimated up to 13% of all primary dentition injuries [4] and up to 3% in the permanent dentition [1]. The choice of treatment procedures depends on the dentition and maturity of the affected teeth and factors

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associated with injury. Long term clinical and radiographic follow-up, demanding treatment with uncertain outcome and possible severe complications including early tooth loss have a high impact on the quality of life of patients' [5].

Available epidemiological data regarding avulsion injuries in children of Eastern Europe are scarce and noncomprehensive. Most of the research was done in specific subpopulations such as schoolchildren, localized geographical sites or within limited age groups.

The aim of this multicenter study was to analyze the epidemiological factors determining the frequency, outcomes and associated factors regarding avulsions of primary and permanent teeth of children treated in the University Dental Clinics in Serbia.

Methods

The study was performed in all the four University Dental Clinics in Serbia. The study group consisted of children aged 1–18 years (n = 2,194) who presented due to dental trauma in the University Dental Clinics in Serbia during the period from January 1, 2003 to June 1, 2010. Retrospective data were collected through dental charts and dental medical records and included: demographics, data regarding etiology of dental trauma (mechanisms, causes and places of injuries), data from clinical examination (injured teeth according to Universal numbering system, number of injured teeth, type of injury, accompanied soft tissue injury and/or facial bone fracture, data concerning first dental treatment, follow-up and complications) and radiographic data (periapical radiographs).

The type of trauma was determined using the Andreasen classification [1].

Etiology of dental trauma was categorized according to current literature data [1, 6, 7]. Mechanisms of dental injuries were classified into: falling, collision and other. Causes of dental trauma were classified as: accident, violence, sports, traffic, play and other. Places of dental trauma were categorized as: home, school/kindergarten, outdoor and other.

According to age and dentition patients were categorized into four groups: the group I (1–3 years), the group II (4–6 years), the group III (7–12 years) and the group IV or adolescents (13–19 years) [8].

Treatment outcome was classified according to the International Association of Dental Traumatology (IADT) values as favorable outcome (absence of symptoms, pulp vitality and continuing in root development) and complications (pain, swelling, abnormal mobility, abnormal percussion sound/pain, radiographic and clinical signs of apical periodontitis, infection related root resorption, arrested root development or tooth extraction due to trauma).

The differences in the means of variables between the groups were tested using both parametric and non-parametric tests depending on the distribution of the variables. Probability values of less than 0.05 were considered to be significant, and values are expressed as frequencies or means ± SD unless otherwise stated. SPSS version 10.1 (SPSS, Chicago, IL) was used for analysis.

Results

A total of 2,194 patients with 4,030 injured teeth (3,077 in permanent, 953 in primary dentition) presented at the University Dental Clinics due to various dental injuries.

Avulsions occurred in 207 (62.3% of boys and 37.7% of girls) patients. The age of subjects at the time of avulsion ranged from 1 to 18 years (mean = 8.6 ± 4.5 years). Mean ages in male and female patients were 8.8 and 8.4 years, respectively. Nearly half of all the patients with avulsions were aged 7 to 12 years (47.9%; n = 99).

A total number of avulsed teeth was 266 (150 permanent teeth, 116 primary teeth). The observed frequency of avulsions was 12% of all dental injuries in primary dentition and 5% in permanent dentition.

The most frequent mechanisms of avulsions were falling (71.9%), and collision (26.1%). A statistically highly significant relationship ($\chi^2 = 10.8$; $p < 0.01$) between the age groups and mechanisms of injury was observed. Collisions were frequent in adolescents (50%) and less prevalent in small children (12.1%) in whom 87.9% of all avulsions occurred as a consequence of falling. Unfavorable outcome was the most frequent in avulsed permanent teeth as a result of unknown mechanism (66.7%), although a statistically significant difference could not be proven.

Tooth avulsion as a result of accidental injury was observed in 41.5% patients and 23.6% during play time. Sport injuries were recorded in 18.4%. Avulsions as a result of violence and traffic accidents were rare, 8% and 6%, respectively. As shown in Table 1, there was a highly significant relationship between the age groups and causes of injuries ($\chi^2 = 65.6$; $p < 0.001$). The most frequent cause of injury in preschoolers was accident. However, in adolescents and teenagers sport injuries and trauma due to violence were most common. Although rare, avulsions as a result of traffic accidence most frequently had unfavorable outcome (92.9%). There was a significant relationship between avulsion outcome and causes of injury in permanent dentition ($\chi^2 = 12.268$; $p < 0.05$).

Table 1

Causes of avulsions in regard to age groups

Age groups (years)	Cause of injury (%)					
	Accidental	Sport	Violence	Traffic	Play	Other
1–3	81.8	–	3.0	–	15.2	–
4–6	48.4	3.2	–	–	48.4	–
7–12	36.4	21.2	3.0	7.1	28.3	4.0
13–18	18.2	36.4	27.3	11.4	2.3	4.5
Total	41.5	18.4	7.7	5.8	23.7	2.9

Regarding the place of injury (Table 2), avulsions most frequently occurred outdoor (56.5%) in all the age groups. A statistically highly significant relationship ($\chi^2 = 45.9$; $p < 0.001$) between the age groups and place of injury was observed, with injuries at home being most frequent in small children (75.8%) and less prevalent in adolescents (9%) in whom 75.0% of all avulsions occurred outdoor. A statistically highly significant relationship between place of injury and avulsion outcome was observed in permanent dentition ($\chi^2 = 22.5$; $p < 0.001$), with unfavorable outcome being most frequent after avulsions at unknown places (100%) and outdoor (69.5%).

Table 2

Place of avulsions in regard to the age groups

Age groups (years)	Place of injury (%)			
	Home	School/Kindergarten	Outdoor	Other
1–3	75.8	3.0	21.2	–
4–6	45.2	6.5	48.4	–
7–12	16.2	18.2	62.6	3.0
13–18	9.1	15.9	75.0	–
Total	28.5	13.5	56.5	1.4

Only one tooth was avulsed in 77.8% of patients, 16.4% of patients had two teeth avulsed, 5% had three teeth avulsed and the remaining 1% had four avulsed teeth. A statistically significant relationship between the number of injured teeth *per* patient and avulsion outcome was observed ($p < 0.05$), with unfavorable outcome being more frequent when traumatic event included more injured teeth besides the one with avulsion (63.0%). The upper incisors were the most frequently avulsed teeth both in primary and permanent dentition (85.9%).

In 70% of the patients avulsions were accompanied by the injury of the surrounding oral and/or facial tissues, while seven of these patients also had fractures of alveolar bone or tooth socket. The most frequent accompanied injury was laceration and contusion of oral mucosa which was observed in 25.6% of patients with avulsions. Concomitant crown fracture was observed in 3% of avulsed teeth.

Regarding presentation and mode of transport of 150 avulsed permanent teeth (Figure 1), adequate media (saline, saliva or cold milk) was used for transport of 11.3% of affected teeth. Inadequate transport media, such as a dry handkerchief or a pocket, was observed in 16.0% of the presented avulsed permanent teeth and 48.7% were lost at the accident site or patients did not try to find avulsed tooth due to lack of knowledge. In 24.0% of avulsed permanent teeth precise transport media could not be determined. Favorable outcome was more frequent in adequately transported teeth that were replanted (70.7%) than in inadequately transported replanted teeth (66.7%), although a statistically significant difference could not be proven.

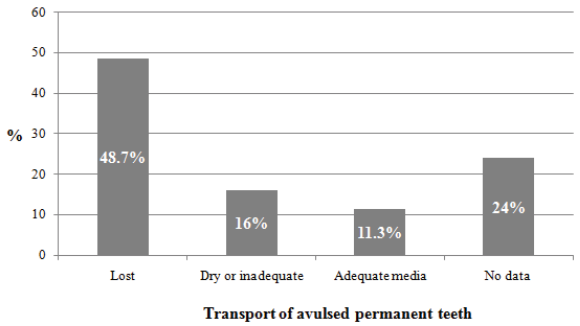

Fig. 1 – Distribution of avulsed permenent teeth according to transport.

Of 150 avulsed permanent teeth in total, 46.7% were replanted. Only 24.6% of replanted teeth were transported in adequate media. The mean time to replantation was 6.9 ± 10.9 hours (ranging from 15 minutes to 48 hours). Only 5.7% of all replanted teeth were presented in emergency dental office within 20 minutes after the injury, while two thirds (67.1%) had extra-alveolar time of more than one hour (Figure 2). No statistically significant difference was found between the time to replantation and outcome.

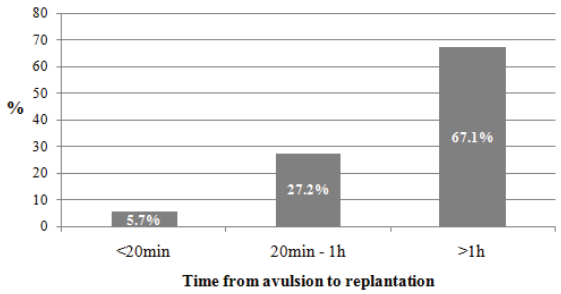

Fig 2. – Distribution of replantiations according to the time from avulsion

A fibre-composite splint was used for immobilization in all 70 replanted teeth. Endodontic treatment including pulp extirpation and filling of the root canal within two weeks after replantation was performed in 92.9% of replanted teeth. Although 36 of all replanted teeth were immature, pulp extirpation and filling of the root canal was not performed only in 8.3% of these. Revascularization with continuity in root development was observed in two of them. The other one showed complication two months after replantation which presented with discoloration, negative pulp sensibility test, percussion tenderness and apical radioluscency, therefore endodontic therapy was necessary. During the follow-up period, no further complications were observed in any of these three teeth. In immature replanted teeth with preliminary endodontic treatment complications were observed in 13.9%. However, two of these teeth were extracted due to occurrence of severe complications.

During the follow-up period, complications were observed in 31.9% of all replanted permanent teeth. Half of all observed complications occurred during the first year after replantation and no complications were observed after five years. The mean time until occurrence of complication was 1.5 years (533 ± 516 days). The most common complication observed in both groups was external inflammatory root resorption (45.5%). Endodontic retreatment was possible in 45.5% replanted teeth with complication. Tooth extraction was observed in 54.5% replanted teeth after occurrence of complication.

Discussion

The results of the present study indicate that avulsions are rare injuries (5% in permanent teeth and 12% in primary teeth). The proposed explanation for avulsions being more prevalent in primary dentition is the fact that the structures of supporting tissues are more elastic in younger children [9, 10]. It has been reported that avulsions in permanent dentition are most common at the age of 7–14 which was also observed in our study [11, 12].

The results of this multicenter study showed that boys were in twice higher risk of severe injuries such as avulsions than girls which could be explained by differences in gender psycho-social characteristics during childhood and adolescence: boys are more prone to risk behavior, play rough games and participate in contact, so-called man sports, which could be explained by higher level of catecholamine in boys [10, 13–15].

The most common mechanism of avulsions observed in present study was falling which is in accordance with known epidemiological data [16, 17]. It was found that most of the injuries occurred accidentally, during leisure time at home which is in accordance with the results of the study conducted by Wood and Freer [10]. The results of the present study also show differences in cause or place of injury according to patients' age, which emphasizes need for age specific targeted prevention strategies [18]. Interestingly, Petrović et al. [19] found that most of avulsions were a consequence of traffic accidents. In our study, avulsions due to violence and traffic accidents were very rare, which correlates with other epidemiological data from literature [20]. This contrast in results might be explained by differences in methodology: in our study, category traffic accidents did not include falls from bicycle, skate or roller shoes. The real cause of injury was estimated through the underlying event, because it was concluded that children usually ride bicycle during leisure and play time. The results of the present study show that unfavorable outcome of avulsion most frequently occur as a result of injury of unknown origin (place and cause). Epidemiological data from the literature suggest that victims of violence tend to give vague and imprecise history about event. Recent epidemiological data show that the incidence of facial and intraoral signs in physical abuse is high, therefore clinicians who treat paediatric dental injuries should be aware of violence as possible underlying cause of injury [1, 21, 22].

It has been observed that avulsions, both in permanent and primary teeth most commonly affect maxillary incisors. The proposed explanation is the position of upper incisors and preventive effect of maxilla during occlusion [20]. In our study avulsions were most frequently single tooth injuries, with two thirds of patients with concomitant facial trauma, which is in concordance with known literature data [23]. Martin et al. [24] observed a significant relationship between the number of injured teeth, severity of injury and late presentation for after-hours treatment. The results of our study statistically significantly show more frequent unfavorable outcome when traumatic event include more injured teeth besides the one with avulsion.

According to current guidelines, replantation is not recommended as treatment of avulsed primary teeth due to potential damage to permanent successors [25]. However, in permanent dentition prompt replantation is considered to be the treatment of choice for avulsions. Even delayed replantation, especially in immature teeth may be considered as a treatment option (although with poor prognosis), because this treatment allows maintenance of alveolar ridge contour space [26–28]. In this study, replantation was carried out in only 69 of 151 affected permanent teeth. The authors from Beijing observed higher replantation rate – 85/120 [20], and low replantation rates were observed in studies by Petrović et al. [19] – 32/62, Tzigkounakis et al. [29] – 27/90 and Kinoshita et al. [30] – 10/32. Since almost half of all avulsed teeth in our study were not even brought to the emergency dental office, it is reasonable to conclude that the lack of knowledge regarding immediate management of avulsions in people present at the site of accident was the main reason for the low observed replantation rate. Since usually these people are parents, teachers and coaches, further efforts are necessary in order to educate public about emergency procedures in case of avulsion injuries.

Timely replantation of the avulsed teeth and extra-alveolar environment are the most important factors influencing healing and favorable outcome. According to current literature data, the time period of dry storage between injury and replantation should not exceed 20 minutes [31]. Barrett and Kenny [32] analyzed the influence of extended extra-alveolar period of more than five minutes on the outcome of replantation. In this study only four of 69 replanted teeth had the extra-alveolar period of less than 20 minutes. Similarly, Diaz et al. [33] reported that only 3% of affected patients in their study were treated within 30 minutes and the most common form of storage was dry. Batstone et al. [34] showed that only 5% of their patients received emergency treatment within three hours, including patients with immediate treatment needs. Similar results in the present study confirm the necessity of developing educational and prevention strategies.

Surprisingly, our data show no statistically significant association between the time of replantation and the outcome. This may in part be explained by the fact that only 26.1% of replanted teeth transported in adequate media, but mainly because 94.2% of all replanted teeth were treated with prophylactic endodontic treatment, disregarding teeth maturity. The main reason for such high rate of endodontic treatment was the fact that most of replanted teeth in our study were transported in inadequate storage medium and/or with extended extra-alveolar time.

The results of our study show that 26.1% of the replanted permanent teeth were placed in adequate storage media during transport, which is higher percentage than in results showed by Petrovic et al. [19] (6.25%). This result might be explained by multicentre character and larger sample size of the present study which included four University Dental Centers in different regions of Serbia. Recommended storage media for avulsed teeth transport are saline, milk or saliva [20]. Tooth rescue box (tissue culture medium) is considered as physiologic medium for transport of avulsed tooth, but is rarely available at the site of accident [1, 28]. Saline, milk and Ringer are considered wet but non-physiologic storage media [3]. Although recent literature data show that saliva might be appropriate for short term storage, and it is the most available, important disadvantages are the presence of oral flora, salivary enzymes and accidental swallowing of the tooth [35].

Current guidelines recommend the use of wire-composite splint in immobilization of replanted teeth [1, 26]. In that way, physiologic functional tooth movements are allowed during the splinting period. Interestingly, the results of our study show exclusive use of fiber-composite splints probably due to the fact that they are better tolerated by patients.

Endodontic treatment within the two weeks after replantation was performed in 65 of 69 replanted permanent teeth in the present study. Thirty two of 35 immature replanted teeth were treated with prophylactic endodontic treatment. Literature data show that timely and appropriate endodontic treatment after replantation prevents occurrence of severe complications such as inflammatory root resorption [36]. However, data from current dental trauma literature show that long term prognosis of endodontic treated immature teeth might be seriously threatened due to a possible occurrence of cervical fractures [1]. The influence of dental treatment on the outcome of injury in replanted teeth could not be determined in this study due to a small number of avulsed and replanated teeth not treated with prophylactic endodontic treatment.

The occurrence of complications after replantation of avulsed teeth reported in recent studies is up to 84.4% [19, 37]. In the present study recorded occurrence of complication was lower (20.3%) with inflammatory root resorption being the most common type of complication (12%). A low rate of observed complications found in our study might be explained by the fact that most of the replanted teeth were treated with prophylactic endodontic treatment within the two weeks after replantation and the observed survival rate correlates with reported rates in treatment of mature permanent teeth [1].

Conclusion

The results of this, the first multicenter study regarding avulsion injuries in Serbian children show that severe injuries with unfavorable outcome most frequently occur outdoor or after injuries of unknown origin (place, cause). Most of patients receive delayed treatment or not the best possible treatment due to late presentation or presentation without the avulsed tooth. This finding emphasizes the need for further efforts in health education of public about necessary actions when tooth injury occurs. Besides preventive measures, continuous education of dental professionals is necessary in order to update their knowledge about emergency management of avulsion injuries. Further longitudinal studies in the region are necessary to acquire precise information about factors related to tooth avulsions, treatment and outcome.

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Scoring system development for prediction of extravesical bladder cancer

Razvoj bodovnog sistema u predviđanju ekstravezikalnog karcinoma mokraćne bešike

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Abstract

Background/Aim. Staging of bladder cancer is crucial for optimal management of the disease. However, clinical staging is not perfectly accurate. The aim of this study was to derive a simple scoring system in prediction of pathological advanced muscle-invasive bladder cancer (MIBC). **Methods.** Logistic regression and bootstrap methods were used to create an integer score for estimating the risk in prediction of pathological advanced MIBC using precystectomy clinicopathological data: demographic, initial transurethral resection (TUR) [grade, stage, multiplicity of tumors, lymphovascular invasion (LVI)], hydronephrosis, abdominal and pelvic CT radiography (size of the tumor, tumor base width), and pathological stage after radical cystectomy (RC). Advanced MIBC in surgical specimen was defined as pT3-4 tumor. Receiving operating characteristic (ROC) curve quantified the area under curve (AUC) as predictive accuracy. Clinical usefulness was assessed by using decision curve analysis. **Results.** This single-center retrospective study included 233 adult patients with BC undergoing RC at the Military Medical Academy, Belgrade. Organ confined disease was observed in 101 (43.3%) patients, and 132 (56.7%) had advanced MIBC. In multivariable analysis, 3 risk factors most strongly associated with advanced MIBC: grade of initial TUR [odds ratio (OR) = 4.7], LVI (OR = 2), and hydronephrosis (OR = 3.9). The resultant total possible score ranged from 0 to 15, with the cut-off value of > 8 points, the AUC was 0.795, showing good discriminatory ability. The model showed excellent calibration. Decision curve analysis showed a net benefit across all threshold probabilities and clinical usefulness of the model. **Conclusion.** We developed a unique scoring system which could assist in predicting advanced MIBC in patients before RC. The scoring system showed good performance characteristics and introducing of such a tool into daily clinical decision-making may lead to more appropriate integration of perioperative chemotherapy. Clinical value of this model needs to be further assessed in external validation cohorts.

Key words:
urinary bladder neoplasms; prognosis; factor analysis, statistical; transurethral resection of prostate; neoplasm staging; hydronephrosis.

Apstrakt

Uvod/Cilj. Stadiranje raka mokraćne bešike je od ključne važnosti u optimalnom lečenju bolesti. Kliničko stadiranje, međutim, nije dovoljno pouzdano. Cilj rada bio je da se izvede jednostavan bodovni sistem u predviđanju patološki uznapredovalog, mišićnoinvazivnog raka mokraćne bešike (MIBC). **Metode.** Logistička regresija i samodopunjujuća metoda korišćena je za izradu celobrojnog skora procenjenog rizika predviđanja patološki uznapredovalog MIBC uz pomoć kliničkopatoloških podataka pre učinjene cistektomije: demografskih karakteristika, inicijalne transuretalne resekcije (TUR) tumora mokraćne bešike [gradus, stadijum, brojnost tumora, limfovaskularna invazija (LVI)], prisustva hidronefroze, abdominalne i pelvične kompjuterizovane tomografije (veličina tumora, veličina baze tumora) i patološkog stadiranja nakon učinjene radikalne cistektomije (RC). Uznapredovali MIBC u hirurškom uzorku definisan je nalazom pT3-4 tumora. Prediktivna tačnost je procenjena površinom ispod *receiving operating characteristic* (ROC) krive. Klinička korisnost je procenjena analizom krive odlučivanja. **Rezultati.** Ova jednocentrična retrospektivna studija uključila je 233 odrasla bolesnika sa BC kod kojih je učinjena RC na Vojnomedicinskoj akademiji u Beogradu. Oboljenje ograničeno na organ utvrđeno je kod 101 (43,3%) bolesnika, dok je 132 (56,7%) imalo uznapredovalo oboljenje. U multivarijatnoj analizi tri faktora rizika bila su tesno povezana sa uznapredovalom bolešću: gradus

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inicijalne TUR [(*odds ratio* (OR) = 4,7)], LVI (OR = 2) i hidronefroza (OR = 3,9). Rezultujući ukupan bodovni skor kretao se od 0 do 15 poena sa kritičnom vrednošću iznad 8 poena, a AUC 0.795, ukazujući na dobru diskriminacionu sposobnost. Model je pokazao odličnu kalibraciju. Analiza krive odlučivanja pokazala je neto korist duž svih pragova verovatnoće i kliničku korisnost modela. **Zaključak.** Sastavili smo jedinstven bodovni sistem koji bi mogao pomoći u predviđanju uznapredovalog MIBC kod bolesnika pre učinjene radikalne cistektomije. Bodovni sistem je pokazao dobre performanse. Primena ovakvog sredstva u svakodnevnom kliničkom odlučivanju mogla bi dovesti do adekvatnije integracije preoperativne hemioterapije. Kliničku vrednost ovog modela treba dalje proceniti eksternom validacijom.

Ključne reči:
mokraćna bešika, neoplazme; prognoza; statistička analiza faktora; resekcija prostate, transuretralna; neoplazme, određivanje stadijuma; hidronefroza.

Introduction

Bladder cancer (BC) is the most common urologic cancer in men, the eighth most common malignancy in women and the fifth most common malignancy worldwide. Although new bladder tumors are frequently superficial (60–75%) in nature, many of those (up to 20%) can progress to advanced disease. On the other hand, an essential number of advanced tumors are diagnosed at initial presentation with no prior history of transitional cell carcinoma (TCC).

Staging of BC is crucial for optimal management of the disease. Radical cystectomy (RC) has been established as the primary treatment for localized or regionally advanced invasive bladder tumors, as well as high-risk superficial tumors resistant to intravesical therapy. The oncological outcome after radical surgery highly depends on the extent of the disease: the 5-year survival rate was in the range of 60–81% in pT2 tumor, 17–47% in pT3-4 tumor, and 22–35% in pN+ tumor [1]. A similar situation is found in choosing appropriate cases for extensive pelvic lymph node dissection (PLND). Because of understaging, these patients did not receive neoadjuvant chemotherapy (NACT) that is associated with a potential benefit for this group of patients. Available data shows an absolute survival benefit from NACT of ≈ 5% for patients undergoing RC. However, only a small number of patients with stage III BC actually receive NACT [2]. Furthermore, predicting extravesical disease also aids in patient selection for bladder-preserving approach [1].

Clinical staging based on physical examination, transurethral resection (TUR) pathology and imaging are the most important factors for predicting pathological stage, but unfortunately, predictions are not perfectly accurate. Despite technological improvements, imaging studies are still inaccurate, both in staging of primary tumor as well as in nodal staging [2]. Consequently, clinical prediction has evolved from physician judgment alone to risk group stratification, to prediction models based on multivariate regression or principal component analysis, to nomograms and a decision tree model [1, 3–8].

Several recent studies have demonstrated that multivariate models are more accurate than most informative single predictors such as any TUR staging variable in isolation, clinical staging alone or than techniques of risk group assignment [7]. For better identification of advanced muscle-invasive BC (MIBC), Karakiewicz et al. [4] had developed two nomograms to predict pT3-4 and pN+ disease. Their models, however, failed to retain favorable discrimination ability in a European series [9]. Furthermore, the risk of pT3-4 tumor and lymph node involvement was underestimated in external dataset [9, 10]. At last, pre-surgical models that can accurately predict which patients are likely to have more extensive disease are sparse.

Based on these considerations, the aim of this study was to examine whether a multivariate model expressed in scoring system could generate more accurate stage predictions. To test this, we developed a prognostic model and scoring system to accurately predict advanced pathologic T stage at cystectomy.

Methods

After obtaining institutional review board approval, we retrospectively reviewed medical records of 248 patients who had undergone radical surgery for BC at the Military Medical Academy, Belgrade, Serbia, over the 11-year study period (from January 2002 through December 2012). For each patient, comprehensive clinical and pathologic information was collected as precystectomy assessment. The patients underwent routine cystoscopic and upper tract evaluation, physical examination, TUR of bladder tumor (TURBT), abdominal and pelvic computed tomography (CT) and chest radiography. Evaluation for the presence of hydronephrosis, if any, was performed in all the patients, as previously described [11]. TUR stage was assigned by the operative surgeon according to the 2002 tumor nodes, metastasis (TNM) system. Lymphovascular invasion (LVI) in TURBT or biopsy specimen was defined as the unequivocal presence of tumor cells within the endothelium-lined space, with no underlying muscular walls [12]. The indications for RC were tumor invasion into the *muscularis propria* or prostatic stroma or Ta, T1, or carcinoma *in situ* refractory to TUR with intravesical chemotherapy and/or immunotherapy. No patient received radiotherapy or chemotherapy before RC. The patients with non-urothelial BC, or salvage RC after failed radiotherapy or neoadjuvant chemotherapy, or incomplete data were excluded. No patient had distant metastatic disease at the time of cystectomy. All the patients underwent RC, pelvic lymphadenectomy and urinary diversion [13]. All surgical specimens were processed according to standard pathological procedures and histopathological slides were reviewed by genitourinary pathologists according to the 1973 World Health Organization grading and 2002 American Joint Committee on Cancer TNM staging.

Outcome measures

The presence of advanced MIBC in surgical specimens was the primary interest of statistical analysis. It was defined as pT3-4 tumor with/without lymph node metastases after pathological review.

Predictor variables

The following predictor variables were chosen *a priori* for the defined outcome: demographic data (age, sex), TURBT findings (grade, stage, multiplicity of tumors, LVI), hydronephrosis, abdominal and pelvic CT radiography (tumor size, tumor base width), and pathological stage after RC.

Statistical analyses

Univariate analysis was initially carried out to search for the variables that were statistically significantly associated with potential risk factors for advanced MIBC. Variables that showed statistically significant relationship ($p < 0.05$) were incorporated in the multivariate model. Multiple logistic regression analysis was applied (with Backward–Wald stepwise) to adjust for possible confounders and to identify and quantify the independent extravesical disease predictors. The regression results were expressed in odds ratios (ORs) with 95% confidence interval (CIs). The stability of the model's effect estimates and check for overfitting examined by using the bootstrap method, as previously described [14]. Briefly, we generated 1,000 samples using bootstrapping methods, and then the medians of the resultant beta coefficients for each variable were used for developing an integer based weighted point system for advanced MIBC. The coefficient for each variable was multiplied by 10 and then the result was rounded off to the nearest integer. Each patient-discharge record was assigned the individual scores by summing the individual risk factor points. The best discriminating power was identified by determining the cut-off points for predicting advanced MIBC as the score giving the best Youden index (sensitivity + specificity - 1) for each scoring system. Eventually, the scoring system was applied to test the rule. Prognostic model validation (calibration) was performed by comparing the observed and predicted event rates for groups of patients. Receiver operating characteristic (ROC) curves were used to quantify discrimination, measures that distinguish between patients who experience the event of interest and those who do not [15]. We determined the sensitivity, specificity, overall correctness of prediction, and positive and negative predictive values for scoring systems. Clinical usefulness was assessed by using decision curve analyses [16]. These analyses estimate a ''net benefit'' for prediction models by summing the benefits (true positives) and subtracting the harms (false positives). The latter are weighted by a factor related to the relative harm of a missed advanced MIBC cancer versus an overrated tumor. Assumption is made that identification of advanced MIBC would lead to treatment with NACT. Net benefit is plotted against threshold probabilities compared with "NACT for all" strategy and "NACT for none". The interpretation of a decision curve is that the model with the highest net benefit at a particular threshold probability should be chosen. All analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL) and R-statistics (the R foundation for Statistical Computing, version 2.3.1) and the statistical significance was set at $p < 0.05$.

Results

This retrospective cohort study design examined clinical and pathological descriptive variables of 233 evaluable patients with BC undergoing RC. The mean patient age was 63.8 ± 9.2 years (range 42–86 years). The population comprised 28 (12%) women and, 205 (88%) men. There were 120 (51.5%) patients with primary RC and 113 (48.5%) with secondary RC. Of all the patients, 109 (46.8%) presented with no, 78 (33.5%) unilateral and 46 (19.7%) bilateral obstruction (hydronephrosis). Preoperative size of dominant tumors was median 4.0 cm, interquartile range (IQR) 3.0 cm (range 1–12 cm). In 33 (14.2%) patients there was one, in 37 (15.9%) two, and in 163 (70%) more than two tumors. Tumor base width on CT was median 3.0 cm, IQR 2.0 cm, (range 1–10.0 cm). TUR was performed in 194 (83.3%) patients, median 2 months, IQR 1 month (range 1–12 months) before RC. A total of 197 (84.5%) patients had TUR grade 3 cancers, and 36 (15.5%) TUR grade 2 cancers. At TUR, 12 (5.2%) had Ta or Tis pathologic stage of disease, 46 (19.7%) had T1 stage, and 175 (75.1%) had T2 stage. LVI in TURBT or biopsy specimens was noted in 162 (69.5%) patients.

Pathological staging of the entire cohort was distributed as follows: 1 (0.4%) patient had residual carcinoma *in situ* (CIS), 12 (5.2%) had T1, 88 (37.8%) had T2, 79 (33.9%) had T3, and 53 (22.7%) had T4 disease. Overall, patients were categorized into organ confined (OC) disease (pathological stage < T3: n = 101, 43.3%) versus pathologically advanced MIBC [stage ≥ T3; n =132 (56.7%)].

The clinicopathological characteristics of the patient cohorts (OC or advanced MIBC) are shown in Table 1. Of note, there were no differences in age, gender, primary or secondary RC, number of tumors between those OC *versus* those who were not.

In univariate analysis, 6 risk factors displayed a significant correlation with advanced MIBC (Table 2). During multivariate analysis that included these 6 parameters as covariates, three sustained their prognostic significance (Table 2). The analysis demonstrated the initial tumor grade, LVI and hydronephrosis had strong prognostic value of advanced MIBC. All variables maintained significance in the bootstrap model; thus, the model was considered to be reliable and not over-fit. The Hosmer and Lemeshow goodness of fit test statistic was $p = 0.285$, thereby demonstrating good fit. The Brier score for a model was 0.1762. The Nagelkerke's R^2 value which indicates the percentage of variation of the outcome explained by the predictors in the model was 0.3767. A coefficient of reliability (Cronbach's alpha) was 0.6619 that was considered acceptable.

Next, a total score was calculated by summing the points from each variable for each patient. The resultant total

Table 1

Baseline patients (n = 233) clinicopathological characteristics in organ confined and muscle-invasive bladder cancer (MIBC)

Characteristics	Organ confined disease	Advanced MIBC	*p*
Age (years), $\bar{x} \pm SD$	63 ± 9.4	64.4 ± 8.9	0.262
Gender: female/male, n (%)	15/86 (14.9)	13/119 (9.8)	0.310
Primary/secondary, n (%)	51/50 (50.5)	62/70 (47)	0.600
Size of tumors (median), cm	3.3	5.2	0.000*
Number of tumors: 1/ 2/ ≥ 3, n (%)	11/18/72 (10.9/17.8/71.3)	22/19/91 (16.7/14.4/68.9)	0.403
Initial tumor grade: 2 or 3, n (%)	30/71 (29.7)	6/126 (4.5)	0.000*
Initial tumor stage: Ta/T1/T2, n (%)	6/30/65 (5.9/29.7/64.4)	6/16/110 (4.5/12.1/83.3)	0.030*
Lymphovascular invasion no/yes, n (%)	47/54 (46.5)	24/108 (18.2)	0.000*
Hydronephrosis: no/unilateral/bilateral, n (%)	70/30/1 (69.3/29.7/1)	39/48/45 (29.5/36.4/34.1)	0.000*
Base width on CT (cm)	2; 2.75	3; 2	0.000*

*** Statistically significant difference ($p < 0.05$); CT – computed tomography.**

Table 2

Analysis of possible and independent predictors for advanced muscle-invasive bladder cancer (MIBC) and point values

Risk factors	Univariate analysis		Multivariate analysis		Bootstrap		Point value
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	B	B	
Size of tumors	1.361 (1.181–1.650)	0.000					
Initial tumor grade	8.872 (3.523–22.339)	0.000	4.697 (1.74–12.682)	0.002	1.547	0.099	2
Initial tumor stage	1.932 (1.194–3.126)	0.007					
Lymphovascular invasion	3.917 (2.170–7.068)	0.000	2.026 (1.026–4.004)	0.042	0.706	0.154	3
Hydronephrosis	4.593 (2.908–7.253)	0.000	3.867 (2.284–6.274)	0.000	1.353	0.254	5
Base width on CT	1.510 (1.260–1.809)	0.000					

CT – computed tomography; OR – odds ratio; CI – confidence interval

possible score ranged from 0 to 15, with a cut-off value of > 8 points. The areas under the ROC curve for the model was 0.818, (95% CI 0.764–0.871), showing the model to have good discriminatory ability. In internal validation, after adjusting for overfitting the scoring system achieved a bootstrap-corrected area under curve (AUC) of 0.795 (95% CI 0.739–0.851) (Figure 1), and the discrimination ability was only slightly decreased (0.023), indicating a successfully built robust model. The sensitivity was 68.9% (95% CI 60–76.7%), the specificity was 72.3% (95% CI 62.5–80.7%), the positive predictive value was 76.5%, whereas the negative predictive value was 64%. In other words, a score of less than or equal to 8 correctly identified OC disease in 73 of 101 patients (72.3%), whereas a score of more than 8 correctly identified advanced MIBC in 91 of 132 patients (68.9%). Graphical assessments of score calibration are presented in Figure 2. The scoring system was well calibrated ($R^2 = 0.825$).

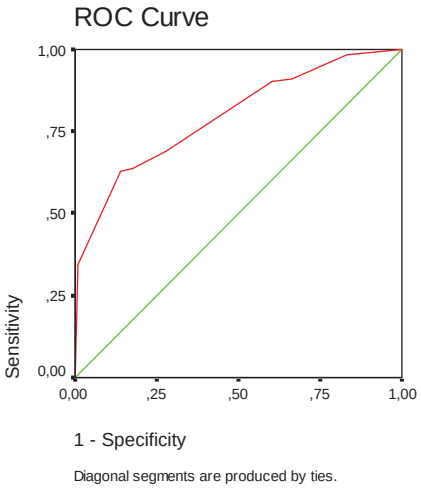

Fig. 1 – Receiver operating characteristic (ROC) curve analysis scoring system for predicted advanced muscle-invasive bladder cancer.

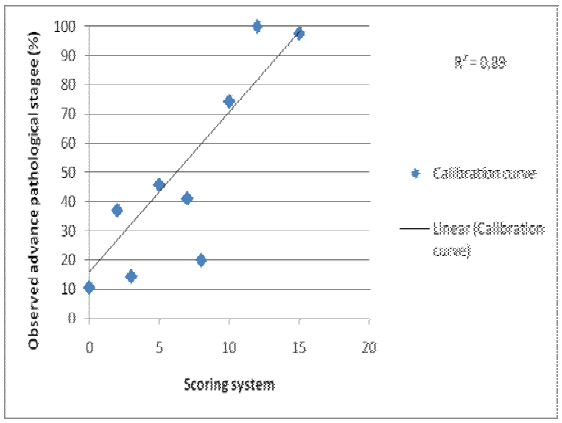

Fig. 2 – Observed *versus* predicted advanced muscle-invasive bladder cancer by score.

In the decision curve analysis (Figure 3), the model predicting advanced MIBC provided a net benefit throughout the entire range of threshold probabilities as compared with the strategy of treating all patients with NACT, or alternatively, treating no one. The graph shows that the final model leads to the highest net benefit (dotted black line) compared to the models including only tumor grade (dotted red line) or only LVI (dotted green line).

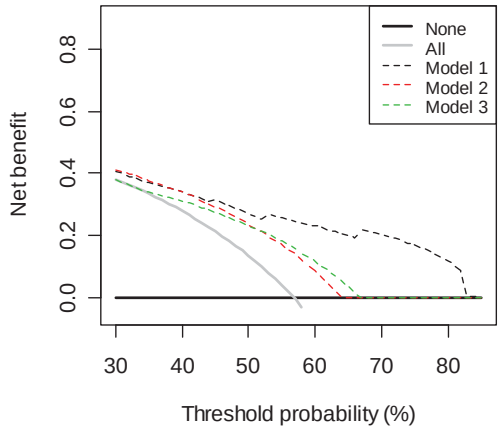

Fig. 3 – Decision curve analysis of the effect of prediction models on detection of advanced muscle-invasive bladder cancer (MIBC). Net benefit is plotted against various threshold probabilities. The model 1 is the final model including initial tumor grade, lymphovascular invasion and hydronephrosis (dotted black line). The model 2 is a model including only tumor grade (dotted red line). The model 3 is a model including only lymphovascular invasion (dotted green line).

Discussion

The most significant prognostic factor in patients undergoing RC for MIBC is the pathologic stage. It has been reported that the rate of clinical understaging is as high as 50% [7, 10]. Consequently, the need to improve pathological stage prediction is of great importance.

In the present study, exceptional approach was used to applying a scoring system, a mathematic tool, without the need for statistical software for interpretation/prediction to achieve an improvement in pathologic stage prediction before RC in the individual patient. By combining known clinicopathological prognostic factors, our model was able to achieve an accuracy of 79.5%. Various measures of model fit (discrimination, calibration) showed a good predictive ability and clinical usefulness in the internal validation.

To date, several clinicopathological factors have been reportedly associated with post-surgical pathological stage and were included in the existing models such as TUR parameters of stage and grade [1–4, 7, 8], LVI [1–3, 6], hydronephrosis [3, 6–8], age [4, 6, 8], female gender [2, 4], CIS [4], histological variants [2], tumor size [7], tumor growth pattern [8], multiplicity of tumors [6, 8], palpable mass [6], number of intravesical treatments [6], NACT [4], primary versus secondary RC [17], oncofetal markers [7]. We found that LVI at TURBT to be strongly associated with advanced MIBC, that in accordance with previous studies, that have determined LVI to be a strong independent predictor of upstaging, poor clinical outcome [18], nodal invasion and survival in patients undergoing RC [19]. Although LVI was less commonly found in TUR samples than in RC specimens, the pathological feature is strongly suggestive of advanced MIBC [20] and pathologists should be encouraged to report LVI in TURBT pathological reports as it has a direct impact on patients staging and prognosis.

Similar to report by Karakiewicz et al. [4], variables of TUR parameters (stage and grade) have reached statistical significance in univariate or multivariate analysis in our model. However, Shariat et al. [21] reported only 35.7% agreement between TUR stage and surgical stage in patients with BC. They reported pathological upstaging in 42% and pathological downstaging in 22% of their patients. It is known that different quality of TUR reportedly leads to variation in clinical staging from 5% to 70% and may adversely affect the adequacy of biopsy specimens and the reproducibility of the current staging models [6]. In addition, different interpretations of histological findings on TUR specimens among pathologists may have an impact on the accuracy of TUR-related variables. Moreover, in this study initial TURBC was not performed in all of our patients and the above noted may explain why this variable did not demonstrate an independent effect in our study. There is a strong correlation between tumor grade and stage, and most poorly differentiated tumors being muscle and deeply invasive at pathological stage. These tumors have not only a risk of invasion, but also a significant risk of recurrence, progression and cancer-specific mortality rates both noninvasive and invasive BC [22]. It is not surprising that the histologic grades of urothelial carcinoma of the bladder in our model are a crucial prognosticator, and have the independent prognostic significance in prediction of advanced MIBC.

Karakiewicz et al. [4] included TUR parameters of stage and grade, the presence of carcinoma *in situ*, patient age and sex, and treatment with neoadjuvant chemotherapy to predict both a pathologic stage of T3 and lymph node-positive disease in 726 cystectomy patients. Their model indicated the accuracy of 76% for patients with advanced T-classification. However, a recent validation study in European patients, demonstrated a notable decrease in model performance: the AUC was 67.5% for pT3-4 disease and 54.5% for pN+ disease [9]. Therefore, our model included additional clinical parameters known to predict pathologic outcome such as hydronephrosis. These findings support those of previous investigators such as Stimson et al. [23], who reported that preoperative hydronephrosis was independently associated with extravesical and node-positive disease at the time of cystectomy. Similar to another report [8], hydronephrosis was the first-tier discriminator in predicting extravesical disease. We found that abnormal imaging was a strong independent predictor and control for other predictors, those patients with hydronephrosis had nearly fourfold increases in the risk of advanced MIBC. The independent prognostic value of hydronephrosis was further confirmed in another series of cT2 disease as predictor of extravesical disease [24]. Incorporating

these factors in our scoring system resulted in the AUC of 0.79, which is statistically better than a model including only variables proposed by Karakiewicz et al. [4] and similar to other reports (0.79–0.85) [1, 3, 6, 7].

In most previous studies on pathologic stage prediction only patients with clinically OC MIBC [1, 3, 6–8] were analyzed, but they were only a subpopulation of patients with BC invading bladder muscles and candidate for RC. However, in our study a broader population was incorporated, and subsequently included patients diagnosed as having clinical T3 and T4 disease considering that clinical prediction is of limited accuracy, and that RC is standard treatment for T3, but also in some T4 disease [25]. Our results are in agreement with recently reported findings [17] that patients who undergo secondary RC (for recurrent/progressive disease after initial bladder sparing modalities) have more favorable pathology at the time of cystectomy and are understaged to a lesser degree than patients who receive a primary RC.

This study has several limitations worth noting. First, the enrolled patients were retrospectively collected in a single tertiary center with a relatively small patient cohort who may influence the results by the selection bias. Second, we examined extravesical disease extension which is a useful intermediate endpoint. However, more clinically significant endpoints are predicting disease outcome or response to therapy and it will be the focus of future studies. Additionally, the study did not include other possible risk factors for advanced disease, such as biomarkers, [7] bimanual palpations [26]. These data were not available in our cohort. In BC, although not yet part of routine clinical assessment, multiple biomarkers have been identified, including urine, immunohistochemical, and abnormal levels of serum oncofetal markers before cystectomy, that in combination with other known clinical prognostic factors could achieve enhanced preoperative prediction of pathologic staging (reported 85% accuracy in predicting extravesical BC) and were associated with adverse pathologic outcome, poor outcome and reduced survival [7]. On the other hand, lack of sufficient data on bimanual palpation could indicate that most current urologists are relying more and more on these pelvic imaging techniques during the clinical staging process and in accordance with the observed decrease in the number of bimanual palpation performed in the last decades. Furthermore, bimanual palpation is a subjective measure, and depends on both the experience of the surgeon and the physical constitution of the patient [26]. Our models are not applicable to patients who were pretreated with radiotherapy or to those harboring pathologies other than transitional cell carcinoma. In addition, we used a bootstrap method internal validation and did not use an external cohort to validate our scoring system. Nevertheless, the prediction model represents another step toward accurately estimating individualized risk of advanced MIBC in a patient population lacking optimal staging procedures.

Conclusion

Using a panel of clinicopathological features obtained before radical surgery, we developed a unique scoring system, simple user-friendly, to assist in predicting advanced muscle-invasive bladder cancer in patients before radical cystectomy. The newly devised formula has the accuracy of 79.5% and has been internally validated. Adoption of such a tool into daily clinical decision-making may lead to more appropriate integration of perioperative chemotherapy, thereby potentially improving survival in patients with bladder cancer. Further external validation in a large cohort is necessary. The clinical value of this model needs to be further assessed in external multi-institutional validation cohorts.

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Stress hyperglycemia in acute myocardial infarction

Stres hiperglikemija u akutnom infarktu miokarda

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Introduction

Hyperglycemia as a response to stress was firstly described by a French physiologist Claude Bernard in 1855 [1]. Since then, a number of studies have shown that stress hyperglycemia (SH) is important in many diseases, e.g. myocardial infarction, apoplexia, sepsis, trauma, and that it correlates with adverse outcome [2–7]. Increased glucose level during stress is evoked by integrated hormonal, cytokine and nervous counterregulatory signals on glucose metabolic pathways and, therefore, presented in the same time with hyperinsulinemia and insulin resistance [1–14]. Unlike the diagnostic criteria for diabetes mellitus (DM), there have been methodological problems with defining SH, and the consensus is clearly needed for the definition of SH in AMI [14]. A proposal is that authors should analyze their database in two ways: both by using quartiles and the best cut-off value of glycaemia for mortality in AMI patients [14].

Even more evidences have accumulated to underline the importance of stress hyperglycemia as a prognosticator in acute myocardial infarction

The Harmonizing Outcomes with Revascularisation and Stents in Acute Myocardial Infarction (HORIZON-AMI) trial, a large-scale prospective study of patients with ST-Elevation Myocardial Infarction (STEMI), treated with primary percutaneous coronary intervention (PCI), demonstrated the independent prognostic value of admission glucose levels on early and late mortality in both patients with and without known diabetes mellitus (DM) [15]. In the retrospective study of 4176 patients without known DM undergoing primary PCI for STEMI, Timmer et al. [16] recently demonstrated the association of elevated glucose level (on admission) and 1-year and long-term mortality and association with larger infarct size. Mladenovic et al. [17] had the similar results in nondiabetic patients with STEMI. Furthermore, in multivariate analysis, in patients without DM, who underwent PCI for the first AMI, SH has proved to be an independent predictor of myocardial salvage index [18] which, assessed by cardiovascular magnetic resonance (CMR), is an independent predictor of clinical outcome [19]. SH was shown to be a good indicator of increased risk for hospital death and predictor of poor outcome in patients with AMI and temporary electrical cardiac pacing, without previously diagnosed DM [20]. High glycaemia on admission predicted increased in-hospital and long-term mortality in patients with STEMI complicated with cardiogenic shock [21]. Importance of SH seem to last for a very long time – even for decades, as demonstrated in the study od Deckers et al. [22]. Namely, mortality was 64%, 71%, and 82% at 20 years in patients with normal, mild, and severe hyperglycemia, respectively. Deckers and coworkers analyzed a large number of patients (11,324),

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of whom 41% had elevated admission blood glucose (ABG) ≥ 7.8 mmol/L (140 mg/dl). The prevalence of hyperglycemia at admission increased by 22% from 1985 to almost 50% in 2008. Additionally, SH is more important than it used to be earlier, because it was a significantly stronger predictor of adverse 30-day outcome after MI in the last decade than 25 years ago. Moreover, among 1,185 consecutive MI patients studied, raised admission plasma glucose (APG) was associated with increased mortality, irrespective of the initial reperfusion strategy, although the relation was more pronounced in the preinvasive era (*p* value for heterogeneity of effects < 0.001) [23].

The presence of stress hyperglycemia association with almost all important clinical events in acute myocardial infarction

SH is related to AMI size, including a high Killip class, low left ventricular ejection fraction (LVEF), cardiogenic shock, requirement for initial cardiorespiratory resuscitation and increased concentrations of cardiac troponin, creatine kinase MB (CK–MB), pro-BNP and lactic acid [24, 25]. Maximum level of CK and CK-MB were significantly higher in patients with acute hyperglycemia [26].

In the group of young patients (18–45 years) with first attack of AMI, initial serum glucose level was the significant independent variable in the prediction of ventricular arrhythmia attack [27]. In addition, in the recent study of 1,258 patients with AMI, admission hyperglycemia (> 10 mmol/L, 180 mg/dL) was associated with a significantly higher prevalence of ventricular fibrillation (VF) and ventricular tachycardia (VT) in non-diabetic patients [28]. The possible mechanisms leading to VF are higher free fatty acid concentrations, as a consequence of hyperglycemia and insulin resistance, that induce arrhythmias by damaging cardiac-cell membranes and by causing calcium overload [28, 29].

Furthermore, SH was shown to be associated with increased prevalence of atrial fibrillation (AF) in AMI, irrespective of DM status, i.e. in both new onset and in previously diagnosed DM, as well as in patients with elevated fasting glucose [30]. The patients with both SH at admission (≥ 8.0 mmol/l, 144 mg/dL) and AF had almost 14.5 times higher in-hospital mortality than the patients who had neither SH nor AF [30]. Besides associations with VT/VF and AF, Dziewierz et al. [31]. demonstrated a connection of admission glycemia and second to third grade atriventricular (AV) block and pulmonary edema in patients with AMI [31]. In DM patients, this association is confirmed for VT/VF and second to third grade AV block, whereas in nondiabetic patients was confirmed for AF and pulmonary edema [31]. In the prospective study of 834 patients with STEMI, the association of SH on admission (> 140 mg/dL, 7.77 mmol/L) and a higher incidence of rhythm disturbances: malignant ventricular tachyarrhythmias including VT/VF, new AV block and bundle branch block was recently demonstrated [32].

Nakamura et al. [33] recently evaluated the association of glucose level and clinical variables during primary PCI in patients with STEMI. They demonstrated that corrected thrombolysis in myocardial infarction (TIMI) frame counts were significantly higher in patients with acute hyperglycemia and were independently associated with plasma glucose level. In AMI, hyperglycemia is a predictor of impaired coronary flow before reperfusion [34]. The presence of acute hyperglycemia was associated with the impairment of epicardial coronary flow after primary stent implantation. In patients with SH at the time of AMI and temporary electrical cardiac pacing larger myocardial necrosis (i.e. higher troponin level) was noted, as well as: more prevalent Killip class > 1, lower LVEF and systolic blood pressure (BP) on admission [27]. Additionally, SH is (in patients without DM) an independent predictor of the extent of myocardial salvage, which is in turn an independent predictor of outcome and the main mechanism by which patients with AMI benefit from reperfusion therapies [18]. Moreover, SH is a marker of left ventricular (LV) remodeling, which may help explain post-infarction transition to LV failure [35]. Additionally, SH correlates significantly with microalbuminuria, which is a sign of endothelial dycfunction [36].

Indeed, nothing in the organism is just black or white, paricularly in such a complex conditions as AMI. We shall not forget that an increased blood concentration is basically an adaptive mechanism for stress („fight or flight situation“). A recent paper reminds us that not all increases of glycemia in hospitalized patients are dangerous [37].

This is also underlined by the recent guidelines for AMI, suggesting less tight glycemia targets and avoiding hypoglycemia, which is very dangerous in this setting.

Recommendations from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism published to summarize accumulated knowledge, and to trace paths for further research

In 2008 the American Heart Association (AHA) statement on hyperglycemia in AMI, suggested definition of hyperglycemia – APG > 140 mg/dL (7.77 mmol/L) [38]. AHA statement recommended, until further data became available, approximation of normoglycemia to be a reasonable treatment goal [suggested range for plasma glucose 90–140 mg/dL (5.0–7.77 mmol/L)], as long as hypoglycemia is avoided [38].

Also, further evaluation (preferably before hospital discharge) was recommended for acute coronary syndrome (ACS) patients with hyperglycemia but without prior history of DM, in order to determine the severity of their metabolic derangement. This evaluation may include fasting glucose and glycated hemoglobin (HbA1C) assessment and, in some cases, postdischarge oral glucose tolerance test (OGTT) [38].

The role of stress hyperglycemia as a prognosticator in acute myocardial infection may be further improved by using more appropriate cut-offs

Despite the fact that association of hyperglycemia with poor outcome was repeatedly demonstrated in patients with AMI, there is a lack of consensus on how to achieve the op-

timal sensitivity and specificity of this prognosticator. A step toward improvement may be to use different cut-off values for SH in AMI patients with and without known (previously diagnosed) DM. It is a logical assumption, given the fact that patients with DM have already impaired glucoregulation. Moreover, patients with DM have a higher average glycemia in comparison with the other AMI patients [39, 40]. In the study of 500 AMI patients, the best Reciever operating characteristics (ROC) curve-derived cut-off value for admission serum glucose concentrations in patients without known DM was 8.55 mmol/L (153.9 mg/dL), with the sensitivity 79% and specificity 87% for mortality [41, 42]. This value corresponds to the cut-offs which have been used in many studies for AMI patients without DM [23, 30, 32, 43, 44]. The best cut-off value in AMI patients with known DM was 18.0 mmo/L (324 mg/dL), which is more than twice higher, and it achieved 64% sensitivity and 75% specificity for in-hospital mortality [41]. As shown in meta-analysis, in the studies with diabetic patients the cut-off was usually 10 mmol/L (180 mg/dL) [43].

However, the same cut-off value for SH in all AMI patients was used in most studies in the last decade. In some rare exceptions the cut-off value was different. In the paper from 1989 by Sewdarsen et al. [45], the cut-off value 11 mmol/L (198 mg/dL) was used for patients with DM, as opposed to 8 mmol/L (144 mg/dL) for patients without DM.

Results of basic investigations on how hyperglycemia worsens outcomes in patients with acute myocardial infarction

Hyperglycemia contributes to poor outcomes in patients with AMI by several mechanisms. Hyperglycemia has a number of immunomodulatory effects. It can lead to significant oxidative stress [46]. By the mechanisms of oxidative stress, hyperglycemia acutely increases cytokine concentrations (interleukin-1β, 6, 8 and 18, tumor necrosis factor-alpha) and exaggerates inflammation [47, 48]. This effect is more pronounced in patients with impaired glucose tolerance [49]. Glucose excursions can further promote inflammation by increasing leukocyte adhesion molecules, inducing nuclear factor kappa B (NF-k β) [50] and promoting the procoagulant state [51, 52]. Recent studies show that TNF-alpha-induced activation of the NF-k β pathway plays a critical role in cardiomyocyte apoptosis [53, 54]. Hyperglycemia-induced myocardial apoptosis is mediated, in part, by the activation of cytochrome c-activated caspase-3 pathway, which may be triggered by reactive oxygen species (ROS) derived from high levels of glucose [55]. Another study also demonstrated that intermittent high glucose concentration enhances apoptosis in human umbilical vein endothelial cells in culture and suggests that variability in glycemic control could be more deleterious to endothelial cells than a constant high concentration of glucose [56].

Moreover, hyperglycemia impairs the polymorphonuclear neutrophil function resulting in decreased intracellular bactericidal activity, opsonic activity and innate immunity [51, 52].

Patients with hyperglycemia have enhanced T-cell activation, both CD4+ and CD8+, as well as a large number of natural killer (NK) cells with known role in plaque instability [48].

Futher, due to insulin resistance patients with hyperglycemia are especially susceptible to thrombotic events by a concurrent insulin-driven impairment of fibrinolysis and a glucose-driven activation of coagulation [57].

Acute hyperglycemia-induced oxidative stress leads to the inactivation of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and consequently abnormal Ca^{2+} signaling and contractile dysfunction [58].

Another study demonstrated that hyperglycemia leads to endothelial dysfunction, increased plasma hyaluronan levels and coagulation activation and indicates a potential role for glycocalyx perturbation in mediating vascular dysfunction during hyperglycemia [59].

Futhermore, acute hyperglycemia abolishes ischemic preconditioning *in vivo* [60].

Putative pathophysiologic mechanisms of stress hyperglycemia effects on worsening the prognosis and the occurence of an ischemic event

SH has the unfavorable independent prognostic role in non-diabetic patients with STEMI, regardless of AMI severity, extension, and treatment [61]. It is still difficult to answer the crucial question for practice: Is SH in AMI a risk marker or a therapeutic target [62]?

Basically, there are 2 ways, relating SH to worse prognosis in AMI: a) SH is a marker of at least 3 major prognostic factors: advanced age, large actual necrosis in AMI (or hemodynamic instability due to superimposed new myocardial necrosis upon already exsisting myocardial damage), and increased catecholamine and sympatethic nervous system activity; b) SH is a mediator (active pathophysiologic factor) which contributes to poor outcome. Probably by both direct and indirect effects SH may cause additional harm in AMI [63]. There are evidences that acute hyperglycemia could be harmful by itself, leading to hemodynamic changes (increased heart rate and blood pressure (BP), important determinats of myocardial oxygen need), in addition to elevation of catecholamines [64, 65]. Moreover, Ishihara et al. [66] were able to demonstrate, using multivariable analysis, a significant correlation between higher glucose and impaired predischarge LVEF, even after adjustment of acute LVEF. This suggests that acute hyperglycemia is causally associated with further deterioration of LV function following reperfusion in AMI [66, 67].

Irrespective whether they reflect SH as a marker or active player in worsening prognosis in AMI, the following mechanisms (some of them overlap importantly) are currently believed to contribute : increased bood concentration of free fatty acids (resulting from a relative insulin deficiency), which produce toxic effects on cardiomyocytes, increase myocardial oxygen need, and depress myocardial contractility [68]; microvascular obstruction (due to plugging of leukocytes in the coronary capillaries and venules, giving raise to platelet-dependent thrombosis in the capillaries, etc.).

Microvascular obstruction was considered the reasonable explanation for the findings on contrast-enhanced cardiovascular magnetic resonance (CMR) [69]. SH is associated with a higher incidence of TIMI < 3 flow in the infarct-related artery after PCI [25] and even in patients with TIMI 3 flow after PCI patients with SH have higher final TIMI frame counts on angiography [70]; endothelial dysfunction [67]; no-reflow phenomenon", for which glycemia was the strongest independent predictor [67, 71]; decrease of collateral blood flow to the ischemic area (by adversely affecting nitric oxide availability) [67, 70]; electrophysiologic disturbances, resulting in arrhythmias [72, 73]; exaggeration of the inflammation by the oxidative mechanism [49, 71, 74]. For example, in-stent restenosis correlated with mean glycemia as well as with oxidative stress and inflammatory markers during the insulin infusion period and intensive glycemic control during PCI halved restenosis at 6 months [75], increased immune response [48, 73], increased apoptosis [61, 67], increase of interstitial fibrosis [61].

In addition to aforementioned mechanisms relating SH to worse prognosis in AMI, the following might help explaining the higher incidence of new ischemic event: prothrombotic state, generated by hyperglycemia [63, 67], which results in part from diminished plasma fibrinolytic activity and effect of tissue plasminogen activator [63]. Also, glycemia is an independent predictor of platelet dependent thrombosis [70]. Moreover, among diabetic patients, those with STEMI and glycemia > 8.5 mmol/L on admission had a poorer response to clopidogrel [76]. Additionally, improved glycemic control reduces platelet reactivity in DM patients after PCI [68]. From therapeutic point of view, it may be important that in ACS patients with hyperglycemia intensive glucose control results in a reduction of platelet reactivity only in the presence of elevated HbA1c levels [77]; decreased nitric oxide bioavailability [78]; possible increased risk for upper gastrointestinal bleeding [79] which may be due to stress ulcer, resulting from decreased gastric mucosal blood flow, increased gastric mucosal permeability with increased acid back-diffusion, and ischemia–reperfusion injury [80].

In line with the aforementioned, non ST elevation acute coronary syndromes (NSTE-ACS) patients with both diagnosed and undiagnosed DM had significantly higher risk for Global Utilization of Streptokinase and Tissue Plasminogen Activator (TPA) for Occluded (GUSTO) coronary arteries moderate or severe bleeding and need for in-hospital transfusion (as comapred to non-diabetics, despite similar age, serum creatinine levels, and rates of invasive procedures and antithrombotic therapy), suggesting that they may be more vulnerable to hemorrhage [81].

Stress hyperglycemia and the major adverse cardiac and cerebrovascular event following primary percutaneous coronary intervention (PPCI)

In PPCI-treated STEMI patients, SH is a marker of both subsequent mortality and more frequent major adverse cardiac and cerebrovascular events (MACE) in general [82–84]. SH was also associated with increased 30-day rates of reinfarction, acute renal injury, target vessel revascularization (TVR) and major bleeding in 3,405 patients in the HORIZONS-AMI trial [15]. In the German Acute Coronary Syndromes [ACOS] Registry, in 5,866 STEMI patients, SH (>150 *vs* <120 mg/dl), was significantly related to increased risk of MACCE (composite of death, reinfarction, stroke, or rehospitalization), adjusted OR 1.31, 95% CI 1.00 to 1.71, $p < 0.0001$ [25].

Tamita et al. [62] studied 275 AMI patients, with the median follow-up interval of 5.3 years. Patients with abnormal fasting glycemia and/or OGTT had a significantly higher ABG as well as more MACE defined as: cardiovascular death, stroke, non-fatal myocardial infarction or ACS, non-TVR either by coronary artery bypass grafting (CABG) or coronary angioplasty and congestive heart failure that required hospitalisation [62].

In a study on 2,482 consecutive STEMI patients, those with SH, but without DM, had the highest risk population for in-hospital mortality and MACE (composite end points including death, reinfarction, and TVR) [85].

In the study of Mather et al. [86] patients with high admission glycemia were significantly more likely to experience clinical MACE, defined as cardiovascular death, recurrent myocardial infarction, coronary revascularization or hospital admission for cardiovascular cause, at any time than normoglycemic patients, Hazard Ratio (HR) 3.82 (95% CI: 1.61, 9.06) [86].

Zhang et al. [84] studied 853 STEMI patients. In-hospital stent thrombosis was also more commonly seen in patients with SH.

In STEMI patients (out of whom 9.5% were treated using PPCI), those who presented glucose ≥ 140 mg/dL (7.7 mmol/L) had higher rates of malignant ventricular tachyarrhythmias, bundle branch block, new atrioventricular block and in-hospital mortality [87].

Among 4,793 STEMI patients (including 12% treated with PCI), MACE (all-cause mortality, cardiogenic shock, and reinfarction) were significantly more frequent in patients with higher admission glycemia [88].

In 6,358 AMI patients without diabetes, SH prior to coronary angiography predicted contrast-induced acute kidney injury (AKI), even after adjusting for confounding variables, most importantly impaired renal function at baseline [89, 90].

The incidence of cardiac failure, arrhythmia, cardiac death, reinfarction, post-infarction angina pectoris, and MACE was higher in 456 non-diabetics AMI patients who had SH (> 11.1 mmol/L *vs* < 7.8 mmol/L) [91].

Mrdovic et al. [92] incorporated SH in the RISK-PCI score. SH was defined as glycemia ≥ 6.6 mmol/L at admisssion. SH was „worthy" one point (out of 20 in total). Thirty-day MACE comprising death, nonfatal reinfarction and stroke was the primary end point. An 18-fold graded increase in the primary end point was observed between patients in a low risk class and those in a very high risk class [92].

MACE (reinfarction or heart failure or mortality) were more frequent at follow up of patients with SH (≥ 190 mg/dL, compared with those with admission glucose levels < 190 mg/L) in the study of Pei-Chi et al. [93].

Therapeutic approach to stress hyperglycemia

SH is a powerful predictor in-hospital morbidity and mortality in AMI, both in diabetic and non-diabetic patients [94, 95]. A 1 mmol/L increase in glycemia above the normal range correlates with a 4% raise in mortality for non-DM patients and 5% for known DM patients [96]. Despite the importance of the problem in general and in everyday practice, we have no firm, evidence-based knowledge whether intensive treatment to lower hyperglycemia in AMI will improve prognosis [96]. The choice of hypoglycemic drugs, treatment tresholds and targets are the subject of a long-standing debate, and opinions have been sometimes diametral, which may translate into substantial differences in results.

Studies on tight glycemia control by insulin

The first evidence toward intensive glycemic control in intensive care unit came from the proof-of-concept Leuven (Belgium) studies in surgical, medical and the pediatric intensive care unit (ICU), assessed causality. All the 3 trials found that insulin usage to target strict normoglycemia 4.44–6.11 mmol/L (80–110 mg/dL) had lad to improved outcome compared with tolerating hyperglycemia to 12 mmol/L (215 mg/dL), which is the renal threshold for glycosuria. Targeting blood glucose around 8 mmol/L (145 mg/dL) seems preferable [97–101]. Such findings were not confirmed by other well-conducted randomised controlled trials (RCTs) in intensive care ICU patients [95].

Normoglycaemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation (NICE-SUGAR), was the largest such international RCT (n = 6,104 of either medical or surgical ICU patients). It demonstrated that tight glycaemic control was associated with higher incidence of severe hypoglycaemia and increased 90-day mortality (24.9% *vs* 27.5% in the control group, OR: 1.14; 95% CI: 1.02 to 1.08; $p = 0.02$; the number needed to harm = 38). Excess deaths were mainly cardiovascular. An intermediate blood glucose target 7.77–10 mmol/L (140–180 mg/dL) was safer than targeting normoglycemia [95, 101 102].

Glucontrol (the Comparing the Effects of Two Glucose Control Regimens by Insulin in Intensive Care Unit Patients) RCT included 1,101 patients from medical/surgical ICUs. It was stopped earlier than planed because the incidence of hypoglycemia (9.8%) was too high and the target glycemic control was not reached [101].

Thus, recent studies in ICUs have not shown improved outcomes in patients allocated to tight blood glucose control, but rather an excess of adverse events related to more frequent hypoglycaemic episodes.

Although there are important similarities between ICU and coronary ICU patients, it is questionable to what extent results could be extrapolated from medical ICU to AMI pateints. The first such, relatively large trial on AMI patients was Diabetes, Insulin Glucose Infusion in Acute Myocardial Infarction (DIGAMI) trial (n = 620 patients). It demonstrated mortality benefit at one year (18.6% *vs* 26.1%), obtained by tight glucose control through *iv* insulin [67, 103]. The subsequent DIGAMI-2 trial (n = 1,253) showed no mortality benefit of a long-term insulin therapy in patients with both AMI and type 2 DM. Morbidity also did not differ among the groups [94, 104]. The oposite results of the two major trials concerning this topic might be the consequence of suboptimal quality of studies [96].

The Hyperglycemia: Intensive Insulin Infusion in Infarction (HI-5) study on 240 AMI patients did not find a reduction in mortality among patients who received insulin /dextrose infusion therapy, but did find a lower incidence of heart failure (12.7 *vs* 22.8%, $p = 0.04$) and reinfarction within 3 months (2.4 *vs* 6.1%, $p = 0.05$) [105].

Causal relation between high glycemia and high morbidity and mortality in AMI is not definitively confirmed, and hyperglycemia might be an epiphenomenon. Conclusive, large trials seem to be very expensive, precluding their conduction in conterporary economic environment – in the sufficent size to provide reliable answers [95]. Thus, recent trials of insulin treatment in AMI patients failed to demonstrate desired reduction in mortality, but showed unwanted, raised incidence of severe hypoglycemia [69]. Recent meta-regression anlysis of the studies from 1965–2011 compared a tight glycemia control strategy (by insulin in most patients) with a less intensive regimen. Total number of patients was 2,113 and mortality was not different between the groups [106].

As most studies of this topic were not optimaly conducted [107] differences in numerous morbidities between strict glycemic control and conventional treatment were not reported in sufficient details (usually only a couple of them), or not significantly different or not felt important in subsequent meta-analyses. This is presumably due to the absence of definitive consensus about criteria for treshold, targets and means to treat hyperglycemia in ACS. For example, as far as morbidity is concerned, in a DIGAMI study, groups did not differ regarding reinfarction, ventricular fibrillation, high degree atrioventricular conduction disturbances or congestive heart failure [108]. Likewise, the combined total event rate (death, stroke, or reinfarction) did not differ significantly among the 3 groups in DIGAMI 2 [104].

Studies using glucose–insulin–potassium infusions

Glucose-insulin-potassium (GIK) infusions were found in AMI to be of no value and even potentially harmful [94, 103, 109, 110]. GIK therapy has not induced any improvement in outcome, although various GIK formulations, treatment duration, routes of administration, etc. were tested [110–112].

Glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction (CREATE-ECLA), is the largest scale international study, which randomized 20,201 patients to 24 h GIK or usual care. The CREATE-ECLA showed that high-dose GIK infusion had a neutral effect on mortality, cardiac arrest, and cardio-

genic shock in STEMI patients [67]. Thus, GIK infusions are not recommended in current clinical guidelines [110]. Timing may be important. For example, the moderate benefit was demonstrated with out-of-hospital GIK administration in comparison with placebo: rates of the composite outcome of cardiac arrest or in-hospital mortality were lower with GIK. Regretably, there was no improvement in 30-day survival [113].

Importance of hypoglycemia, including iatrogenic one

An association of increased mortality and morbidity with hypoglycemia also has been demonstrated [40, 103, 114].

In a recent meta-regression analysis (which involved 2,113 patients), Chatterjee et al. [106] found in the tight glucose control group significantly higher rate of hypoglycemia. Even without achieving target glycemic control, relative risk (RR) was very high (13.40, 95% CI 3.69–48.61; $p < 0.01$), absolute risk increase was 12% and a number needed to harm was 9 (95% CI 6.8–9.8).

Intensive glycemic control also failed to improve CHF, arrhythmias and reinfarction rates. Meta-regression revealed that mortality with intensive glycemic control was worse with increased duration of therapy ($p = 0.001$, for trend). Therefore, benefit of tight glycemic control in AMI patients with type 2 DM is limited, but risk of serious hypoglycemia is significant [106]. Hypoglycemia relates to prolonged hospital, greater cost of hospitalization, and higher mortality both during hospitalization, and after discharge [115]. Several mechanisms may contribute. For example, hypoglycaemia may exacerbate myocardial ischaemia and may cause dysrhythmias [115, 116]. Hypoglycemic episodes provoke sympathetic nervous system activation and catecholamine surge, leading to arrhythmia, myocardial ischemia, and sudden death. Hypoglycemia can be particularly dangerous in pateints with cardiac autonomic neuropathy [117]. Hypoglycemia is related to prolongation of QT and reentrant arrhythmias, often quoted as crucial for the "dead in bed" syndrome [114]. Too rapid rate of glycemia reduction could be a factor in adverse CVD outcomes [117]. Hypoglycemia can provoke an increase in blood viscosity and coagulation, vasoconstriction by increased secretion of endothelin, platelet activation and aggregation, increased release of inflammatory mediators and cytokines. Hypoglycemia promotes free fatty acid metabolism and reduces glycolysis, with increased cardiac oxygen consumption and with a possible direct toxic effect on cardiomyocytes [118]. Spontaneous hypoglycemia, may be even more dangerous than iatrogenic hypoglycemia [119]. Even in stable CAD patients, under elective procedure, hypoglycemia had an almost three-fold higher risk of MACE (including in-stent restenosis and TVR) at 3 years [118]. Clinical significance of asymptomatic hypoglycemia has not been sufficiently elucidated. A possible difference in spontaneous *vs* drug-induced hypoglycemia also needs to be additionally evaluated [88].

A word of caution is needed considering methodology. Many point-of-care (POC) systems do not account for the patient's hematocrit or degree of oxygenation, both of which may produce errors in glycemia measurement. Thus, both in anemic and in hypoxic patients, falsely high glycemia readings may occur [120].

Recently, a new, promissing therapeutic approach for hyperglycemia was proposed, namely, glucagon-like peptide infusion, which exerts insulinotropic and insulinomimetic actions, with a low risk for hypoglycemia [73, 121, 122].

Glycemic threshold for therapy

Sufficient evidence is missing to strongly recommend any specific treatment to manage hyperglycemia in an ACS patient other than trying to keep glycaemia within reasonable levels (usually defined by consensus) [110]. A well-designed RCT in ACS is obviously needed to determine glucose treatment thresholds and targets [102]. On the basis of the balance of current evidence, it is prudent to treat hyperglycemia > 180 mg/dL (10 mmol/L), to change the recommendation for the use of insulin to control glycemia in NSTE-ACS from a more stringent to a more moderate target range, and to avoid hypoglycemia [102]. Similar approach is suggested for ICU patients. Continuous insulin therapy should be started in the ICU, when ABG levels are ≥ 10.0 mmol/L (180 mg/dL) and in those with previous DM when preprandial glucose levels are ≥ 7.77 mmol/L (140 mg/dL) during follow-up [123]. Insulin therapy is the treatment of choice for hyperglycemia in ICUs, initiating continuous intravenous infusion when ABG is > 10.0 mmol/L (180 mg/dL) [110]. American Diabetes Association's Standards of Medical Care in Diabetes recommended in 2010 initiating insulin therapy in critically ill patients with blood glucose > 10 mmol/L (> 180 mg/dl) and to target a blood glucose range of 7.8–10.0 mmol/L (140–180mg/dl) [96, 124].

Targets for hyperglycemia therapy

Guidelines for ACS recommend nowdays less strict glycemia control than a few years earlier. Until more data become available the treatment target should be to avoid severe hyperglycemia [glucose concentration > 10–11 mmol/L (> 180–200 mg/dL)] as well as hypoglycemia [< 5 mmol/L (< 90 mg/dL)] [103].

A strategy of "strict, but not too strict" glucose control in STEMI seems to be a practical approach. In the acute phase, it is reasonable to maintain a blood glucose concentration ≤ 11.0 mmol/L (≤ 198 mg/dL), but absolutely avoid hypoglycemia [94, 107]. It is reasonable to use an insulin-based regimen for hospitalized patients with UA/NSTEMI to achieve and maintain glucose levels < 10 mmol/L (< 180 mg/dL), while avoiding hypoglycemia [102]. The recommended blood glucose target is 7.7–10 mmol/L (140–180 mg/dL) for most patients [123].

More recent guidelines recommend a more lax control of glycemia in critically ill patients: between 8–10 mmol/L (144–180 mg/dL) [125].

The aim of glycemic control in the acute phase should be a glucose level < 11.0 mmol/L (< 198 mg/dL), while avoiding fall of glycaemia < 5 mmol/ L (< 90 mg/dL).

Treatment of hyperglycemia in an ICU with a strategy of "strict, but not too strict": glycemic target is 7.77–10 mmol/L (140–180 mg/dL) for most patients, rather than a more stringent target of 6.11–7.77 mmol/L (110–140 mg/dL) [110].

Insulin infusion as the recommended way to treat hyperglycemia in AMI/ACS

Current guidelines suggest a dose-adjusted insulin infusion with monitoring of glycemia in some patients [94]. In the first instance, we should consider a dose-adjusted insulin infusion with regular monitoring of blood glucose levels [107]. Continuous insulin infusion is the currently recommended first-line therapy for patients with AMI and acute hyperglycemia, but it takes time to achieve optimal glucose levels by the time of reperfusion [93]. Glycemia should be monitored every hour until the target range is reached, and then every 2 h. Following the acute period (usually the initial 24 h), continuous therapy is stopped and subcutaneous insulin (usually long-acting analogs) started [110].

For patients with type 2 DM and ACS, insulin is not required beyond the first 24 h – unless clinically required for the management of their DM. Immediate intensive blood glucose control should be provided to patients with AMI and DM or marked hyperglycaemia (> 11.0 mmol/L). This should last for at least 24 h [126].

The aforementioned is in line with advices for other hospitalized pateints. The 2011 American College of Physicians guideline suggests a target blood glucose level of 7.8 to 11.1 mmol/L (140 to 200 mg/dL) if insulin therapy is used in surgical ICU/medical ICU patients. However, firm evidence is missing whether such target-driven glucose control in AMI has meaningful clinical benefits [127].

Dose-adjusted infusions of insulin for 24 h have been recomended for hyperglycemia treatment in all recent AMI/ACS guidelines. Precise suggestions are missing in these and few other related contemporary guidelines [94, 102, 103, 107, 127, 128]. The best contemporary guideline addressing treatment of hyperglycemia in an ICU is writen by Jacobi et al. [129]. A valid insulin therapy includes usage of a reliable insulin infusion protocol, frequent blood glucose monitoring, avoidance of finger-stick glucose testing through the use of arterial /venous glucose samples, and dextrose replacement for hypoglycemia prevention and treatment [129].

Continuous insulin infusion (1 unit/mL) therapy should be initiated after priming new tubing with a 20-mL waste volume. Insulin may be mixed with 0.9% sodium chloride, lactated Ringer's injection, Ringer's injection, or 5% dextrose. Insulin may be prepared in glass container. If insulin-induced hypoglycemia (< 3.89 mmol/L, 70 mg/dL) occurs, insulin infusion should be stopped and 10–20 g of hypertonic (50%) dextrose should be administered [129]. Glycemia should be measured in 15 min with further dextrose administration as needed to achieve glycemia > 70 mg/dL (3.89 mmol/L), with a goal to avoid iatrogenic hyperglycemia [129].

Insulin use in any patient with hyperglycemia is fraught with problems. Insulin is stil often administered incorrectly (e.g., the use of subcutaneous "sliding scales") [95].

Recommended approaches to detect diabetes mellitus and impaired glucose tolerance in acute myocardial infarctiion patients

DM is another characteristic associated with high risk for adverse outcomes after ACS [102]. For adequate treatment, it is important do detect DM and impaired glucose tolerance (IGT), which are prevalent in AMI. An estimated 20% of ACS patients are known to have DM, a further 25% have undiagnosed DM and 40% with IGT. Thus, up to 85% of ACS patients have some degree of dysglycemia at presentation, which persists in a significant proportion of patients at 3 months [96]. Therefore, it is reasonable to measure HbA1c and fasting blood glucose in all patients without known DM, who developed hyperglycemia during the acute phase. If equivocal, an oral glucose tolerance test (OGTT) may be needed after discharge. This should preferably be measured 4 days after the acute phase [94]. OGTT is suggested because either high ABG, fasting plasma glucose or HbA1c, in AMI patients without DM are not sensitive enough to uncover previously undiagnosed abnormal glucose tolerance or DM [130]. Likewise, ACS patients with HbA1c ≥ 6.5% on admission may be considered diabetic while, in those without known DM and HbA1c < 6.5%, OGTT should be performed 1–4 weeks after ACS [123]. One should offer all patients with hyperglycaemia after ACS, but without known DM, tests for HbA1c levels before discharge and fasting blood glucose levels no earlier than 4 days after the onset of ACS. These tests should not delay discharge. The Center for Clinical Practice at NICE (UK) does not recommend routinely OGTT to patients with hyperglycemia after ACS and without known DM, provided that HbA1c and fasting glycemia are within the normal range [107]. The European guidelines on DM, pre-DM, and cardiovascular diseases (CVD) recommend an OGTT in patients with established CVD [130, 131].

Goals for the next period

While there is a significant evidence that hyperglycemia is associated with increased mortality and morbidity in AMI, further studies are warranted to guide management in patients with AMI and acute hyperglycemia [67, 132].

Moreover, international consensus statement is needed about: which glucose concentration is the most useful (admission, fasting,...) as a prognosticator in AMI; which cut-offs values of the admission glycemia should be recommended for DM and non-DM pts; should HgbA1C and OGTT be used in routine glucose metabolism evaluation in AMI (and, if yes, when), having cost-effectiveness in mind; algorithm for the treatment of SH.

Conclusion

Rapidly accumulating evidence confirms the relation of stress hyperglycemia both with mortality in acute myocardial infarction patients and with major adverse outcome measures. Precise recommendations regarding the target glucose concentration in acute myocardial infarction have already

been published. New approaches (such as using different cut-off values for patients with and without known diabetes mellitus) may help optimizing utility of stress hyperglycemia in critical illnesses. However, some important questions remain to be answered in near future, as they are relevant to everyday clinical practice.

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CURRENT TOPIC

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Is there enough evidence for routine use of drug-eluting stents in acute myocardial infarction with ST segment elevation?

Da li ima dovoljno dokaza za rutinsko korišćenje stentova obloženih lekom u akutnom infarktu miokarda sa ST elevacijom?

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myocardial infarction; acute disease; drug-eluting stents; stents; thrombosis; treatment outcome.

Ključne reči:
infarkt miokarda; akutna bolest; stentovi, lekom obloženi; stentovi; tromboza; lečenje, ishod.

Introduction

Primary percutaneous coronary intervention (PPCI) is defined as angioplasty with or without stent implantation, with no prior or concomitant fibrinolytic therapy [1]. PPCI compared with medical therapy is a method of choice in the treatment of acute myocardial infarction with ST-segment elevation (STEMI), significantly reducing mortality and reischemia [1]. Implantation of bare metal stent (BMS) on the culprit lesion in STEMI is associated with a reduced incidence of target vessel revascularization (TVR), but is not associated with a reduced mortality and reinfarction compared to primary balloon dilatation of a culprit lesion [2]. Although some authors question the rate of restenosis of BMS in STEMI due to different patophysiological process, plaque rupture and formation of thrombus, restenosis in STEMI patients occurs in more than 20% of patients [2, 3]. The advantage of drug-eluting stents (DES) compared to BMS to prevent coronary restenosis in a variety of patients is proved in elective procedures [4, 5], while in the treatment of STEMI is still controversial, due to the lack of randomized studies with a duration of follow-up more than one year. Thus, at the moment there is a debate weather DES should be used in PPCI routinely.

STEMI is an independent stent thrombosis (ST) predictor both for BMS and DES especially when the complex lesions are treated (ostial and bifurcation lesions) [6–9]. This can be explained by prothrombotic state, hemodynamic changes (cardiogenic shock), stent apposition and insufficient expansion of the stent. Also, it is shown on autopsy that DES postpones endothelization of ruptured plaque, which is a primary cause of late ST, with persistent fibrin deposition compared to BMS [10]. Because of a longer duration of arterial healing of ruptured plaque (> 1 year) compared to stable plaque [8], safety of DES in STEMI patients cannot be determined in short term studies. Observational studies have indicated the existence of an increased risk in emerging late and very late (> 1 year) ST associated with the use of the first generation of DES [11], especially for indications that are different from those approved by the U.S. Food and Drug Administration (FDA) ("off-label" DES indications), which includes STEMI [12]. However, recent results from randomized trials, meta-analysis and registries, with short and intermediate duration of follow-up have demonstrated that the selection of the second generation of DES in STEMI is safe since there is no difference in mortality and reinfarction compared to the BMS group with a significant reduction in TVR.

Randomized studies using sirolimus-eluting stents

In a randomized prospective study, Single High Dose Bolus Tirofiban and Sirolimus Eluting Stent *vs* Abciximab and Bare Metal Stent in Myocardial Infarction (STRATEGY) [13], with follow-up of 8 months, the primary objectives (death, myocardial infarction, stroke and TVR) were significantly lower in the sirolimus-eluting stents (SES) group than in the BMS group (18% *vs* 32%, $p = 0.04$) primarily due to less TVR in the SES group compared to the BMS group (7% *vs* 20%, $p = 0.01$). The limitations of the study were: involvement of single center, small sample size (even though only 12% of consecutive patients were not enrolled in the study) and the same choice of stent and glycoprotein IIb/IIIa. To overcome the above limitations, the same group of authors presented the Multicentre Evaluation of Single High-

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Dose Bolus Tirofiban *vs* Abciximab With Sirolimus-eluting Stent or Bare Metal Stent in Acute Myocardial Infarction Study (MULTISTRATEGY) trial [14], where a sample of 745 patients were first randomly assigned to either abciximab or tirofiban and then to SES or BMS. After 8 months, major adverse cardiac events (MACE), composite of death of any cause, reinfarction, and clinically driven TVR, was significantly different in the SES compared to the BMS group (7.8% *vs* 14.5%, $p = 0.004$), also due to lower rate of TVR (3.2% *vs* 10.2%, $p < 0.001$) [14]. Composite endpoint of death, reinfarction and ST was comparable between the two groups at the end of 8 months follow-up [14]. ST did not differ significantly between the two groups even though dual antiplatelet therapy was given in SES group for at least 3 months and sensitive classification (definite/probable/possible) of ST was used [14].

Similar results were seen in the randomized Trial to Assess the Use of the Cypher Stent in Acute Myocardial Infarction Treated with Balloon Angioplasty (TYPHOON) study, which included 712 STEMI patients with a follow-up period of 12 months [15]. The primary endpoint-target vessel failure (defined as target-vessel-related death, reinfarction or TVR) was significantly reduced in the SES than in the BMS arm (7.3% *vs*14.3%, $p = 0.004$) which was again primarily due to reduced rates of TVR in the SES compared to the BMS group (5.6% *vs* 13.4%, $p < 0.001$) [15]. The rate of acute and subacute ST did not differ between the two arms after the first year of follow-up [15]. Recently the same group of authors presented that after 4 years there were no significant differences in definite or probable ST, freedom from reinfarction and cardiac death in the SES group compared to the BMS group, while freedom from target lesion revascularization (TLR) was significantly better in the SES group [16].

Randomized trials using paclitaxel-eluting stents in STEMI

The Paclitaxel-eluting Stents *vs* Bare Metal Stents in Myocardial Infarction with ST-segment Elevation (PASSION) study that included 619 STEMI patients randomized to paclitaxel-eluting stent (PES) or BMS [17], with a 12-month follow-up period, did not show a statistically significant difference in primary events (death, myocardial infarction, TVR) between PES and BMS groups (12.8% *vs* 8.8%, $p = 0.12$) [17]. TVR between the PES and BMS groups was not statistically significant (5.3% *vs* 7.8%), probably due to a low percentage of patients with diabetes (11%), more limited selection of angiographic characteristics (larger vessel diameter) and the absence of angiographic follow-up. The percentage of ST was not statistically significant between the PES and BMS groups after one year [17]. At 5 years, the occurrence of the composite of cardiac death, recurrent myocardial infarction, or TLR was comparable in the PES and BMS arm (18.6% *vs* 21.8%, $p = 0.28$), as also the incidence of definite or probable ST (4.2% *vs* 3.4%, $p = 0.68$) [18].

Safety and efficacy of PES stents in STEMI has been proved so in far the largest published randomized trial Harmonizing Outcomes with Revascularization and Stents in AMI (HORIZONS-AMI), involving 3006 STEMI patients [19]. After 12 months, the PES group compared to the BMS group had a significantly lower TLR (4.5% *vs* 7.5%, $p = 0.002$), and TVR (5.8% *vs* 8.7%, $p = 0.006$). The mortality and ST was similar between the PES and BMS group [19]. At 3 years, the major findings from the stent part of the trial were that the PES seemed safe in STEMI with a significantly lower ischaemia-driven TVR in the PES arm [20]. There were no significant differences in the rates of death, reinfarction, stroke [20]. ST was similar in both groups – around 5% [20]. In the intravascular ultrasound substudy of HORIZONS-AMI, it was shown that acute stent malapposition was similar in PES and BMS treated lesions, but late acquired stent malapposition was more common in PES treated lesions and it was due to positive remodeling and plaque/thrombus resolution [21]. However, either acute stent malapposition or late acquired stent malapposition were not associated with adverse cardiac events at one year [21]. A recent optical coherence tomography study shows that PES significantly reduces neointimal hyperplasia, but results in higher rates of uncovered and malapposed stent struts and different healing response of the ruptured plaque at a 13-month follow-up [22]. Still, studies are needed to determine the relationship between these optical coherence tomography observations and long-term adverse clinical events.

Randomized trials using the second generation of drug-eluting stents in STEMI

Since the first generation of SES and PES raised safety concerns after the first year [23, 24], second generation of DES brought novel improved biocompatible and biodegradable polymers [25], new antiproliferative agents and designs which might increase biocompatibility therefore improving long term efficacy and safety profile. Thus, patients with STEMI might benefit from the second generation of DES [26].

Drug Elution and Distal Protection in Acute Myocardial Infarction (DEDICATION) trial included 626 patients from the two centers [27]. The primary endpoint was the loss of luminal diameter in the infarct-related lesion determined using quantitative coronary angiography at 8 months. Stents implanted in the DES group were SES in 47%, PES in 40% and zotarolimus-eluting stents in 13% [27]. While the primary endpoint was in favor of DES (late lumen loss 0.06 ± 0.66 mm *vs* 0.47 ± 0.69 mm, $p < 0.001$), there was a strong tendency toward a higher cardiac death in the DES group (4.2% *vs* 1.6%, $p = 0.09$) [27]. TLR was lower in the DES arm (5.1% *vs* 13.1%, $p = 0.001$), while ST rates were similar in the two groups (2.0% *vs* 2.6%, $p = 0.72$) [27]. Inclusion criteria in this trial were less strict with a higher rate of patients with complex lesions, older patients, more stents per patient implanted and stented longer segments of the coronary arteries compared to the other studies [27]. After 3 years the rate of all-cause mortality was not statistically different while the cardiac death was significantly higher in DES group (6.1% *vs* 1.9%, $p = 0.01$) which was contrary to previous studies [28]. MACE was still significantly higher in the BMS arm (18.2% *vs* 11.5%, $p = 0.02$) due to a higher TVR [28].

The Evaluation of the Xience-V Stent in Acute Myocardial Infarction (EXAMINATION) trial presented novel data with the second generation of cobalt-chromium everolimus-eluting stent (CoCr-EES) in STEMI [29]. The author stated that "all-comers" design of the study with wide inclusion and less exclusion criteria will be a representative sample for "real world" population. Patients were randomized 1:1 on either CoCr-EES or BMS [29]. There were no differences in primary endpoint (all-cause death, reinfarction or revascularization), cardiac death and reinfarction between the two groups. Although the study failed to reach its primary endpoint, there were benefits in using EES since TVR and TLR were significantly lower in CoCr-EES compared to BMS arm, while definite ST was significantly higher in the BMS group (1.9% *vs* 0.5%, $p = 0.01$) [29]. However, this trial was not powered to show differences of ST and thus whether those findings are real or attributable to chance remain uncertain [29, 30]. Still, these results are similar to the result of recent meta-analysis that CoCr-EES had also reduced ST compared with BMS [30]. These results support the safety and efficacy of CoCr-EES in a representative sample of STEMI patients especially in preventing the early ST rate with the use of second generation DES [29]. A recent nonrandomized study, which evaluated the safety and effectiveness of the second generation of CoCr-EES in patients with acute myocardial infarction (AMI) with a patient without AMI showed at 1 year low clinical event rates in these two groups [31]. Comparing with elective procedures, the rates of ST at one year were 1.08% vs. 0.85% and late ST (30 days-1 year) were 0.31% *vs* 0.47%, (AMI *vs* non-AMI, all p = ns) [31]. Even though the sample size of AMI patients was small, low ST rates associated with CoCr-EES use in both non-AMI and AMI patients in this study are consistent with previous randomized controlled trials such as Clinical Evaluation of the XIENCE V Everolinus Eluting Coronary Stent System (SPIRIT) IV [32] and EXAMINATION [31].

A recent meta analysis has presented that CoCr-EES is associated with a significant reduction in definite ST compared with BMS and other first and second generation DES including PES, SES, resolute zotarolimus and phosphorylcholine polymer-based zotarolimus eluting stent at a 1-year follow-up [25]. In the same meta-analysis it was presented that only CoCr-EES showed a significant reduction of definite ST compared with BMS at a 2-years follow-up [25]. The authors stated that the results are consistent with the result of experimental studies, which compared EES with BMS, showing that a lower rate of ST in EES is due to the design and material (reduced stent strut thickness, use of a cobalt-chromium and platinum-chromium alloys instead of stainless steel) and durable, fluorinated and thromboresistant polymer [25]. It is of note, that even SES were also associated with significantly lower 1-year rates of definite ST compared to the BMS, but it was not maintained at 2 year follow-up [25]. However, larger and adequately powered randomized trials with longer follow-up in STEMI setting are needed to eliminate concerns of safety of these devices compared to BMS and with other DES.

The latest Comparison of Biolimus Eluted From an Erodible Stent Coating With Bare Metal Stents in Acute ST-Elevation Myocardial Infarction (COMFORTABLE AMI) trial included STEMI patients randomly assigned on a 1:1 basis to treatment with biolimus-eluting stents (BES) from a biodegradable polylactic acid polymer or BMS [33]. The primary endpoint of the study was the device-oriented composite of cardiac death, reinfarction, and ischemia-driven TLR at 1 year [33]. Major adverse cardiac events at 1 year occurred in 4.3% of patients receiving BES and 8.7% patients receiving BMS ($p = 0.004$) [33]. It was due to lower risk of target vessel reinfarction ($p = 0.01$) and ischemia driven TLR ($p = 0.001$) in patients receiving BES compared with those receiving BMS. The rates of cardiac death were not significantly different while ST occurred in 5 patients treated with BES and 12 patients ($p = 0.10$) treated with BMS [33]. The second generation DES–BES with biodegradable polymers provide controlled drug release with subsequent degradation of polymer contrary to durable polymer coatings for drug release of the first generation DES, which might be a trigger for the late ST [26, 34]. This might improve long-term clinical outcomes beyond 1 year by reducing the risk of ST by 80% compared to the first generation DES [26, 33, 34].

Similar to patients with stable angina, no randomized studies have demonstrated the effectiveness of DES in lowering the rates of cardiac death and myocardial infarction compared to BMS arms in STEMI patients. Also, current trials do not bring enough evidence concerning benefits of DES compared to BMS in STEMI patients as in the elective procedures when DES is proved to be more effective (long lesions, small vessels, diabetic patients). On the other hand, these randomized studies clearly indicate the safe use of DES in STEMI patients and a reduced TVR in the DES group, without a significant difference in cardiac death, reinfarction and ST after 1-year follow-up. Meta-analyses of these randomized studies also present that DES significantly reduce TVR compared to BMS, without an increase in death, reinfarction, or ST within 1 [35] and 2 years of the index procedure [25, 26, 37]. However, a long-term analysis at 3 to 5 years after the procedure showed that the use of the first generation DES in STEMI is associated with an excess of very late thrombotic complications [23] which occurred more likely in the DES group compared to the BMS arm.

The second generation DES might overcome very late thrombotic complication due to novel improved biocompatible and biodegradable polymers, new antiproliferative agents and designs, as a result patients with STEMI might benefit from these devices [25, 26, 34, 38]. Both Examination and Comfortable trials are not statistically powered neither have long-time follow-up to provide definite answer about safety of second generation DES. To be statistically powered to detect the difference in low-frequency events such as very late ST between available DES, there is a need for randomized trial which would include as many as 10,000 patients in STEMI setting. Consequently, the results from observational data, meta analysis and randomized trials between different DES devices in stable angina and acute coronary syndrome presented that second generation DES are more effective and

with increased safety compared with either BMS or the first generation of DES, which should lead to greatly improved outcomes in patient with AIM [24–26, 34, 39–41].

Conclusion

The efficacy of DES compared to BMS in reducing in-stent restenosis and repeat intervention within one year was shown in many randomized studies, registries and meta-analysis, therefore further studies comparing the efficacy of DES to BMS might not be needed in the setting of AMI. Although no significant difference in mortality, reinfarction and ST was shown in DES compared to BMS, late safety issues with DES are mostly related to the first generation of DES. Observational data, meta-analysis and randomized trials with the second generation of DES devices have showed better efficacy with increased safety compared with either BMS or the first generation of DES leading to the conclusion that the second generation of DES should be the "first choice" in STEMI setting.

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CASE REPORTS

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Paraganglioma of the thyroid gland: A case report

Paragangliom štitaste žlijezde

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Abstract

Introduction. Thyroid paraganglioma is a very rare malignant neuroendocrine tumor. Immunohistochemical features of thyroid paraganglioma are helpful for the diagnosis. **Case report.** A 69-year-old female came to hospital with the presence of a growing thyroid nodule of the left lobe. Ultrasonic neck examination showed 5 cm hypoechoic nodule in the left thyroid lobe. Thyroid scintigraphy showed a big cold nodule in the left lobe. Computed tomography (CT) scan showed left lobe thyroid tumor with tracheal deviation on the right site. Extended total thyroidectomy was done. Intraoperative consultation with the pathologist confirmed thyroid cancer. The pathologist diagnosed thyroid paraganglioma on the base of immuohistochemical investigation. This thyroid paraganglioma was positive for neuron-specific enolase, chomogranin A, synaptophysin, and S-100 protein highlighted the sustentacular cells. Tumor cells were negative for thyroglobulin, epithelial membrane antigen, cytokeratin, calcitonin, and carcinoembryonic. After the surgery the patient was treated with chemotherapy, peptide receptor radionuclide therapy, and permanent TSH suppressive therapy. The patient was followed with measurements of thyroid hormone and serum neuron–specific enolase, chromogranin A level, every 6 months. Gastroscopy, colonoscopy, chest and abdomen CT scan as well as further tests (chest x-ray, ultrasound of the neck, and whole body octreotide scintigraphy) were done. No primary neuroendocrine tumor in digestive sistem or in the chest was found. After more than 3 years the patient has no evidence of the recurrent disease. **Conclusion.** Radical resection of thyroid paraganglioma, followed by chemotherapy and peptide receptor radionuclide therapy, should be considered the treatment of choice in patients with thyroid gland paraganglioma.

Key words:
neuroendocrine tumors; thyroid gland; paraganglioma; diagnosis; surgical procedures, operative; drug therapy.

Apstrakt

Uvod. Tiroidni paragangliom je rijedak maligni neuroendokrini tumor. Imunihistohemijske odlike ovog tumora bitne su za dijagnozu. **Prikaz bolesnika.** Bolesnica, stara 69 godina, javila se ljekaru zbog progresivno rastućeg čvora u lijevom režnju štitaste žlijezde. Ultrazvučni nalaz je ukazao na čvor veličine 5 cm u lijevom režnju, hipoehogene strukture sa mikrokalcifikatima. Scintigrafski nalaz ukazao je na hladan čvor u lijevom režnju. Kompjuterizovana tomografija pokazala je tumor lijevog režnja štitaste žlijezde koji je potiskivao dušnik u desnu stranu. Urađena je totalna tireoidektomija. „Intraoperativna konsultacija" sa patologom ukazala je na karcinom lijevog režnja štitaste žlijezde. Na osnovu imunohistohemijskih pretraga preparata, patolog je postavio dijagnozu tireoidnog paraganglioma. Tumorske ćelije bile su pozitivne na neuron-specifičnu enolazu, hromogranin, sinaptofizin i S-100 protein, a negativne na tireoglobulin, kalcitonin, epitelni membranski antigen, citokeratin i karcinoembriogeni antigen. Nakon hirurškog liječenja sprovedena je hemioterapija, trajna tireostimulišući hormon (TSH) supresivna terapija i protein usmjerena radioterapija. Bolesnica je kontrolisana svakih 6 mjeseci, uz određivanje nivoa TSH, serumske neuron specifične enolaze i hromogranina A. Urađene su i gastroskopija, pankolonoskopija, kompjuterizovana tomografija grudnog koša i abdomena, scintigrafija cijelog tijela oktreotidom, i nijedan nalaz nije ukazao na postojanje primarnog neuroendokrinog tumora digestivnog trakta ili grudnog koša. Nakon više od tri godine kod bolesnice nema znakova postojanja recidiva bolesti. **Zaključak.** Totalna tireoidektomija sa postoperativnom hemioterapijom i ciljanom peptid-receptor radionuklidnom terapijom jeste metoda izbora u liječenju bolesnika sa tireoidnim paragangliomom.

Ključne reči:
neuroendokrini tumori; tireoidna žlezda; paragangliom; dijagnoza; hirurgija, operativne procedure; lečenje lekovima.

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Introduction

Thyroid paraganglioma is a rare malignant tumor, arising from the neural crest-derived paraganglia of the autonomic nervous sistem [1]. Extra-adrenal paraganglia which are histochemically non-chromaffin, are related to the parasympathetic system and are located primarily in the head and neck region, the superior mediastinum and retroperitoneum [2, 3]. There are 25-case reports on this tumor described in the literature [4]. Paragangliomas of the head and neck region account for 0.012% of all head and neck tumors. They are malignant in 4–16% cases [5]. The carotid body and glomus jugulare account for more than 80% of the cases. In the head and neck region, paraganglia are presented as paired orbital, jugulotympanic, and very rare in the laryngs, trachea and the thyroid [6]. It is typically presented with fast tumor growth progression and presented neck tumor. The neuroendocrine lesions of the thyroid are few and include C-cells lesions (C-cell hyperplasia and medullary carcinoma), mixed C-cell and follicular-derived tumors, paraganglioma, intrathyroid adenoma, and metastasis to the thyroid from neuroendocrine carcinoma arising elsewhere.

Thyroid paraganglioma arise from inferior laryngeal paraganglia, which can be found within the thyroid capsule [7].

All reported cases of paraganglioma of the thyroid occurred in women, and manifesteted as a solitary nodus, and ranged in size from 1.5 to 10 cm [8]. They can be confined to the thyroid or in some cases can exhibit infiltration into surrounding tissue.

Surgical treatment is the basic treatment for thyroid paraganglioma. Additional treatment includes thyroid stimulating hormone (TSH) suppressive therapy by L-thyroxine, chemotherapy, and peptide receptor radionuclide therapy. Although debates on radicalism of surgical treatment have lasted to this day, total thyroidectomy is the most widely accepted surgical treatment.

We reported a patient surgically treated for thyroid paraganglioma.

Case report

A 69-year-old female was sent to the Department for Endocrine Surgery, Clinical Center of Montenegro due to neck tumor. Although the patient knew about the nodule in the left lobe for 1 year, over the last month she noticed painless growth of a nodule. Inspection of the neck showed neck deformity on the left side, and palpatory 5 cm wide fixed painless tumor of the left thyroid lobe. There was no evidence of cervical lymph nodes enlargement. Laryngoscopy showed left laryngeal nerve pulsy. We evaluated a nodule by fine-needle aspiration (FNA) of the thyroid as follows: THY 5, malignant tumor.

Ultrasonography examination of the neck showed hypoechoic heterogeneous 5 cm large and irregular contour nodule, with calcification in the central part. There was no enlargement of the cervical lymph nodes. Chest X-ray showed no metastases. There was a normal thyroid hormone and calcitonin level. Thyroid scintigraphy showed a cold nodule in the left lobe. CT scan showed a left thyroid lobe tumor, 5 cm wide, no tracheal infiltration (Figure 1).

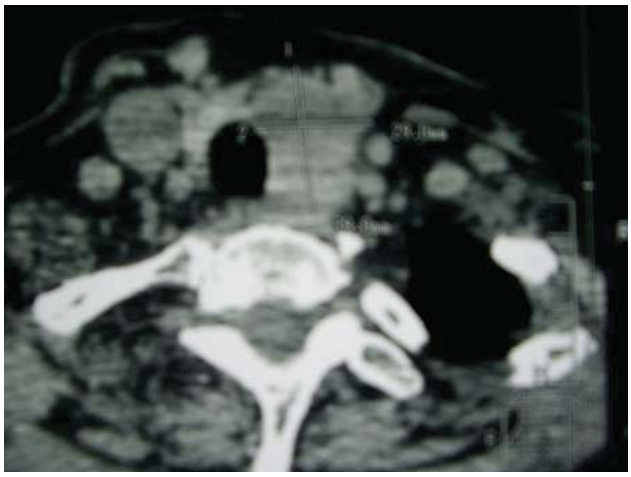

Fig. 1 – Computed tomography scan of thyroid paraganglioma.

During the surgery a left thyroid lobe tumor was found, infiltrating strap muscles on the front left neck side. The tumor also infiltrated the left jugular vein and the front part of the trachea. Extended total thyroidectomy with tangential tumor resection from trachea was performed (Figure 2).

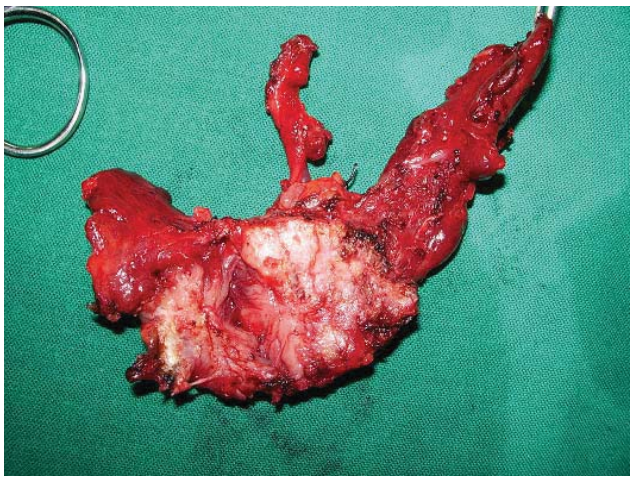

Fig. 2 – Thyroid paraganglioma – total thyroidectomy.

The entire thyroid gland was removed with the left strap muscles, left jugular vein. Debulking was very difficult, due to the presence of a firm neoplasm that spread beyond the gland capsule with infiltration into surrounding tissues. A central neck dissection was made and one lymph node with metastatic disease was found. There was no evidence of postoperative hypoparathyroidism, or respiratory insufficiency. The first postoperative day the patient was treated at Intensive Care Unit, and the following 5 days at the Department for Endocrine Surgery. The following day the patient went home in good condition.

The pathologist diagnosed an invasive neuroendocrine carcinoma of the thyroid. Convencional histology was per-

formed on formalin-fixed and paraffin-embedded tissue blocks, 4 micrometer sections were cut, deparaffinized in xylene and stained with haematoxylin and oesin (H&E). Light microscopy of this tumor revealed the hallmark nesting growth pattern with chief and sustentacular cells seen in thyroid paraganglioma arrising in sites other than the thyroid (Figure 3) There were prominent vascularity and thin fibrous

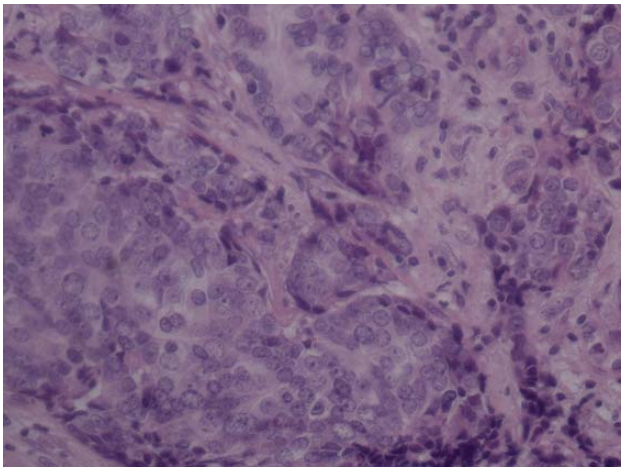

Fig. 3 – Cellular detail showing nests of tumor cells embraced by sustentacular cells (H&E, 40 ×).

septa separating nests of tumor cells. Some cells also exhibited isolated bizarre cells and mitoses, by examining well-fixed histological section. In 2 cervical lymphonode we found metastases of this tumor. This thyroid paraganglioma was positive for neuron-specific enolase, chromogranin A, synaptophysin, and S-100 protein highlights the sustentacular cell (Figure 4). Ki 67 was expressed in 25% of tumor cells. Tumor cells were negative for thyroglobulin, epithelial membrane antigen, cytokeratin, calcitonin, and carcinoembryonic antigen (CEA). These immunohistochemical features of thyroid paraganglioma were helpful for differentiating it from medullary carcinoma.

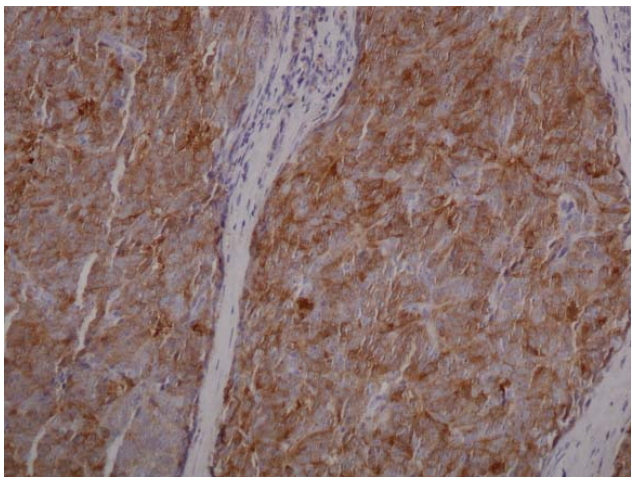

Fig. 4 – Tumor cells evident by chromogranin A immunostaining (20 ×).

Gastroscopy, colonoscopy, chest and abdomen CT scan as well as further tests (chest x-ray, ultrasound of the neck, and whole body octreotide scintigraphy) were done with no primary neuroendocrine tumor found in the digestive system or in the chest.

One month after the surgery the wound was healing well, with no evidence of local recurrence. There was no evidence of hypoparathyroidism or laryngeal nerve palsy. The patient continued on L-thyroxin suppressive therapy with TSH level 0.05 mU/L.

Two months after the surgery the patient had 6 courses of chemotherapy. One year later, octreoscan body scintigraphy showed normal finding, and tumor marker finding with low chomogranin A serum level. One year after the surgery the patient had peptide receptor radionuclide therapy. There was no evidence of local recurrent disease on body octeoscan scintigraphy.

The patient was regularly controlled for 3 years with no evidence of local recurrent disease.

Discussion

Medullary carcinoma is a typical neuroendocrine tumor of the thyroid gland. Other neuroendocrine lesions of the thyroid are C-cell hyperplasia, intrathyroidal nodules and tumors that display neuroendocrine features including hyalinizing trabecular neoplasms, insular carcinoma, true paraganglioma, parathyroid lesions, and tumors metastatic to the thyroid [9]. All the 25 reported cases of paraganglioma of the thyroid occurred in women, manifested as a solitary node [10]. They can be confined to the thyroid or some cases can exhibit infiltration into surrounding tissue. Most thyroid paraganglioma are confined within the thyroid capsule, but in 3 cases the neoplasm was locally invasive and infiltrated through the tracheal wall [11]. Also, in the presented case the tumor spread beyond the thyroid capsule with infiltration of the left laryngeal recurrent nerve, and strap muscule. Light microscopy revealed the hallmark nesting (Zellballen) growth pattern with chief and sustentacular cells seen in paraganglioma arising in sites other than the thyroid [12]. Paraganglioma stains positive for chomogranin A, synaptophysin and S-100 protein. Not surprisingly, the diagnosis of thyroid paraganglioma is rarely established preoperatively by FNA biopsy or intraoperatively by frozen section. The histopathological features usually suggest medullary carcinoma [13]. This is due to the clustering of cells with granular cytoplasm and a richly vascularized stroma. In contrast of medullary carcinoma, paraganglioma tends to exibit S-100 protein staning in sustentacular cells composed at the periphery of the cell nest and they lack staining for cytokeratin, CEA and calcitonin. Our paraganglioma stained positive for neuron-specific enolase, chromogranin A, sinaptophysin, and S-100 protein. Differential diagnosis includes 2 main entites, namely hyalinizing trabecular adenoma of thyroid and medullary carcinoma [14]. Tumor cells of medullary carcinoma also can express a variety of other hormons besides calcitonin, such as corticotropin, melatonin-stimulating hormone, serotonin and gas-

trin [15]. The gold standard for the diagnosis of medullary carcinoma is immunostaining for calcitonin [16]. Medullary carcinoma also stains positive for CEA.

About 6% of patients with thyroid cancer present with life-threatening tumor invasion of the trachea. After the complete tumor resection, 5-year and 10-year survival rates of 40–75% can be achieved [17]. An incomplete tumor resection has a negative effect on the prognosis. A tangential tumor resection (shaving) is indicated if no transmural invasion of trachea occurrs.

The aim of enlarged surgical treatment in thyroid carcinomas is to guarantee respiratory and alimentary functions as well as symptomatic benefits, to obtain local control of the disease and recovery of the adjuvant therapeutic options, such as metabolic and conventional radiation [18].

Conclusion

Malignant thyroid paraganglioma is a rare tumor. The pathologist can diagnose thyroid paraganglioma by examining well-fixed histological section with immunohistochemical investigation of chromogranin A, neurone-specific enolase, and protein S-100. Thyroid paraganglioma tumor cells are negative for thyroglobulin, and calcitonin. This tumor usually invades the thyroid capsule. Invasive tumor presents a delicate surgical problem because of radical surgical eradication. The prognosis of thyroid paragangliloma appears to be favourable, provided that surgical excision is complete. Total thyroidectomy, followed by chemotherapy and peptide related radiotherapy should be considered the treatment of choice.

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CASE REPORT

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Visceral hybrid reconstruction of thoracoabdominal aortic aneurysm after open repair of type A aortic dissection by the Bentall procedure with the elephant trunk technique – A case report

Visceralna hibridna rekonstrukcija torakoabdominalne aneurizme aorte nakon otvorene rekonstrukcije aortne disekcije tipa A procedurom Bentall uz pomoć tehnike *elephant trunk*

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Abstract

Introduction. Reconstruction of chronic type B dissection and thoracoabdominal aortic aneurysm (TAAA) remaining after the emergency reconstruction of the ascending thoracic aorta and aortic arch for acute type A dissection represents one of the major surgical challenges. Complications of chronic type B dissection are aneurysmal formation and rupture of an aortic aneurysm with a high mortality rate. We presented a case of visceral hybrid reconstruction of TAAA secondary to chronic dissection type B after the Bentall procedure with the 'elephant trunk' technique due to acute type A aortic dissection in a high-risk patient. **Case report.** A 62 year-old woman was admitted to our institution for reconstruction of Crawford type I TAAA secondary to chronic dissection. The patient had had an acute type A aortic dissection 3 years before and undergone reconstruction by the Bentall procedure with the 'elephant trunk' technique with valve replacement. On admission the patient had coronary artery disease (myocardial infarction, two times in the past 3 years), congestive heart disease with ejection fraction of 25% and chronic obstructive pulmonary disease. On computed tomography (CT) of the aorta TAAA was revealed with a maximum diameter of 93 mm in the descending thoracic aorta secondary to chronic dissection. All the visceral arteries originated from the true lumen with exception of the celiac artery (CA), and the end of chronic dissection was below the origin of the superior mesenteric artery (SMA). The patient was operated on using surgical visceral reconstruction of the SMA, CA and the right renal artery (RRA) as the first procedure. Postoperative course was without complications. Endovascular TAAA reconstruction was performed as the second procedure one month later, when the 'elephant trunk' was used as the proximal landing zone for the endograft, and distal landing zone was the level of origin of the RRA. Postoperatively, the patient had no neurological deficit and renal, liver function and functions of the other abdominal organs were normal. Control CT after 6 months showed full exclusion of the aneurysm from the systemic circulation without endoleak and good flow through visceral anastomosis. **Conclusion.** In patients with comorbidities, like in the presented case, visceral hybrid reconstruction of chronic dissection type B with TAAA could be the treatment of choice.

Key words:
aortic aneurysm, thoracic; vascular surgical procedures; treatment outcome; comorbidity.

Apstrakt

Uvod. Rekonstrukcija hronične disekcije tipa B i aneurizme torakoabdominalne aorte (TAAA), zaostale nakon urgentne rekonstrukcije ascendentne aorte i luka aorte zbog akutne disekcije tipa A, predstavlja jednu od najvećih izazova u hirurgiji. Komplikacije hronične disekcije tipa B su nastanak aneurizme aorte i njena ruptura sa visokom stopom smrtnosti. Prikazana je visceralna hibridna rekonstrukcija TAAA nakon hronične disekcije tipa B i procedure Bentall tehnikom *elephant trunk* zbog akutne aortne disekcije tipa A, kod bolesnika sa visokim rizikom od peroperativnog mortaliteta. **Prikaz bolesnika.** Bolesnica, stara 62 godine, primljena je u našu ustanovu radi rekonstrukcije Crawford tipa I TAAA nakon hronične disekcije. Bolesnica je imala akutnu aortnu disekciju tipa A ranije, zbog čega je operisana. Učinjena je procedura Bentall tehnikom *elephant trunk* sa zamenom valvule. Na prijemu bolesnica je imala koronarnu bolest (in-

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farkt miokarda, dva puta u protekle tri godine), zastojnu srčanu bolest sa ejekcionom frakcijom od 25% i hroničnu opstruktivnu bolest pluća. Na kompjuterizovanoj tomografiji (KT) aorte TAAA je prikazana sa maksimalnim prečnikom od 93 mm u descendentnoj grudnoj aorti nakon hronične disekcije. Sve visceralne arterije poticale su iz pravog lumena sa izuzetkom celijačne arterije (CA), i završetak hronične disekcije nalazio se ispod ishodišta gornje mezenterične arterije (GMA). Bolesnica je podvrgnuta prvo otvorenoj hirurškoj rekonstrukciji GMA, CA i desne bubrežne arterije (DBA). Postoperativni tok protekao je bez komplikacija. Endovaskularna rekonstrukcija TAAA sprovedena je kao druga procedura jedan mesec kasnije, pri čemu je za proksimalnu zonu fiksiranja endografta korišćena procedura *elephant trunk*, a za distalnu zonu fiksiranja nivo odvajanja DBA. Postoperativno, bolesnica nije imala neurološki deficit, a funkcija bubrega, jetre i ostalih abdominalnih organa bila je normalna. Kontrolna KT nakon šest meseci pokazala je potpuno isključenje aneurizme iz sistemske cirkulacije bez *endoleak*-a i dobar protok krvi kroz visceralne anastomoze. **Zaključak.** Kod bolesnika sa komorbiditetima, kao što je kod prikazane bolesnice, visceralna hibridna rekonstrukcija hronične disekcije tipa B sa TAAA može predstavljati terapiju izbora.

Ključne reči:
aneurizma, torakalna; hirurgija, vaskularna, procedure; lečenje, ishod; komorbiditet.

Introduction

Open surgical repair of extensive thoracic aortic aneurysms and thoracoabdominal aortic aneurysm (TAAA) in elderly and in patients with comorbidities remains unsatisfactory due to considerable mortality and morbidity. After introducing the 2-stage approach to complex aortic aneurysm reconstruction [1], and thoracic endovascular aortic repair (TEVAR) as a minimally invasive procedure [2], the mortality rate higher than 31% in TAAAs repair has been decreased [3]. When TAAAs involving the visceral aorta or in cases with chronic type B aortic dissection of TAAA, encouraging results revealed with combining lesser open abdominal operation to bypass the visceral and renal vessels with the placement of an endovascular stent graft [3, 4]. This combination of endovascular exclusion with visceral revascularization for treatment of TAAAs involving the visceral aorta, termed the visceral hybrid procedure.

We presented a case of visceral hybrid the TAAA reconstruction secondary to chronic dissection type B after the Bentall procedure with 'elephant trunk' technique due to acute type A aortic dissection.

Case report

A 62 year-old woman was referred to our institution for repair of the Crawford type I TAAA secondary to chronic dissection. The patient had undergone repair of acute type A aortic dissection by the Bentall procedure with the elephant trunk technique and valve replacement 3 years before in other medical institution, when the left subclavian artery was ligated.

Computed tomography (CT) of the aorta, performed in our institution revealed TAAA with a maximum diameter of 93 mm in the descending thoracic aorta secondary to chronic dissection. The true lumen of distal part of descending aorta was with the maximum diameter of 12 mm. CT scan also showed that all the visceral arteries were originated from the true lumen with exception of the celiac artery (CA), and the end of chronic dissection was below the origin of superior mesenteric artery (SMA). The origin of the left renal artery (LRA) was 2 cm below the origin of the right renal artery (RRA). The distal, normal infrarenal aorta (without dissection) was 25 mm in diameter (Figure 1).

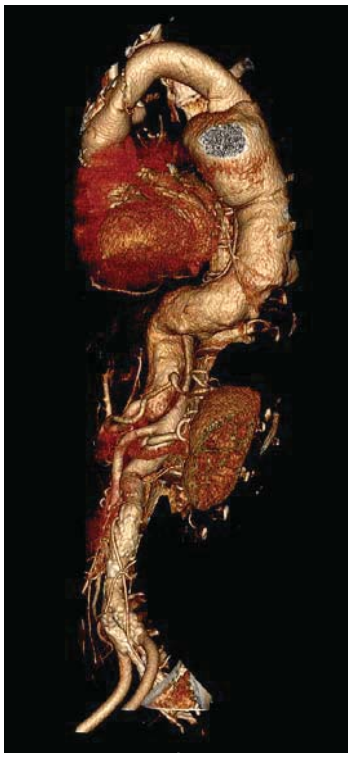

Fig. 1 – Computed tomography scan before the operation: chronic type B dissection with thoracoabdominal aortic aneurysm.

On admission, the patient had a lot of comorbidities: coronary artery disease (myocardial infarction, 2 times in the past 3 years), congestive heart disease with ejection fraction of 25%, and chronic obstructive pulmonary disease.

Due to the patient condition we decided to make visceral hybrid TAAA reconstruction like 2-stage procedures, with 3-vessel reconstruction (SMA, CA, RRA).

Open surgical visceral reconstruction was performed as the first procedure in general anesthesia with thoracic epidural analgesia. The distal aorta and the origins of the visceral arteries were exposed through transperitoneal abdominal ap-

proach. For the visceral bypass debranching we used a Dacron® bifurcated graft of 16 × 8 mm, and a Dacron® limb of 8 mm for the RRA which was anastomosed end-to-side with a bifurcated graft. All visceral anastomoses between grafts and arteries were done by end-to-side anastomosis with proximal ligation of the origin of the arteries after Doppler sonographic confirmation of flow through anastomosis. Ischemia of the right kidney during RRA reconstruction was 16 min, SMA reconstruction lasted 12 min and celiac trunk reconstruction 16 min. The visceral bypass grafts were anastomosed end-to-side to the normal distal part of the aorta. Postoperative course of the patient was without complications.

CT scan one month after the first operation showed chronic dissection type B with TAAA without increasing the maximum diameter and orderly flow through the reconstructed visceral arteries (SMA, CA, RRA) (Figure 2).

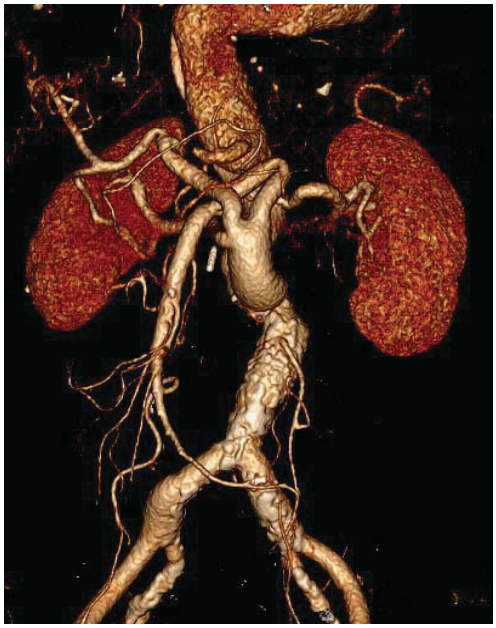

Fig. 2 – Control computed tomography scan after the 3 vessels visceral debranching.

The second endovascular stage procedure was performed after control CT. In general anaesthesia the right femoral artery was exposed and a 22 French delivery system was inserted. Through the percutaneous right brachial artery approach a 5 French pigtail in the aortic arch was placed. Aortography was performed through this pigtail catheter to determine the level of the proximal lending zone, the level of distal lending zone and the level of origin of the LRA. After administration of 5,000 IU of heparin sodium, the endovascular two stent-grafts (TGE 343420; W. L. Gore , Flagstaff, Arizona, USA) were inserted over the guide wire with overlapping of two stent-grafts in the lenght of 5 cm. The proximal lending zone of the stent-graft was 28 mm Dacron graft from the elephant trunk procedure (Figure 3). The distal landing zone of the stent-graft was the level of the origin of RRA where the aortic diameter was 27 mm. Control aortography was performed to verify aneurysm exclusion and to show free perfusion of the stent-graft. Finally, all catheters were removed and arteriotomy of the right femoral artery was sutured. Postoperative course was without complications. The patient was discharged on the day 5 postoperatively, with no neurological deficits. Renal function, liver function and functions of the other abdominal organs were normal.

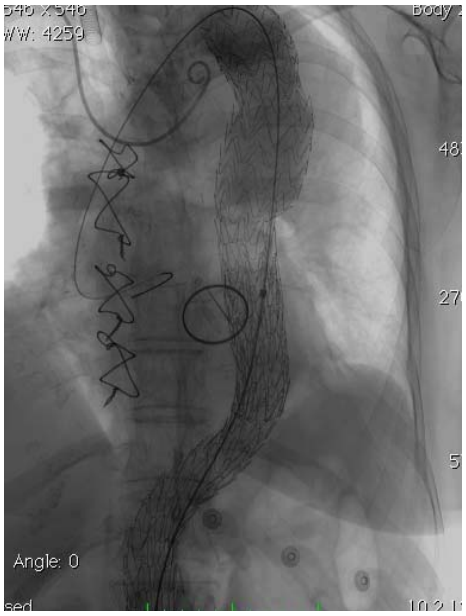

Fig. 3 – Endovascular reconstruction of thoracoabdominal aortic aneurysm with 2 stent-grafts.

On control examination 6 months after endovascular procedure, CT scan showed full exclusion of the aneurysm from the systemic circulation without endoleak and good flow through the visceral anastomosis. Also, CT scan showed complete thrombosis of the false lumen and aneurysm (Figure 4).

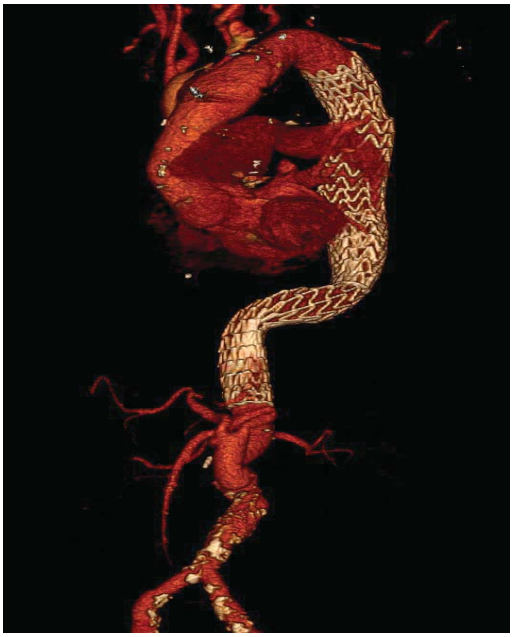

Fig. 4 – Control computed tomography scan 6 months after the endovascular repair.

Discussion

TAAAs as well as extensive thoracic aortic aneurysms that involve the ascending aorta, aortic arch and descending aorta require challenging repairs associated with the high rate of morbidity and mortality. Since the introduction of the 2-stage approach to reconstruction of complex aortic aneurysms by Borst et al. [1], the elephant trunk technique has become the standard surgical treatment for reconstruction of these aneurysms. In the 2-stage approach to acute type A aortic dissection treatment, the first step includes aortic valve replacement and reconstruction of ascending, arch and descending segments of aorta with a graft, so that the portion of the graft is left suspended within the lumen of the proximal descending thoracic aorta (elephant trunk) for a subsequent distal aortic reconstruction [1]. The second stage could be completed with open reconstruction, but more desirable with endovascular repair, especially in high risk patients [5, 6]. In this approach, the elephant trunk is used as the landing zone for the endograft. The key features of this technique are safer aortic clamping, reduced aortic clamp time, reduced risk of ischemic consequences and lower cumulative mortality rate [5].

There is a significant incidence of comorbidities in patients with lesions of thoracic and/or thoracoabdominal aorta [7, 8]. After initially described by Dake et al. [2], TEVAR was accepted in many medical centers as a minimally invasive approach, especially in high risk patients. It was reported that TEVAR provides a reduction of operative mortality rates nearly 75% as compared to open surgical repair where the mortality rate was described up to 31% [3, 9]. However, total endovascular grafting of more extensive TAAAs was limited by the presence of the visceral and renal arteries. The visceral hybrid procedures of TAAA includes open visceral vessels debranching and their revascularization, and endovascular exclusion of TAAA. The extent of visceral reconstruction include 1–4 vessels. Complications after open visceral debranching are acute renal disease, ischemic colitis and paraplegia in the most patients. Mortality rates are higher when 3 or more vessels are reconstructed [10]. However, visceral graft patency range from 85% to 97% in more than a year follow-up in large series [10–12]. This minimally invasive surgical approach offers a possible alternative treatment option for patients who are unfit for the open repair or who have unfavourable anatomy for total endovascular repair [13]. Our patient was well-favored for visceral debranching and reconstruction because of the end of chronic dissection in the level of visceral vessels. Further advantages of visceral hybrid repair of TAAA include avoiding the need for thoracotomy, supracoeliac aortic clamp, left or full heart bypass and extensive tissue dissection associated with open repair [14]. Black et al. [3] showed a series of 25 patients with TAAA who underwent visceral hybrid reconstruction. They identified an elective mortality of 17% and no paraplegia with the procedure. Zhou et al. [15] performed 31 hybrid procedures in high-risk patients that included 15 patients with TAAA and with hybrid visceral debranching aortic repair. The perioperative mortality rate in their series was 3.2%, without postoperative paraplegia. Unlike total endovascular reconstruction for chronic aortic dissection when it is possible that false lumen dissection retrograde still blood fills, in hybrid visceral repair of TAAA with chronic dissection type B the false lumen is always excluded and there is no problems with retrograde flow. Although this kind of treatment is most suitable for patients with comorbidities, there is still significant morbidity of 30% and mortality rate of 13% to 23% [3, 16].

In our patients with a large number of concomitant diseases open TAAA reconstruction would be accompanied with a high risk and difficult to perform. Because of the involvement of the origin of SMA, CA and RRA by dissection total endovascular TAAA reconstruction was not appropriate to do. Visceral hybrid TAAA reconstruction has proven to be the best solution in this patient due to minimal invasive reconstruction and less trauma.

Conclusion

Open surgical repair of extensive thoracic aortic aneurysms and thoracoabdominal aortic aneurysm remains the surgical approach in medical centers worldwide, but in elderly and patients with comorbidities this approach carries considerable mortality and morbidity rates. Total endovascular reconstruction of chronic dissection type B with thoracoabdominal aortic aneurysm is not always possible to perform. In such cases, visceral hybrid reconstruction is the treatment of choice. In future larger series should demonstrate the true value of such surgical treatment as an alternative method for open surgical reconstruction.

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CASE REPORT

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Identification of *Clostridium septicum* in a tubo-ovarian abscess: A rare case and review of the literature

Identifikacija bakterije *Clostridium septicum* u tuboovarijalnom apscesu

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Abstract

Introduction. Tubo-ovarian abscess (TOA) is a conglomerated mass of pelvic organs including the tube, the ovary, and the bowel. The most commonly isolated organisms from TOAs are *Escherichia coli* (*E. coli*) and *Bacteroides species*. **Case Report.** We reported a case of *Clostridium septicum* (*C. septicum*) infection from a ruptured TOA with atypical clinical features. Culture of intra-abdominal free fluid obtained during surgery yielded *C. septicum*. VITEK II (bioMérieux, France) automated system was used for advanced identification of the bacteria. Parenteral clindamycin in combination with an aminoglycoside was used. The patient was discharged 19 days after the surgery and was clinically asymptomatic 6 months after the surgery. **Conclusion.** The differential diagnosis of TOA caused by *C. septicum* can be difficult, due to the lack of the symptoms. Tissues infected with *C. septicum* can become necrotic. A combination of early, adequate antibiotic therapy and surgery is the key point of the treatment.

Key words:
pelvic inflammatory disease; abscess; rupture; clostridium septicum; gynecologic surgical procedures.

Apstrakt

Uvod. Tuboovarijalni apsces (TOA) predstavlja konglomerat koji zahvata karlične organe: jajovod, ovarijum i debelo crevo. Najčešće izazivači TOA su *Escherichia coli* (*E. coli*) i *Bacteroides species*. **Prikaz bolesnika.** Prikazali smo bolesnicu sa infekcijom izazvanom bakterijom *Clostridium septicum* (*C. septicum*), nastalom nakon rupture tuboovarijalnog apscesa (TDA) sa atipičnom kliničkom slikom. Iz kulture iz slobodne abdominalne tečnosti uzete tokom hirurškog zahvata, izolovana je *C. Septicum*. Automatizovani sistem VITEK II (bio Mérieux, France) korišćen je za brzu identifikaciju bakterija. Bolesnici je ordinirana parenteralna antibiotska terapija: klindamicin u kombinaciji sa aminoglikozidom. Otpuštena je iz bolnice 19. dana posle hirurškog zahvata i bila je bez kliničkih simptoma u narednih 6 meseci. **Zaključak.** Diferencijalna dijagnostika TOA uzrokovanog *C. septicum* može biti teška, zbog izostanka simptoma. Tkiva inficirana sa *C. septicum* mogu nekrotizovati, a kombinacija rane, adekvatne antibiotske terapije i hirurgije ključna je u lečenju TOA.

Ključne reči:
karlični organi, zapaljenske bolesti; apsces; ruptura; clostridium septicum; hirurgija, ginekološka, procedure.

Introduction

Pelvic inflammatory disease (PID), which is characterized by a polymicrobial infection in the upper genital tract, can include endometritis, salpingitis and tubo-ovarian abscess (TOA). It is generally caused by sexually transmitted infections (STIs) such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* that spread upward from the lower genital tract, infecting and causing inflammation of the uterus, fallopian tubes, and ovaries [1]. Anaerobic, and facultative bacteria are the other microorganisms in the pathogenesis of PID [2]. PID is one of the most common causes of hospitalization for gynecologic disorders among woman in reproductive age. In the United States, 770,000 cases of PID are diagnosed every year [3]. The annual cost of PID and its consequences is estimated to be $ 4.2 billion [4]. PID is usually sexually active women's disease. It may be the cause of sepsis and mortality in untreated cases. A TOA can be detected in about 15% of women with PID. TOA develops in up to 34% of patients hospitalized for PID [5].

An end-stage process of acute PID is TOA. It is a conglomerated mass of pelvic organs including tube, ovary and bowel. Severe acute PID usually generates this disease. TOA could be defined in 18–34% of patients with PID [5]. Risk

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factors for TOA are similar to that of PID and include multiple sexual partners, intrauterine device and low socioeconomic status [6]. Since the disease is commonly caused by the STIs, intercourse with a partner having infection is the most important risk factor in TOA formation. However, gynecologic surgery, genital malignancy, *in vitro* fertilization treatment, and perforated appendicitis have also been shown to cause TOA [7].

The association of *Clostridium species*, which are gram-positive, anaerobic spore former, usually found in the soil and gastrointestinal tract, with post-traumatic and surgical wound complications is well-known. A total of 80–90% of all clostridial infections occur due to *C. perfrigens*. *C. septicum* is isolated at 4–20% of clostridial infections [8]. Spontaneous *C. septicum* infections are rare and associated with cyclical neutropenia, diabetes mellitus and immunosupression. A strong association of spontaneous *C. septicum* infection with haematological and colorectal malignancies is known. Cerebral abscess and aortic-ring abscess due to *C. septicum* have been also reported before [9, 10]. A survival rate of only 35% of patients with *C. septicum* has been reported regardless of the presence of an occult malignancy *versus* a clinically evident malignancy. It is mandatory to carry out a systematic and aggressive approach to the treatment of these patients [11]. A ruptured TOA and septic shock with *Clostridium perfringens* in a postmenopausal woman was reported [12]. *Clostridium sordellii* and *Clostridium perfringens* are responsible for a toxic shock after medical and spontaneous abortions [13].

We reported a rare case of *C. septicum* infection from a ruptured TOA with atypical clinical features.

Case report

A 38 year-old Caucasian woman, para II, gravida II, was referred to our Department with a pelvic mass. She presented with a 10-day history of nausea, vomiting and right lower abdominal pain. She had a regular menstrual cycle, her history revealed two unremarkable cesarean sections. She had a total thyroidectomy. The patient did not have a personal and familial history of gastrointestinal disorders. She was not receiving immunosuppressive therapy. She had not received treatment for cancer. She had her intrauterine device (IUD) removed two months before after having it for several years.

On physical examination, there was right lower quadrant tenderness. There was guarding of muscle. She had white vaginal discharge. A large, immobile, 12 × 10 cm semisolid mass at the right adnexal region was palpated *via* bimanual pelvic exam. There was a fixed retroverted uterus. The uterosacral ligaments and parametrium were not tender. The patient was afebrile. Vital signs were in normal ranges. Laboratory investigations revealed a high white blood cell count of 32,900/μL, 90% of which comprised segmented neutrophils and a high trombocyte count of 722,000/μL. Serum C-reactive protein (CRP) level was found to be high. It was 48.1 mg/dL. Blood urea nitrogen (BUN) was 29 mg/dL; serum creatinine was 2.88 mg/dL; and uric acid was 8.6 mg/dL. Our ultrasonographic examination showed a 125 × 72 mm semisolid, heterogenous mass at the right adnexal region (Figure 1). There was fluid in the pouch of Douglas.

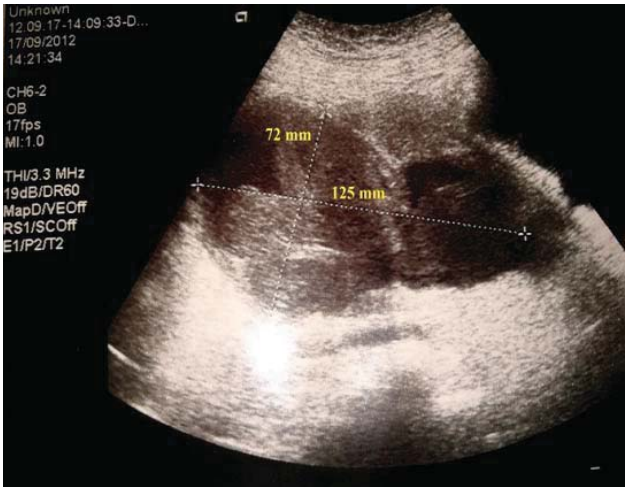

Fig. 1 – Preoperative transvaginal ultrasonographic view of the pelvis.

The serum levels of CA-125, CA-19-9, carcinoembryonic antigen (CEA) and α-fetoprotein (AFP) were 15.11, 14.30, 1.63 and 1.61 U/mL, respectively. The patient's serum β-human chorionic gonadotropin (hCG) level was 0.1 IU/L. She was negative for hepatitis B surface antigen. Antibody testing for HIV and HCV was done on serum specimens. Tests were negative. Clindamycin was used in a combination with an aminoglycoside by the parenteral route. The patient underwent laparotomy with a preoperative diagnosis of pelvic abscess. Mid-line laparotomy was performed. About 2,000 mL of infected fluid and pus were located inside the abdominal cavity. After draining, it was seen that the right tubo-ovarian abscess had already ruptured and adhered to the ileum, sigmoid colon and rectum. A sample of the peritoneal free fluid was taken. Dense adhesions between the mass and other pelvic structures were seen. Right tubo-ovarian complex was removed. Right salphingo-oophorectomy was performed. Samples of free fluid were cultured onto chocolate agar, blood agar, and eosin methylene-blue lactose agar. Two preparate were made and evaluated with gram staining at the same time. Anaerobic culture media was put in a jar and oxygen-free environment was provided with using dry process gas packet (AnaeroGen – Oxoid, Basingstoke, UK veya GENbox-bioMérieux, Lyon, France). Then chocolate agar was placed in the waxy jar. All of the media were incubated at 35–37°C for 48 hours. There was not bacterial overgrowth in blood culture. Bacteria that did not live or grow in the presence of oxygen was accepted as anaerobic bacteria. The structure of the colony was analyzed. Gram stain was performed to help identify colonies isolated from cultures. Also VITEK II (bioMérieux, France) automated system was used for advanced identification of bacteria. Large gram-positive, spore forming, obligate anaerobic, rod-shaped and motile bacteria (*C. septicum*) were obtained from intraabdominal free fluid (Figure 2).

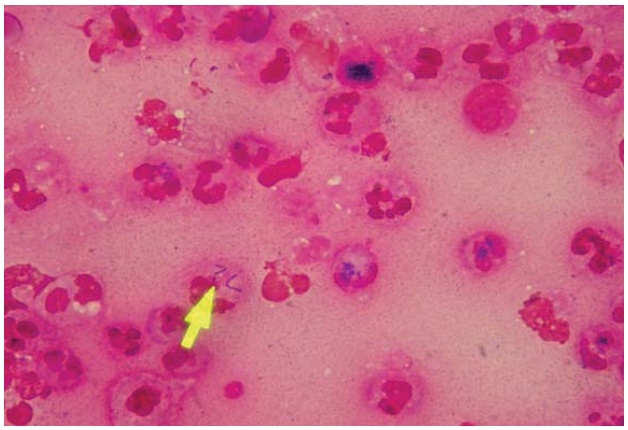

Fig. 2– *Clostridium septicum* obtained from intarabdominal free fluid (dry process gas packet).

Combined upper and lower gastrointestinal endoscopy was performed after detection of *C. septicum* in order to eliminate a potential neoplasm of the gastrointestinal tract. The computed tomography (CT) scan of the chest, abdomen and pelvis was done. No tumor was visualized. Postoperative CT scans also showed contrast enhancement in the previous operation site (Figure 3). The patient was discharged 19 days after the surgery, and was clinically asymptomatic 6 months after the surgery.

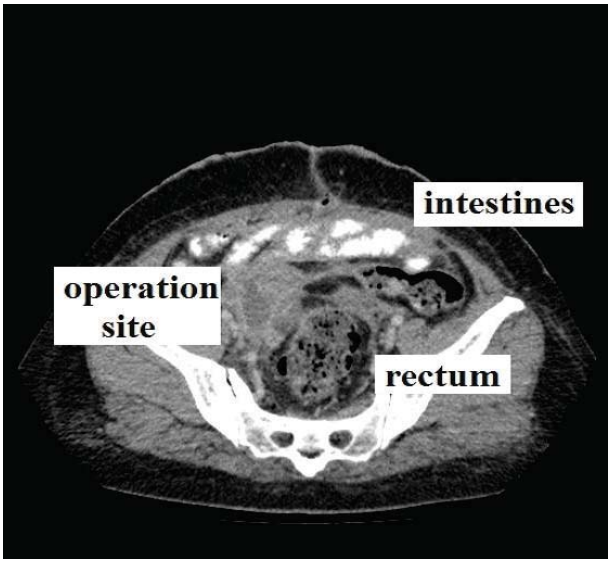

Fig. 3 – Postoperative computed tomography (CT) scan of the operation site.

Discussion

Various gynecological and nongynecological pathologies can be presented as pelvic masses. Infectious diseases should be considered in differential diagnosis. Following an acute salpingitis, fallopian tubes become distended with purulent material creating a TOA. PID can lead to tubal scarring and stenosis which become distended as the tubal secretions accumulate within the tubal lumen. This process leads to the formation of hydrosalpinx and presents as a tubular pelvic mass. Both of these conditions can be chronic or acute and should be always suspected in any woman with a history of malodorous cervical discharge, pelvic pain and fever [14]. A purely clinical approach using the findings of lower genital tract inflammation (leukorrhea) associated with pelvic organ tenderness will identify the vast majority of women with PID [15].The uterosacral ligaments and parametrium in our patient were not tender. There was no fever, malodorous cervical discharge, no the history of PID so it was quite difficult to diagnose preoperatively.

Infections due to *Neisseria gonorrhoeae*, like those resulting from *Chlamydia trachomatis*, are a major cause of PID in the United States [16]. But *Neisseria gonorrhoeae* and chlamydia are rarely recovered from an abscess. The most commonly isolated organisms from TOAs are *E. coli* and *Bacteroides species* [17]. There is an association between the presence of the vaginal flora bacteria and/or actinomycetes and TOA in women who use IUDs. There is an increased risk of ascending infection. IUD tails facilitate the transfer of bacteria from the vagina/cervix to the upper genital tract [18]. The presented patient used to have IUD but she had her IUD removed 2 months ago. No vaginal bacteria or actinomycetes in the specimens could be detected.

C. septicum causes myonecrosis through the release of exotoxins such as the alpha toxin, lethal toxin and hemolytic toxin. They were initially believed to be non-pathogenic. On the other hand, alpha toxin of *C. septicum* is necrotic and lethal. Bacteremic infections with *C. septicum* are associated with a mortality of 68% and should be treated as a medical emergency [19]. Owing to its' anaerobic nature *C. septicum* can be detected in areas of decreased blood flow. *C. septicum* infections are often detected in individuals with the recent history of trauma, surgery, peripheral vascular disease, diabetes, colon cancer, skin infections or burns and septic abortions [20]. The diagnosis of *C. septicum*-associated large bowel malignancy may be delayed or missed. Clinical manifestations are commonly nonspecific, mimicking more common disorders. At times, no clinical clue to a colon malignancy is present. Some clinicians may be unaware of the association. Bacterial sepsis may be the initial feature of previously undiagnosed and unsuspected large bowel carcinoma [21]. There are some atypical presentations of *C. septicum* infections that have been reported in recent years. The possible mechanism of these unusual presentations is generally hematogenous seeding of microorganism and defective circulation. Halak et al. [22] reported a *C. septicum* infection at aortic graft. The patient had an abdominal aortic aneurysm reducing blood flow in the affected areas. It was reported that myonecrosis caused by *C. septicum* in an immunosuppressed patient with no colon cancer, but rather colonic mucosal inflammation produced by *C. difficile* [23]. *C. septicum* infection may be detected in the orbita, brain, aorta and lower limb [24–30]. *E. coli* may be a concomitant or predisposing factor [25]. A rare mechanism is direct extension of infection, such as from incarcerated internal hernia [29]. In 2009 Wagner et al. [12] reported a ruptured tubo-ovarian abscess and septic shock with *Clostridium perfringens* in a postmenopausal woman. The hysterectomy specimen of that patient revealed endometrial carcinoma [12]. A fatal case of *Clostridium sordellii* septic shock

syndrome associated with medical abortion was reported [31]. Endomyometritis and toxic shock associated with *Clostridium sordellii* and *Clostridium perfringens* after medical and spontaneous abortion is well known [13]. Necrotising endomyometritis due to *C. sordellii* infection may be notable for lack of fever, haemoconcentration and a profound leukocytosis [32]. These properties are remarkably similar to those of ruptured TOA due to *C. septicum*. Ruptured TOA due to *C. septicum* is an extremely rare phenomenon. After evaluation, malignancy could be definitively ruled out in our patient. The presented patient, however, was not treated with immunosuppressive drugs. There was no evidence of immunosuppression.

There are few large randomized trials guiding appropriate clinical management of TOA, including antibiotic selection and timing of surgical management and drainage (Table 1) [33]. Radiographic size, leukocyte count, age, and parity are associated with operative or procedural treatment of TOA [34]. Originally, treatment of TOA was thought to perform bilateral oophorectomy and hysterectomy. Medical management with broad spectrum antibiotics is nowadays generally considered as the initial management for unruptured TOAs [2]. The Center for Disease Control (CDC) and Prevention Sexually Transmitted Diseases Treatment Guidelines recommend inpatient intravenous antibiotics for at least 24 hours. No specific inpatient antibiotic regimen was suggested. Upon discontinuation of parenteral therapy, the CDC recommends clindamycin or metronidazole to be used with doxycycline for a total of 14 days of treatment [35]. The best treatment alternative for ruptured TOA is surgery. Parenteral antibiotic therapy cannot be adequate for complete healing. Patients with larger abscesses usually need surgery [34, 36]. Dewitt et al. [36] reported a 60% failure rate of antibiotic treatment for abscesses with dimension of ≥ 10 cm. After intravenous antibiotics terapy, we performed salphingooophorectomy in the presented patient.

Table 1

Recent cases of atypical presentations of *Clostridium septicum* infections

References	Localization	Predisposing disease	Concominant infection
Halak et al. [22]	Aortic graft	Abdominal aortic aneurysm	None
Gnerlich et al. [23]	Colon	End-stage renal disease and immunodeficiency syndrome	*C. difficile* colitis
Fejes et al. [24]	Orbita	Colon tumour and lymphatic malignancy	None
Williams et al. [25]	Brain abscess	Haemolytic uraemic syndrome	*E. coli* 0157
Annapureddy et al. [26]	Aorta	Atheromatous disease of the aorta	None
Rewa and Smith [27]	Lower limp compartment syndrome	Non-Hodgkin's lymphoma	None
Ge and de Virgilio [28]	Aorta	Sigmoid colon adenocarcinoma.	None
Granok et al. [29]	Lung pleura	İncarcerated internal hernia	None
Burnell et al. [30]	Prosthetic joint of knee	Colonic malignancy	None

Conclusion

C. septicum may cause pelvic inflammatory disease and turbo-ovarium abscess in patients without any predisposing factor. Diagnosis of turbo-ovarium abscess due to *C. septicum* is very hard. Lack of fever and malodorous cervical discharge, no history of pelvic inflammatory disease, no pain on movement of cervix are diffuculties. The infected issues with *C. septicum* can become necrotic. A combination of early adequate antibiotic therapy and surgery is the key point of treatment. Although there was no malignancy in the presented patient, malignancy should be kept in mind for all kinds of infecions due to *C. septicum*.

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The contribution of Serbian doctors to the development of physical exercise in the Kingdom of Serbia

Doprinos srpskih lekara razvoju fizičkog vežbanja u Kraljevini Srbiji

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Key words:
history of medicine; serbia; world war I; military personnel; exercise.

Ključne reči:
istorija medicine; srbija; prvi svetski rat; lekari, vojni; vežbanje.

Introduction

After gaining independence at the Congress of Berlin (1878), and the declaration of the Kingdom of Serbia (1882), young Serbian country began to develop strongly economically. Frequent and turbulent political changes during this period also caused some changes in cultural and educational life of the country. Many Serbian intellectuals then came back to free Serbia in order to transfer knowledge and experience from advanced and larger European countries.

During the 19th century, different forms of physical exercising rapidly developed in Europe. In Germany "Jan's turners" gymnastics was practiced; in Sweden, the main method was "Ling's system of health gymnastics", while in England modern sport was expanding with the establishments of sport clubs; in France were organized associations in which the system of "Amoros training" was used, and in the Czech Republic and other Slavic countries was gradually introduced the "sokol" system of exercising [1].

All of the mentioned large systems of physical exercising were expanding their influence on other European countries, intertwining with each other, thus creating the most efficient system of physical exercising. These influences were coming to Serbia from the middle of the 19th century, through young intellectuals, educated abroad. With their return to Serbia they brought back experiences they had on physical exercising, often opening private schools or civil societies for physical exercising. These were mostly schools for adopting fencing, gymnastics or swimming abilities. By the 80s of the 19th century, the school with the most success and perseverance in the emergence and the development of physical exercising was "School of painting Steva Todorović", which worked intermittently from 1857 to 1876.

Contribution of Dr. Vladan Đorđević to sports

Several years after the end of the Serbian-Turkish war in October 1881, the Serbian doctor, and at that time the chief of medical services at the Ministry of Internal Affairs, Dr. Vladan Đorđević, advocated the establishment of a new association for physical exercising (Figure 1) [2]. He wanted the realization of that idea to get the widest support, so that the society can have the continuity the former societies could never be praised about having. Dr. Đorđević addressed, at the beginning of realization of the idea, Belgrade professors of gymnastics and fighting, namely: Stevan Todorović, Professor of physical exercise in "Realka"; Ferdo Mihoković, Professor of gymnastics and fighting at the Military Academy, and Ljubomir Ilić, Professor of gymnastics in Belgrade Gymnasium. He requested from them to teach the students without charge, to make the society able to pay for the services of teachers. When he received a positive response from the group of professors, Dr. Đorđević addressed the citizens with these words:

"Allow me, for just a moment, to remind you of one circumstance, which in our humble opinion deserves our full attention, and this is a factor in the way the physical development is being nurtured today. No doubt you are, just like the undersigned, convinced that the gymnastics, when arranged systematically by age, sex and national customs, is one of the strongest, if not the strongest lever for improving the physical, and because of this, the moral health of the individuals and the entire nations. However, until now, there has been paid a poor amount of attention to that systematic way of physical exercising. Apart from the Military Academy, where this has been systematically done from the start of the school, and the Gymnasium which sometimes had, and sometimes didn't have a gymnastics teacher. Although the name Gymnasium origins from gymnastics, there are barely

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Fig. 1 – Dr. Vladan Đorđević (1844–1930).

three or four real gymnastics teachers in whole of Serbia, and even they have no place besides the academy to train their students. But even if gymnastics would be continuously done in all schools of Serbia, from elementary schools in villages to high schools in Belgrade, and if these schools had everything needed to do the physical exercises continuously and uninterrupted even trough winter, it still wouldn't be enough for the gymnastics to benefit all the people as much as they can. The whole rural population engaged in commerce, crafts, arts and science, mostly spends their life after school with very little physical exercise, or spends it with one-sided physical straining, which are harmful to their health. In countries that are more advanced than our country, there are countless gymnastics affiliated societies with hundreds of magnificent Gymnasiums, where all the classes of citizenship have the opportunity and the resources to continue, for the rest of their life, the work they started at school, and to adjust it to every age, and to later occupation etc. There are countries, such as today's Germany Empire, where the numerous gymnastics associations which compile a vast network are spread through all the countries the German people was torn to, and which haven't only done a great job at improving the physical health of the entire nation, but were also useful to the ideas of civil community and its targets, because of their annual assemblies. For the entire 50 years of their practice, gymnastics associations of German people, have done, for the national mission, almost the same as the German school and literature. They have, by improving the physical strength of the people, at the same time worked on bringing together, mutual understanding and fraternizing of the most separated provinces of German people in terms of language, and life overall. This line of thought led to the belief that it would be about time to do some serious work on popularization of physical exercising and fighting in our country. By leaving it up to the administration of civil education to take care of gymnastics in schools, we reckon that the citizenship and the officialdom in Belgrade should take the initiative for the establishment of a large association for physical exercising and fighting, which would on one side give the opportunity to those who are no longer in school to use the benefits of physical exercising, and on the other side it would educate a large number of capable teachers of gymnastics, through which would this association be able to set up gymnastics associations in every city in Serbia. Since the professors and the gymnastics teachers in Belgrade stated their preparedness to be professors and teachers of physical exercising and fighting without pay, in the possible Belgrade gymnastics association, as long as society is unable to pay the experts for that, and since the High Government of His Majesty charmingly accepted this idea of establishing such a society in Belgrade, and promised their help to ensure social uprising, the necessary statutes for the establishment of association have been made, and the realization of this idea can now start.(…)" [3].

With this letter, which to this day has a use value, Dr. Vladan Đorđević approached renewing and initiating physical exercising in the Kingdom of Serbia. Luckily for him, but us also, the starting of the "Belgrade Association for Gymnastics and Fighting" was successful, and experienced continuity. After the final establishment of the association in 1882 they changed its name to "Belgrade Gymnastics Association Soko", with the acceptance of the "sokol" idea. An interesting fact is that the opinion of Dr. Đorđević, represented when he was sending letters to Serbian citizenship, shortly before forming the association, was transformed into a statute.

The aim of this association was: to work on improving physical strength and health of its members, by using physical exercising and excursions; to ensure the creation of similar associations in Serbia, and to stand in connection with them; to in addition to strengthening and toughening of the children of our country, spread awareness of the Serbian community, and in that way achieve the unification of the fragmented Serbhood in the best way, and as soon as possible [4].

With the change of the name, and the adaptation of "sokol" exercising, a part of the membership was dissatisfied with the change in the "course" of the association, so they founded a civil gymnastics association "Dusan the Mighty" in the following year of 1892. Members of the newly formed society were against accepting the Czech "Sokol" system of exercising, because it was, in their opinion, created by Czech as a resistance to germanization, while Serbia as an independent country did not have the need to induct such a system [5]. Both associations got their branches in the Kingdom of Serbia, and the territory outside the kingdom that was inhabited by Serbian people, so that they soon formed alliances of these associations. Their work was independent until the final unification on February 2, 1910. This joint alliance of "sokol" associations, "Dusan the Mighty" worked vigorously on uniting all Serbian "sokolism", essentially and in supporting parts: the suit, the sign, the salute, the slogan, the name, the song and the commands.

Dr. Vladan Đorđević did various functions in "Belgrade Association for Gymnastics and Fighting", where he was president from 1889 to 1891. He was at the position of the vice president from 1886 to 1888. After the change of the associations name, at one period of the time, another famous Serbian doctor, Milan Jovanović Batut, found himself at the position of president, and held the presidency from 1896 to 1897. Because of his merits in the development of the Olympic thought, Dr. Jovanović was an honorary member of the Serbian Olympic Committee, in addition to the famous names of the social life of that time: Dr. Vladan Đorđević, industrialist Đorđe Vajfert, retired General Boža Janković, university rector Sima Urošević and others [6].

Participation of Serbian "sokols" from the Kingdom of Serbia along with Serbian "sokols" from Vojvodina, Slavonia, Bosnia, Herzegovina and Croatia on second Croatian rally in Zagreb on August, 12–16 1911, meant a great deal to the development of the whole Serbian "sokol" movement, and the realization of the common desire to live in one country.

Confirmation of the importance of gymnastics for a nation, (in the proclamation he specifically spoke of the German people) which Dr. Vladan Đorđević stated in his address to the citizens materialized after 30 years in the Serbian nation. In the year of 1881 the Serbs were without a single association for physical exercise, and after thirty years of work in the field of physical exercise, the same people appeared united on the "Svesokol" rally in Zagreb. The energy that Dr. Vladan Đorđević invested at the startup of physical exercise in Serbia was beneficial, and gave big profits.

Vladan Đorđević was born in Belgrade on November 21, 1844. He finished the Gymnasium in Belgrade, and medicine in Vienna, where he specialized surgery. At first he worked as military doctor, then the Head of the Surgery Department of the Military Hospital, and later as chief of the Ministry of Internal Affairs, and the personal physician of King Milan Obrenovic (1854–1901). In the Serbian-Turkish wars, from 1876 to 1878, he was the head of the military corps, and in the Serbian-Bulgarian War in 1885, he was the chief of the High Command Medical Corps. Dr. Đorđević is one of the founders of the Serbian Medical Association (1872) and the Serbian Association of the Red Cross. In addition to professional medical papers, he wrote dramas, short stories and historical papers. He died on August 31, 1930 [5].

Contribution of Dr. Vojislav Subotić to sports

Vojislav Subotić was the son of the Serbian poet and politician Jovan Subotić. He was born in Novi Sad in 1859. He finished the elementary school in Zagreb, while his gymnasium studies began in Sremski Karlovci and ended in Novi Sad. He began his medical studies in Vienna, but he cut them short to take part in the Serbian-Turkish War in 1876–1878. As a volunteer he took part in the battles on the Drina. After having participated in the war, he continued his studies in order to be promoted to doctor at the age of 22.

He began his practice in Zemun in 1884 when was appointed city physician and the primary doctor. He later founded the first surgical department of the Zemun hospital. During the Serbian-Bulgarian War, still as a Zemun surgeon, he entered the Kingdom of Serbia and treated the Serbian wounded. At the invitation of Serbian Medical Corps, although a young surgeon, he opened a surgical department in Belgrade in 1889, in former Palilula Hospital (now the building of the Serbian Medical Association). In the year of 1907 a new general hospital on Vracar was built, and the first head of the Surgical Department was Dr. Voja Subotić. The same year he organized the first meeting of the Serbian surgeons, and in 1911 he organized the Congress of the Yugoslav surgery which was attended by 132 participants [7].

In wars from 1912 to 1918 Dr. Subotić worked as military surgeon. In the Balkan Wars, he worked in the background, in Belgrade, where the wounded were arriving from the battlefield. During the World War I he worked as a reserve medical colonel in Belgrade, and then in Niš. Not wanting to fall into slavery, he evacuated over Albania, even though his health condition was difficult because of the stenocardia attacks, which followed him throughout the rest of his life. Feeble and weakened, he no longer had his surgical department, so he went to Paris and London, where he worked (1916–1918) at interlial commission as our delegate, using his many acquaintances in international medical circles. In London he delivered a lecture on epidemic typhus in Serbia 1914–1915.

In 1916 he constructed a rail for immobilization of the thigh, and showcased it at the Paris Academy of Medicine, because of which, among other important favours to our medical services, he got elected member of the Paris surgical association in 1916. In early 1918 he returned to Corfu and made himself available to the Minister of the Military, from where he went to Thessalonica. There he was offered safe work in the background of Salonika front, but he refused. He asked to work on the front and chose the Second Army, under the command of Marshal Stepa Stepanović, and he worked there with his students. The experience of Dr. Subotić and his advices often helped in saving a large number of seriously wounded. When he crossed the Salonika Front with his students and colleagues, he worked in "field surgical hospital" in Dragomance near Bitola.

About this hospital, Marshal Stepa Stepanović said:

"This hospital, thanks to unique organization and improvisation of all kinds of things, responded best to its task. It will serve as a model and a historic lesson in history, on how a field surgical hospital should look [7]."

Dr. Vojislav Subotić gained a reputation of an associate war surgeon, with his reports and accomplishments in work, because he was the initiator and the carrier of the epoch of modern surgery in our country. He was not randomly chosen at that time for a member of the Association of War Surgeons of USA and England.

The idea for the establishment of the Faculty of Medicine came back in 1898. But it was only after the World War I ended, February 20, 1920, that administrative functioning of the Faculty of Medicine in Belgrade started. For the Dean of the newly formed Faculty was elected Professor Dr. Milan Jovanović Batut, and the first vice dean became Professor Dr. Subotić. In the following year, 1921, he was elected Dean.

For his contribution to the medical science and surgical practice, Dr. Vojislav Subotić received for life the highest awards, medals and recognition. In addition, he was a member of the German, the French, and the International Surgical Association, Pest Medical Association, and a member of the Paris Academy of Medicine. Dr. Subotić died in December, 1923 in Belgrade, at the age of 64.

In addition to the immeasurable contributions to Serbian medicine Dr. Subotic was a great representative of the idea of physical exercise. On May 1, 1897, he founded the fight association "Serbian Sword" [8] with the aim of propagating the knightly sport, fencing and saber combat. One of the founders and the first teacher in the association was Sarl Duse [9]. From 1891 he was engaged as a Professor of combat at the Military Academy of Belgrade. Professor Duse finished the Brussels School of Fencing (*Ecole Normale de Escrime*) founded in 1885 in Brussels, Belgum and was the first in Serbia to begin training students by the French method. The association was aimed exclusively to the development of fencing among young people, and gave the best competitors to army and the public from 1898 to 1909. "Serbian sword" owned a very beautiful and arranged gym, where competitions were often organized. A great lover of fencing was also Dr. Vojislav Subotić. He was, in addition to Dragomir Nikolajević, Bogoljub Dinić and Sublieutenant Aleksandar Josifović, one of the best students in the Association "Serbian sword" [10].

The photographs from the personal archives of Colonel Nikolajević, about a century old, show the trainings in which teacher Duse and his students, Dr. Subotić and Captain Nikolajević participated (Figure 2). With his participation in one of the oldest associations for physical exercise in the Kingdom of Serbia, Dr. Subotić made a significant contribution to the general popularization and acceptance of physical exercise among Serbian people. Thanks to the work of the fight association "Serbian sword" and the engagement of Dr. Vojislav Subotić, among others, it has come to an increased interest of the youth in the sport of fencing.

Contribution of Dr. Laza Popović to sports

Dr. Laza Popović was born in 1877. After finishing the Serbian Orthodox Great Gymnasium in Sremski Karlovci he studied medicine in Vienna, where he was promoted to general practice doctor in 1901. During his studies he met a significant number of colleagues from the Czech Republic, who introduced him to the idea of "sokol". The "Sokol" association in Sremski Karlovci was founded on his initiative on February 19, 1904 [5]. He was the founder and editor of the "Serbian Soko" magazine, which was published from February 1907. The magazine was printed in Serbian monastic print shop in Sremski Karlovci. Although it was the gazette of "sokol", it also gave information about all Serbian associations of physical exercise. The editorial board strived to make the relations, between the knightly associations "Dusan the Mighty" and "Sokols", better. When reporting on the activities of the Belgrade's "Dusan the Mighty", it is emphasized:

"There was a large audience from the most elegant and high Belgrade circles present, and there were also many Sokols, thus documenting their desire to maintain fraternal relations between the two gymnastics associations [11]."

The "Sokol" association of Sremski Karlovci organized on the regular basis the Vidovdan "Sokol" rallies where "sokols" from all of Serbia would gather. At the second "Svesokol" rally in Zagreb in 1911, at the initiative of Dr. Laza Popović, the Alliance of Serbian "Sokol" associations, which disregarded the country borders at that time, and gathered all the Serbian "Sokols" in one alliance, was established [12].

Fig. 2 – Teacher Sarl Duse (left), Vojislav Subotić (middle) and Dragomir Nikolajević (right).

When the World War I begun, Dr. Popović was accused, along with a group of "sokols" from the Austro-Hungarian territory, of high treason by the Austro-Hungarian state and was sentenced to fourteen months of imprisonment. In the same process also were sentenced Dr. Srđan Budisavljević (head of the Serbian "Sokols" in Zagreb) to eight months in prison, Milan Metikosa (the leader of the Kraina

region) to ten months of prison, Milan Todorović (the leader of the Fruska Gora region) to six months in prison, and Đuro Gavrilović (the leader of the Serbian "Sokols"in Zemun) to thirteen months in prison. The indictment charged them, with the development of political ideas in the "Socol" Society, a connection with the "National Defense" organization, and action towards planning of the annexation of a part of the Austro-Hungarian Monarchy to the Kingdom of Serbia [12]. After the conviction, the University of Vienna took away Dr. Popović's diploma. After the war ended, he was promoted again in Prague in 1918. With the end of the war he became the chief physician and the head of X-ray laboratory in Zagreb hospital. He was the first president of the Society for Radiology, founded in 1927.

Dr. Laza Popović, with his persistent effort in "sokolizm", influenced the acceptance of the "Sokol" ideas, and the development of the fraternal and national sence of the "sokol", from the territory of Austro-Hungarian Monarchy, the Kingdom of Serbia, and countries overseas where Serbs live. Although he was not involved as a physician in the medical service in the army of the Kingdom of Serbia, he made a significant contribution and gave encouragement to the Serbian "sokols", to join the army of the Kingdom of Serbia in the Balkan Wars, and World War I, and thus support the joint Serbian and "sokol" thing. For the mentioned reasons, Dr. Laza Popović found himself in the company of doctors from the Serbian military medical service, who have made outstanding contributions to the development of physical exercise to the end of World War I.

Contribution of Dr. Miloš Borisavljević to sports

Dr. Miloš Borisavljević was born in Ivanjica in 1855. He went to school in Ivanjica and Kragujevac, and finished medicine in Moscow. He spoke and wrote in Russian, French and German. Miloš Borisavljević belonged to the generation of Serbs who had participated in several wars. At the age of 20, he gained his first war experience as a scribe at the headquarters of the Užice brigade, on Javor, in the Serbian-Turkish Wars 1876–1878. With the decree of King Milan Obrenović he was admitted for a military doctor, in 1883, and served as the head of the military hospital in Kruševac, Kragujevac, Niš and Zaječar. Towards the end of the 19th century he became the director of one of the oldest medical institutions in Serbia, the Military Hospital in Niš. From 1901 to 1904 Dr. Borisavljević was the chief of the Army of the Kingdom of Serbia. From 1902 he parted in the Balkan Wars, and later in World War I. Although he was already in the sixth decade of his life, he shared with the army the sweet fruits of victory, but also the bitter fruit of defeat and the exhausting retreat through the Albanian mountains.

He was the active member of the Serbian Association of Red Cross since 1885. At the last meeting of the Association before World War I, on July 11, Dr. Borisavljević was elected President. After the retreat of Serbian Army to Corfu, as the President of the Serbian Association of the Red Cross he sent a famous proclamation to friends of Serbian people in the whole world:

"The history of the world does not remember a greater tragedy, than the one the Serbian people have suffered. In an unequal battle, Serbian army, while defending, and soaking every inch of their country with their blood, had to leave their homeland. The people have drunk even that bitter cup of temptation stoically, and the Serbian soldiers, naked, hungry, cold and haggard, haven't, even then, betrayed their allies. Serbian association of Red Cross was left without any resources, to be able to continue to provide human and Christian support, and therefore we are asking for help from everyone who has Serbian cause and Serbia in their heart." [13]

After this appeal of Dr. Borisavljević and the Serbian association of Red Cross in exile, live actions were let to help collect contributions in money, clothing, hospital and medical supplies for the wounded Serbian soldiers, refuges, prisoners…

Dr. Borisavljević sent another call for help just after the War in 1918. This time also, the support from all over the country, and from foreign countries did not lack. At the meeting of the Serbian Association of the Red Cross in 1921, they changed their name to the Red Cross Association of the Kingdom of Serbs, Croats and Slovenes, and Dr. Borisavljević wished best of luck to the new management in the future work.

This true patriot and enthusiast for the Serbian cause, did not spend his valuable time exclusively on the purposes of the military medical service and the work in the Serbian Red Cross association, but also in the development of the "Sokol" movement in the Kingdom of Serbia.

The annexation crisis that followed the annexation of Bosnia and Herzegovina into Austro-Hungarian Monarchy, in October 1908, sparked a national discontent of Serbian people, who started to gather and unite around this problem. The joint demonstrations were attended by the members of the "Sokol" movement and the members of the knightly societies "Dušan the Mighty". Certainly. This political crisis accelerated the realization of the unification of the "Sokol" Association and the Knightly Society "Dušan the Mighty" into the union of "Sokol" association "Dušan the Mighty". Final unification into one association took place in early 1910, and at the second assembly of the union of the "Sokol" Society "Dušan the Mighty", in February 1911, medical services Lieutenant Colonel Miloš Borisavljević was selected for one of the vice presidents. The assembly elected the honorary president Steva Todorović and Dr. Miloš Borisavljević for the official representatives of this union in the "Svesokol" Alliance of the Slavs. At the same meeting it was decided that the members of this union can also be the members of the Olympic club [1]. At the assembly of the Belgrade "Sokol" Association "Dusan the Mighty", in January 1910, instead of the former president Steva Todorović, the managerial position went to Dr. Miloš Borisavljević. All the abovementioned functions in Belgrade or the Federal Association "Dušan the Mighty" confirm the high level of respect Dr. Borisavljević enjoyed among the members of the association. Participation in the development of physical exercise in the Kingdom of Serbia confirms the versatility of the medical Colonel Miloš Borisavljević.

Conclusion

After gaining independence at the Congress of Berlin (1878), and the declaration of the Kingdom of Serbia (1882), started the strong economic development of young Serbian country. In addition to the economic, there was also a noticeable cultural development, which was most often encouraged by the Serbs educated in advanced countries of Europe. As Serbia moved toward full independence, various societies for physical exercise appeared. Societies that appear in the first three decades of the second half of the 19th century were not able to maintain their continuity. From 1881 a significant role in advent and development of physical exercising among Serbian people was taken by Serbian doctors. As highly educated persons they recognized the importance of physical activity from the health point of view, but also the positive aspects of socialization and the national gathering in the societies of physical exercising. The establishment of the "Belgrade Association for Gymnastics and Fighting", initiated by Dr. Vladan Đorđević in late 1881 was the initial energy that produced more federations and a large number of societies for physical exercising in the next 30 years. The participation of Vojislav Subotić in the development of the "Serbian Sword" Association gave a lot of energy in the emergence and the development of the sport of fencing among Serbian people. Dr. Miloš Borisavljević held high functions in the united association of Serbian "Sokol" Aociety "Dušan the Mighty", while Dr. Laza Popović was a leader of the Fruška Gora region, and one of the most important figures in the initiation and the development of "sokolism" among Serbs in the Austro-Hungarian Monarchy. For all the persons whose contributions were analyzed in the text, it is characteristic that they were great patriots, as they proved by their participation in several wars as members of the military medical services. Dr. Laza Popović was not it the medical services of the Serbian Army, but he proved his patriotism with great zeal that costed him imprisonment by the Austro-Hungarian Monarchy. Their high positions in the Serbian society helped the realization of various sports ideas and content, popularization of physical exercise, and the overall national liberation that was realized within the Kingdom of Serbs, Croats and Slovens.

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

Farmakologija renin-angiotenzin sistema

Naslov: Farmakologija renin-angiotenzin sistema
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Izdavač: Medicinski fakultet, Banja Luka, Republika Srpska, BiH
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Lekovi koji blokiraju renin-angiotenzin sistem (RAS) predstavljaju, bez sumnje, jedno od najvećih dostignuća na polju kardiovaskularne farmakoterapije u poslednje tri decenije. O njihovoj aktuelnosti i značaju govori podatak o trenutnom broju registrovanih predstavnika dve glavne grupe blokatora RAS-a širom sveta: inhibitora enzima koji katalizuje pretvorbu angiotezina I u snažni vazokonstriktor angitenzin II (engl. *angiotensin converting enzyme inhibitors* – ACEI) i blokatora angiotenzinskih AT1 receptora preko kojih angiotenzin II ostvaruje vazokonstrikciju i druge efekte koji dovode do funkcionalnih i strukturnih poremećaja u kardiovaskularnom sistemu. Nedavno objavljeni podaci o najpropisivanijim lekovima u SAD u prošloj 2013. godini (http://www.medscape.com/viewarticle/825053) pokazuju da se na 6. mestu nalazi valsartan, lek iz grupe blokatora angiotenzinskih AT1 receptora, tzv. sartana, što potvrđuje napred navedeno.

Iako se o ACEI i sartanima u domaćoj stručnoj literaturi dosta pisalo tokom proteklih godina, u knjizi "Farmakologija renin-angiotenzin sistema", međutim, po prvi put je dat sveobuhvatni prikaz fiziološke i patofiziološke uloge pojedinih komponenti RAS-a, istorijata istraživanja ovog sistema, otkrića lekova koji ga blokiraju, njihove kliničke primene, kao i pravaca novih istraživanja u toj oblasti. Ako se ima u vidu da je knjigu napisao naš poznati farmakolog prof. dr Rajko Igić, koji je najveći deo svog plodnog naučnoistraživačkog rada posvetio, upravo, izučavanju farmakologije RAS-a, onda možemo biti sigurni da je pred nama štivo koje dolazi iz "prve i prave ruke".

Knjiga sadrži sedam poglavlja, ne računajući Predgovor, Uvod i Zaključna razmatranja: 'Istorijat renin-angiotenzin sistema', 'Komponente renin-angiotenzin sistema', 'Kalikrein-kinin sistem', 'ACE inhibitori', 'Blokatori angiotenzinskih receptora', 'Inhibitori renina' i 'Inhibitori vazopeptidaza', potkrepljena sa 92 literaturna navoda. Pojedina poglavlja sadrže slike i tabele kojima se dodatno pojašnjavaju navodi u tekstu.

Na samom početku knjige, odmah posle sadržaja, dat je Spisak tabela i slika, sa oznakom stranice na kojoj se nalaze, tako da se pojedini prilog može odmah pronaći i pogledati bez prelistavanja cele knjige. Posle tog spiska, sledi Spisak skraćenica koje se pominju u tekstu, što omogućava njegovo lakše razumevanja. Ovde treba napomenuti da bi možda bilo zgodnije da je Spisak skraćenica dat pre Spiska tabela i slika budući da se i u naslovima tih priloga pominju pojedine skraćenice. Na kraju knjige naveden je bogat Indeks pojmova koji se pominju u tekstu, s navođenjem stranice na kojoj se nalaze, što pojednostavljuje snalaženje u knjizi i olakšava njeno iščitavnje.

U knjizi su, u posebnom poglavlju pod nazivom Prilozi, dati osnovni podaci o bolestima u čijoj patogenezi značajnu ulogu igra poremećaj RAS-a, i čija se terapija danas bazira, velikim delom, na blokatorima tog sistema. To su: arterijska hipertenzija, dekompenzacija srca, infarkt miokarda i apnea u snu. Iako je sam autor u Uvodu naglasio da su ovi prilozi "dati s namerom da nelekari lakše prate pojedine segmente knjige", mišljenja sam da će oni biti od pomoći i studentima medicine i tek svršenim lekarima kao mali repetito-

rijum o najvažnijim aspektima navedenih bolesti i njihovog lečenja.

U knjizi je data i kratka biografija autora sa spiskom njegovih publikacija koje se odnose, prvenstveno, na istraživanja RAS-a, tako da čitaoci, ukoliko žele, mogu da pronađu i izvorne radove autora i ocene njegov doprinos izučavanju pojednih komponenti ovog sistema.

Knjiga je pisana lepim i ujednačenim stilom, tabele i slike su jasne i pregledne, sa svim neophodnim objašnjenjima. Gde god je bilo potrebno, dodatna objašnjenja za neke navode u tekstu data su u fusnotama, tako da, praktički, ništa nije ostalo nerazjašnjeno. Ovakav pristup pisanju knjige svakako je rezultat višedecinijskog iskustva autora kao profesora farmakologije na dodiplomskim i poslediplomskim studijama na medicinskim fakultetima nekoliko univerziteta na prostoru bivše SFRJ, urednika i recenzenta većeg broja stručnih i naučnih medicinskih časopisa, organizatora brojnih kurseva o načinima saopštavanja rezultata naučnoistraživačkog rada u biomedicini, koji itekako dobro zna kako treba pripremiti stručni tekst da bi bio lak i razumljiv čitaocu. Zbog toga ovu knjigu s lakoćom mogu čitati i iskusni lekari i univerzitetski profesori, i njihovi studenti i mlađe kolege i svatko od njih može u njoj da pronađe ponešto što će upotpuniti njegovo znanje o RAS-u i lekovima koji na njega deluju, a, svakako, i dati podsticaj da se uključi u istraživanja novih mogućnosti modulacije pojedinih komponenti tog sistema.

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

Srpski vojni sanitet u Prvom svetskom ratu

Urednici: Aleksandar S. Nedok, Branislav Popović i Veljko Todorović
Izdavač: Medija centar „Odbrana“
Mesto i godina izdanja: Beograd, 2014.
Tiraž:700
ISBN: 978-86-335-0422-5

Glavni motiv grupi od deset autora da napišu monografiju o radu srpskog vojnog saniteta u Prvom svetkom ratu bila je želja i potreba da se na objektivan, nepristrastan, analitičan način, na osnovu izvornih dokumenata i naučnih dokaza, osvetle i protumače dostupne činjenice. Dodatni motivi bili su i dva aktuelna jubileja: 175 godina od kontinuiranog postojanja srpskog vojnog saniteta i 100 godina od početka Velikog, odnosno Prvog svetskog rata.

Monografija sadrži 429 stranica, 77 fotografija i 416 podataka citirane literature. Većinom su navođeni originalni dokumenti: naredbe Vrhovne komande i Generalštaba srpske vojske, izveštaji jedinica i ustanova, dnevnici i literalni zapisi učesnika rata, pisani dokumenti stranih armija. Monografija ima sedamnaest poglavlja u kojima su obrađene najvažnije teme o radu srpskog vojnog saniteta u Prvom svetskom ratu.

Najveći pojedinačni doprinos pisanju monografije dao je primarijus naučni savetnik dr med. nauka Aleksandar Nedok. On je obradio organizaciju, formaciju i rad saniteta u toku celog Prvog svetskog rata, posebno ističući rad saniteta u toku najvećih bitaka, u toku epidemije zaraznih bolesti, povlačenja srpske vojske preko Crne Gore i Albanije, tokom boravka na Solunskom frontu i u ofanzivi za oslobođenje zemlje.

General-major u penziji magistar filozofije Milisav Sekulić je napisao poglavlje o vojno-političkom položaju Srbije pre i u toku Velikog rata, a opisao je i najveće ratne operacije srpske vojske.

General-major u penziji primarijus dr Branislav Popović analitički je obradio sanitetske gubitke u srpskoj vojsci, ulogu i značaj saniteta u lečenju i rehabilitaciji ranjenika i bolesnika i njihovom ponovnom povratku u jedinice. On je napisao i poglavlje o radu Srpskog Crvenog krsta i Kola srpskih sestara.

Brigadni general u penziji doc. dr Veljko Todorović je utvrdio da je srpski vojni sanitet u Prvom svetskom ratu bio moderno organizovan, u duhu vremena, slično kao u najvećim evropskim armijama toga doba. Sanitetska služba je bila jedinstvena, sačinjavale su je vojnolekarska, apotekarska i veterinarska služba, a taj model organizacije se održao i danas, stotinu godina nakon tog vremena.

Pukovnik primarijus dr med. nauka Mile Ignjatović, načelnik Klinike za abdominalnu i endokrinu hirurgiju Vojnomedicinske akademije (VMA), detaljno je predstavio rad hirurške službe, ističući njena velika dostignuća i uspehe i navodeći najzaslužnije pojedince (Vojislav Subbotić, Roman Sondermajer, Lazar Genčić, Jordan Stajić, Mihailo-Mika Petrović, Čeda Đurđević, Leon Koen, Nikola Krstić), koji su spadali u najveće evropske ratne hirurge toga vremena. Nisu zaboravljeni ni istaknuti evropski hirurzi, koji su radili u srpskom sanitetu kao dobrovoljci, članovi stranih medicinskih misija (Sergej Kvintilijanovič Sofoterov, Arijus van Tienhoven i mnogi drugi).

Pukovnik prof. dr Dragan Mikić, načelnik Prvog odeljenja u Klinici za infektivne i tropske bolesti VMA i kapetan dr Miroslav Kojić, infektolog, napisali su deo o prevenciji, dijag-

nostici i lečenju zaraznih bolesti u srpskoj vojsci u Prvom svetskom ratu. Detaljno je obrađeno 11 zaraznih bolesti, sa posebnim osvrtom na epidemiju „tri tifusa“, koja je bila uzrok smrti oko 35.000 srpskih vojnika, isto toliko ratnih zarobljenika i oko 200.000 stanovnika Srbije.

Artiljerijski ppukovnik u penziji, magistar političkih nauka Luka Nikolić je napisao tekst o stranim medicinskim i humanitarnim misijama u Srbiji, koje su znatno pomogle, kadrovski i materijalno, oslabljenom srpskom sanitetu u toku celog rata. Posebno su bile brojne ruske, engleske, škotske, francuske i američke misije, a mnogi njihovi pripadnici su se istakli nadljudskim radom, požrtvovanošću i hrabrošću, što je primer i današnjim generacijama zdravstvenih radnika u Srbiji i u njihovim matičnim zemljama. Luka Nikolić je napisao i zapažen rad o lečenju, rehabilitaciji, a kasnije i vojnoj obuci oko 52.000 srpskih vojnika u Bizerti i francuskim bolnicama u severnoj Africi.

Pukovnik u penziji prof. dr Dragan Stupar prikazao je rad apotekarske službe u toku Velikog rata, koja je u granicama objektivnih mogućnosti snabdevala vojsku, sanitetske jedinice i ustanove lekovima i zavojnim materijalom, često pribegavajući iznuđenim, ali veoma funkcionalnim improvizacijama.

Ppukovnik doc. dr Radivoje Anđelković, načelnik Odeljenja za veterinarstvo Uprave za vojno zdravstvo Ministarstva odbrane opisao je organizaciju rada, najvažnije aktivnosti i probleme veterinarske službe. Istaknuta je značajna pomoć u kadrovima, opremi i sanitetskom materijalu koja je dobijena od strane Francuske, pogotovu u toku formiranja Solunskog fronta.

Na osnovu radova svih autora može se istaći da su najveći uspesi i dostignuća srpskog vojnog saniteta u Prvom svetskom ratu:

– ratna hirurgija i ratna hirurška doktrina koje su bile na nivou najboljih u evropskim armijama. Koncept zbrinjavanja ratne rane nije suštinski promenjen ni u današnje vreme, bez obzira na veliki napredak hirurgije i medicine uopšte;

– fleksibilna organizacija, koja se prilagođavala opštoj vojnoj situaciji u srpskoj vojsci i na ratištima;

– izvršena je prva transfuzija krvi u Srbiji (dr Nikola Krstić);

– obavljena je prva vazdušna sanitetska evakuacija u Srbiji, na početku 1916. godine, u završnoj fazi povlačenja srpske vojske iz Srbije. Borbenim avionima koji su dobijeni na poklon od Francuske, u nekoliko navrata obavljena je evakuacija teških ranjenika i bolesnika iz Srbije do Grčke.

– Visoka etika i moral sanitetskog osoblja, koje nije nikada napustilo svoje ranjenike i bolesnike, čak ni u situacijama kada nisu mogli da se povuku sa sanitetskom etapom ili ako bi bili zarobljeni.

– Svetao primer rada srpskog vojnog saniteta je i lečenje zarobljenih neprijateljskih vojnika. Odrednica iz „Privremenog uputa za ratnu sanitetsku službu“, koje je bilo u stvari pravilo sanitetske službe srpske vojske za rad u ratu, da „svu pomoć dužan je sanitetski personal ukazati isto tako neprijatelju, kao i svom vojniku“ potpuno je poštovana, iako su postojali brojni problemi u vezi zdravstvenog zbrinjavanja sopstvenih ranjenika i bolesnika.

O tome kolika je predanost srpskog vojnog saniteta svojoj časnoj dužnosti govori i podatak da je u toku Prvog svetskog rata, od ukupno 594 lekara, apotekara i veterinara, umrlo i poginulo ukupno 143 (svaki četvrti, odnosno 25%). Umrlo je i 25 stranih lekara iz humanitarnih misija. Najčešći uzrok smrti je bilo oboljevanje od pegavog tifusa.

Osnovni nedostatak u radu srpskog vojnog saniteta u toku Prvog svetskog rata je u tome što nije poklonjena odgovarajuća pažnja preventivno-medicinskoj zaštiti od zaraznih bolesti, posebno u toku epidemije tri tifusa.

Na osnovu svega navedenog može se zaključiti da je u toku Prvog svetskog rata, i pored ozbiljnih organizacionih, kadrovskih i materijalnih problema, srpski sanitet časno obavio postavljene zadatke i dao značajan doprinos jednoj od najvećih pobeda srpske vojske u njenoj istoriji.

Knjiga o srpskom vojnom sanitetu u Velikom ratu biće od velike koristi ne samo istoričarima, lekarima i studentima medicine, nego i svim profesionalnim oficirima. Ne može se shvatiti jedinstvo i kompleksnost vojne organizacije, način njenog funkcionisanja, sistem rukovođenja i komandovanja, ako se dobro ne poznaje i ne razume najhumaniji deo tog sistema, veoma značajan u miru, ali i u vanrednim situacijama, uključujući i uvek moguće ratne opasnosti. Neraskidiva povezanost saniteta sa drugim segmentima vojske i sistema odbrane je prirodna, zasniva se na uzajamnoj zavisnosti i egzistencijalnog je karaktera. Toga mora da budu svesne sve državne i vojne strukture, jer kada god to nije bilo tako (a bivalo je), to se loše odražavalo i na vojsku i na sanitet, a nekada i na celu državu.

U ovoj monografiji je, kroz rad saniteta, prikazan samo deo teške i neravnopravne borbe malog i nejakog, ali ponositog slobodarskog naroda za svoju slobodu i opstanak. Prikazano je kolika je cena plaćena u ljudskim životima i materijalnim dobrima. Poslata je poruka „gradu i svetu“ da sloboda nema cenu i da zbog toga ne treba ni pokušavati da se oduzme onima koji znaju da je cene, kojima je to bila osnovna i najveća vrednost i koji su je u svojoj dosadašnjoj istoriji više puta skupo plaćali.

Ova monografija šalje još jednu poruku: civilizacija ne treba nikada da doživi tako veliku nesreću da bi se shvatilo šta je to rat, šta su i kolike njegove posledice i koliko duboke rane ostavlja kod svih, čak i najbezazlenijih njegovih direktnih ili indirektnih učesnika. Toga treba da su svesne današnje, ali i sve buduće generacije. Snagu, veštinu i kreativnost treba koristiti za dobrobit i izgradnju lepšeg i humanijeg sveta.

Brigadni general u penziji doc. dr Veljko Todorović
Pukovnik prof.dr Dragan Mikić

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Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

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Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2014. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

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Godišnja pretplata za 2014. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

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