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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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Zika virus (ZIKV) disease is a mosquito-borne disease caused by ZIKV which in general causes a mild febrile illness with maculopapular rash. However, recent data suggest a possible association between ZIKV and autoimmune, neurological and neurodevelopmental disorders such as Guillain-Barré syndrome and microcephaly in the fetuses and newborns from the mother exposed to ZIKV during the two first trimesters of pregnancy. *Aedes* mosquitoes are considered as main vectors. Before 2007 viral circulation and a few outbreaks were documented in tropical Africa and in some areas in Southeast Asia. Since 2007 several islands of the Pacific region have experienced outbreaks. In 2015, ZIKV disease outbreaks were reported in South America for the first time, and now it is considered as an emerging infectious disease.
The World Health Organisation states that the ZIKV is experiencing "explosive" growth worldwide, potentially threatening over half of the world's population (see Editorial, p. 225-7).

Bolest prouzrokovana virusom Zika (ZIKV) prenosi se ubodom komarca, a manifestuje se opštim blagim febrilnim stanjem sa makulopapularnim osipom. Noviji podaci, međutim, sugerišu moguću povezanost ZIKV sa pojavom autoimunskih, neuroloških i neurorazvojnih poremećaja kao što su Guillain-Barré-ov sindrom i mikrocefalija kod fetusa i novorođenčadi čije su majke bile izložene ZIKV tokom prva dva tromesečja trudnoće. Smatra se da su komarci roda *Aedes* glavni prenosioci ovog virusa. Pre 2007 prisustvo ZIKV zabeleženo je u tropskim područjima Afrike i nekim regionima jugoistočne Azije, a posle 2007. i na pojedinim ostrvima pacifičkog regiona. U 2015. godini bolest prouzrokovana ZIKV zabeležena je najpre u Južnoj Americi, ali sada se već smatra globalnom opasnošću. Svetska zdravstvena organizacija upozorila je na „eksplozivno" širenje virusa sa potencijalnim ugrožavanjem zdravlja više od pola svetske populacije (vidi Uvodnik, str. 225–7).

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

The Author and the Reviewer of the Year 2015 award by the *Vojnosanitetski Pregled*

Priznanja Autor i Recenzent godine Vojnosanitetskog pregleda za 2015.

Silva Dobrić

Institute of Scientific Information, Military Medical Academy, Belgrade, Serbia

This year, the Vojnosanitetski Pregled (VSP), an official journal of physicians and pharmacists of the Serbian Army, 21st consecutive time and 4th consecutive time awarded the Author of the Year prize and the Reviewer of the Year prize, respectively, on the Day of the Military Medical Academy in Belgrade, that is on March 2, because the Editorial Office of the Journal is situated in this institution.

As a reminder, these awards were established in order to highlight the importance of publishing in scientific journals for the expansion and improvement in medical science, but also to raise the prestige and influence of the journal in which the results of scientific research and professional work are published. In addition to authors, whose role in the process of creation of a scientific articles is indisputable, a reviewer also has a major impact on its final appearance and quality, and this is the reason for awarding the prize to reviewers, as well.

The Author of the Year, and the Reviewer of the Year prizes were first awarded in 1996 for the year 1995, and in 2013 for the year 2012, respectively.

The Author of the Year prize is given according to the criteria established in the mid nineties, when this prize was awarded for the first time (Table 1). These criateria take into account the category of articles and the order of authors in the byline, with the original article and the first place among the authors (scored only the first three authors as the most responsible for the creation of an article) provide the highest score. When it comes to the selection of reviewers, in addition to the number of reviews performed in the previous year, their quality (detailed review of each segment of an article), as well as review completion in a specified time limit are also taken into account.

On the basis of the above-mentioned criteria, the Author of the Year prize by the VSP for 2015 is awarded to Prof. Nebojša Krunić, DMD, PhD, from the Faculty of Medicine, University of Niš: He published three original articles in the VSP in 2015, being the first author in one, and the first coauthor in the other two (Table 2).

Ove godine, 21. put zaredom dodeljuje se priznanje Autor godine i 4. put zaredom priznanje Recenzent godine časopisa Vojnosanitetski pregled (VSP) koji će biti uručeni 2. marta na svečanosti obeležavanja Dana Vojnomedicinske akademije (VMA) u Beogradu, instutucije u kojoj je smeštena Redakcija časopisa.

Podsećanja radi, ova priznanja ustanovljena su sa ciljem da se istakne značaj objavljivanja radova u naučnim časopisima za širenje i unapređenje medicinske struke i nauke, ali i za podizanje ugleda i uticaja časopisa u kojima se objavljuju rezultati naučnoistraživačkog i stručnog rada. Pored autora, čija je uloga u procesu nastajanja naučnog i stručnog članka nesporna, veliki uticaj na konačni izgled i kvalitet članka koji se objavljuje, svakako ima i recenzent, pa otuda i priznanje recenzentima.

Priznanje Autor godine VSP-a prvi put je dodeljeno 1996. godine za 1995. godinu, a priznanje Recenzent godine 2013. za 2012. godinu.

Izbor Autora godine VSP vrši se prema kriterijumima ustanovljenim još sredinom devedesetih godina prošlog veka, kada je prvi put i dodeljeno ovo priznanje (Tabela 1). Oni uzimaju u obzir vrstu članka i redosled autora, pri čemu originalni članak i prvo mesto među autorima (boduju se samo prva tri autora kao najzaslužnija za nastanak jednog rada) donose najveći broj bodova. Kada je u pitanju izbor Recenzenta godine, pored broja urađenih recenzija u prethodnoj godini, u obzir se uzima i njihov kvalitet (detaljan osvrt na svaki segment rada) i poštovanje zadatog roka u kome je trebalo izvršiti recenziju.

Na osnovu naprednavedenih kriterijuma, priznanje Autor godine VSP-a za 2015. godinu pripalo je prof. dr Nabojši Kruniću, sa Medicinskog fakulteta Univerziteta u Nišu, kome su u prošloj godini u VSP-u objavljena 3 originalna članka, od kojih je u jednom bio prvi autor, a u druga dva prvi koautor (Tabela 2).

Correspondence to: Silva Dobrić, Institute of Scientific Information, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone.: +381 11 3609 311.

Table 1

Criteria for author and article scoring in the *Vojnosanitetski Pregled/* Kriterijumi za bodovanje autora i članaka u VSP

Article category/ Kategorija rada/	Score/ Broj bodova		
	first author/ prvi autor	second author/ drugi autor	third author/ treći autor
Original article/ Originalni članak/	12	6	3.6
Preliminary report/ Prethodno saopštenje	5	2.5	1.5
General review/ Pregledni članak	10	5	3
Current topic/ Aktuelna tema	8	4	2.4
Case report/ Kazuistika	4	2	1.2
History of medicine/ Istorija medicine	5	2.5	1.5
Editorial/ Uvodnik/	5	2.5	1.5

Table 2

Articles of Prof. Nebojša Krunić published in the *Vojnosanitetski pregled* in 2015/ Članci prof. dr Nebojše Krunića objavljeni u Vojnosanitetskom pregledu u 2015.

No./ Br.	Article category/ Kategorija članka	Authors and title of article/ Autori i naslov članka
1	Original article/ Originalni članak	*Igić M,* ***Krunić N****, Aleksov Lj, Kostić M, Igić A, Petzrović MB, Dačić S, Igić S, Igić A.* Determination of vertical dimension of occlusion by using the phonetic vowel „O“ and „E“. Vojnosanit Pregl 2015; 72(2): 123-131.
2	Original article/ Originalni članak	***Krunić N****, Kostić M, Petrović M, Igić M.* Oral health-realated quality of life of edentulous patients after complete dentures relining. Vojnosanit Pregl 2015; 72(4): 307-11.
3	Original article/ Originalni članak	*Kostić M,* ***Krunić N****, Najman S, Nikolić Lj, Nikolić V, Rajković J, Petrović M, Igić M, Ignjatović A.* Artificial saliva effect on toxic substances release from acrylic resins. Vojnosanit Pregl 2015; 72(10): 899-905.

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The Reviewer of the Year prize by the VSP for 2015 goes to Assoc. Prof. Slobodan Obradović, from the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade, who was the first laureate of this award when in 2013 the first time it had been allocated for 2012. In 2015 Prof. Obradović reviewed 23 papers submitted to the VSP.

On behalf of the Publisher, the Editorial Board and the Editorial Staff of the VSP, as well as in my own name, I congratulate Professors Nebojša Krunić and Slobodan Obradović on their awards, the Author of the Year 2015 and the Reviewer of the Year 2015 by the VSP, respectively, in the hope that they will continue their fruitful cooperation with our Journal in the future.

Their concise *Curricula vitae* are given beneath.

The short *Curriculum vitae* of the Author of the Year 2015 by the *Vojnosanitetski pregled* – Prof. Nebojša Krunić, DMD, PhD

Prof. Dr. Nebojša Krunić was born on August 4 1962 in Niš. He graduated at the Faculty of Medicine (Department of Dentistry), University of Niš, in 1988. He successfully passed the specialist exam in dental prosthetics in 1996. In 1998, he defended his master thesis entitled “The application of laser in the treatment of mechanically altered soft tissues in mobile laminate dental prosthesis”. He defended the doctoral dissertation “The influence of the tested parameters of the burred teeth and dental cement on retention of fixed dental prostheses“ in 2002. The narrow specialization of his professional and scientific work is dental prosthetics.

Priznanje Recenzent godine VSP-a za 2015. godine, ide u ruke prof. dr Slobodanu Obradoviću sa Medicinskog fakulteta VMA Univerziteta odbrane u Beogradu, koji je bio i prvi laureat ovog priznanja, kada je 2013. godine prvi put ono dodeljeno za 2011. godinu. U 2015. godini, prof. Obradović je recenzirao ukupno 23 rada.

U ime Izdavača, Uredništva i Redakcije VSP-a, kao i u svoje lično ime, čestitam prof. dr Nebojiši Kruniću i prof. dr Slobodanu Obradoviću na priznanjima Autor godine, odnosno Recenzent godine VSP-a za 2015, uz nadu da će nastaviti ovako plodnu saradnju sa našim časopisom i ubuduće.

U nastavku su date kratke biografije Autora i Recenzenta godine VSP-a za 2015.

Kratka biografija Autora godine Vojnosanitetskog pregleda za 2015. godinu – prof. dr Nebojša Krunić

Prof. dr Nebojša Krunić rođen je 4. 8. 1962. godine u Nišu. Diplomirao je na Medicinskom fakultetu (odsek Stomatologija) Univerziteta u Nišu 1988. godine. Na istom fakultetu položio je specijalistički ispit iz stomatološke protetike 1996. godine sa odličnim uspehom. Magistarsku tezu pod nazivom „Primena lasera u terapiji mehanički alteriranih mekih tkiva mobilnom pločastom zubnom protezom“ odbranio je 1998. godine. Doktorsku disertaciju pod nazivom „Uticaj ispitivanih parametara brušenih zuba i dentalnog cementa na retenciju fiksnih zubnih proteza“ odbranio je 2002. godine. Uža oblast njegovog stručnog i naučnog rada je stomatološka protetika.

Prof. Nebojša Krunić, DMD, PhD, the Author of the Year 2015 by the *Vojnosanitetski Pregled.*
Prof. dr sc. stom. Nebojša Krunić, Autor godine Vojnosanitetskog pregleda za 2015. godinu.

He has been working at the Faculty of Medicine in Niš, Department of Dentistry (Dental Prosthetics Service) since January 1995 when he became a teaching assistant, and in 2014 he became a Professor. He is the author and coauthor of 50 scientific papers in the field of dental prosthetics, out of which 10 papers were published in SCI listed journals. He participated in conferences (33) and lectures (5) in numerous local and international scientific gatherings. He is also a coauthor of 3 monographs (The application of lasers in orofacial surgery – Prosveta, Niš, 2004; The dysfunctions of temporomandibular joint – Prosveta, Niš, 2004; Acrylate polymers in dentistry – Naisprint, Niš, 2014).

Starting from 2006, he is a member of the Editorial Board of the journal Acta Stomatologica Naissi. He is a proofreader at the journal Acta Facultatis Medicae Naisensis and in 2003 he became a member of the Republic of Serbia Work Group for the creation of Standards in dental protection.

U radnom odnosu na Medicinskom fakultetu u Nišu, na Klinici za stomatologiju (Služba za stomatološku protetiku) nalazi se od januara 1995. godine, kada je izabran u zvanje asistenta pripravnika, dok je za redovnog profesora izabran novembra 2014. godine.

Autor je i koautor u 50 naučnih i stručnih radova iz oblasti stomatološke protetike, od čega je 10 u časopisima sa SCI liste. Učestvovao je saopštenjima (33) i pozivnim predavanjima (5) na brojnim domaćim i inostranim naučnim skupovima. Takođe, koautor je 3 monografije (Primena lasera u orofacijalnoj regiji – Prosveta, Niš, 2004; Disfunkcije temporomandibularnog zgloba – Prosveta, Niš, 2004; Akrilatni polimeri u stomatologiji – Naisprint, Niš, 2014).

Od 2006. godine stalni je član uređivačkog odbora časopisa *Acta Stomatologica Naissi.* Recenzent je časopisa *Acta Facultatis Medicae Naissenis*. Od 2013. godine član Republičke radne grupe za izradu Standarda u oblasti stomatološke zdravstvene zaštite.

The short *Curriculum Vitae* of the Reviewer of the Year 2015 by the Vojnosanitetski pregled – Prof. Slobodan Obradović, MD, PhD

Prof. Dr. Slobodan Obradović, Assoc. Prof. of Internal Medicine at the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade, was born on August 16 1968 in Zagreb, Croatia. He graduated at the Faculty of Medicine, University of Belgrade in 1994 with the average mark of 9.74, in a period 1995–1999 and in 2002 specialized in internal medicine and cardiology, respectively, in the Military Medical Academy, Belgrade.

Since the end of 1999, Prof. Dr. Slobodan Obradović has been working at the Clinic for Urgent Internal Medicine, Military Medical Academy, Belgrade, where has been the Head since October 2013.

Prof. Dr. Slobodan Obradović defended both his master's thesis and PhD dissertation in the Military Medical Academy, related to the field of hemostasis in acute coronary syndromes and percutane coronary intervention. Prof. Dr. Slobodan Obradović was chosen for Assist. Prof. and Assoc. Prof. of Internal Medicine in 2004 and 2011, respectively (Military Medical Academy).

Kratka biografija Recenzenta godine Vojnosanitetskog pregleda za 2015. godinu – prof. dr Slobodan Obradović

Prof. dr Slobodan Obradović, vanredni profesor za predmet Interna medicina na Medicinskom fakultetu VMA Univerziteta odbrane u Beogradu, rođen je 16.8.1968. godine u Zagrebu, Hrvatska. Diplomirao je na Medicinskom fakultetu Univerziteta u Beogradu 1994. godine sa prosečnom ocenom 9,74. U periodu 1995–1999. specijalizovao je internu medicinu na VMA, a supspecijalizaciju iz kardiologije završio je 2002. godine.

Od kraja 1999. godine do danas zaposlen je na Klinici za urgentnu internu medicinu VMA, gde od oktobra 2013. godine obavlja dužnost načelnika Klinike.

Magistrirao je i doktorirao na VMA iz oblasti hemostaze u akutnom koronarom sindromu i perkutanoj koronarnoj intervenciji. Za docenta iz uže naučne oblasti Interna medicina na VMA izabran je 2004. godine, a za vanrednog profesora 2011. godine.

U periodu 2008–2012. rukovodio je projektom „Matične

Prof. Dr. Slobodan Obradović was a 3-year (2008–2012) leading investigatior of the project "Stem Cells in Ischemic Heart Disease Treatment" on which he published a few scholarly papers including a chapter in the book "Stem Cells in Clinic and Research" (Gholamrezanezhad A., editor, Rijeka: In Tech; 2011).

ćelije u lečenju ishemijske bolesti srca" iz koga je objavljeno nekoliko naučnih radova, a 2011. godine i poglavlje „*Stem Cell Therapy in Myocardial Infarction*" u knjizi „*Stem Cells in Clinic and Research*" (Gholamrezanezhad A, editor. Rijeka: In Tech).

Assoc. Prof. Slobodan Obradović, MD, PhD, the Reviewer of the Year 2015 by the *Vojnosanitetski Pregled.*
Prof. dr sc. med. Slobodan Obradović, Recenzent godine Vojnosanitetskog pregleda za 2015. godinu

The results obtained in that project were included in the meta-analysis that was published in Circulation Research in April, 2015. This article was proclaimed as the best article in the journal last year.

Prof. Dr. Slobodan Obradović has been doctorial dissertation advisor/mentor of numerous PhD students in cardiology, being very active in advising young researchers. With Siniša Rusović, MD, and Prof. Dr. Branko Gligić, Prof. Dr. Slobodan Obradović is the author of the monograph "Pulmonary thromboemboly – case reports" published in 2011 reporting on 32 cases treated in the Clinic for Urgent Internal Medicine, Military Medical Academy, Belgrade, and accounting for the major diagnostic and therapeutic procedures in this condition.

Prof. Dr. Slobodan Obradović published numerous scientific articles on hemostasis, percutane coronary intervention, acute coronary syndrome and stem cells transplantation in cardiology. A period of 2009–2011 was marked by presidency of Prof. Dr. Slobodan Obradović in the Group for Thrombosis and Hemostasis, Serbian Association of Cardiologists. At the moment he is a member of several advisory bodies (councils) for anticoagulant and antiplatelet drug use in Serbia. Also, it is worth to mention, Prof. Dr. Slobodan Obradović has been a member of the VSP Editorial Board since 2010.

Exceptional creative ability of Prof. Dr. Slobodan Obradović is supported by the simple fact that in spite of very demandable professional and scientific research engagement he finds time to write, not only scholarly articles and reviews, but also short stories and lyrics usually on everyday medical practice.

Takođe, rezultati ovih istraživanja ušli su u metaanalizu koja je objavljena u časopisu *Circulation Research* u aprilu 2015. godine. Taj rad je proglašen za najbolji rad tog časopisa u 2015. godini.

Mentor je više doktorata iz oblasti kardiologije i, kao takav, izuzetno aktivan u podizanju naučnoistraživačkog podmlatka. Zajedno sa dr Sinišom Rusovićem i profesorom Brankom Gligićem autor je monografije „Plućna tromboembolija kroz prikaze slučajeva" koja je objavljena 2011. godine. U njoj su prikazana 32 bolesnika lečena na Klinici za urgentnu medicinu VMA, uz navođenje najvažnijih dijagnostičkih i terapijskih postupaka u ovom stanju.

Objavio je veći broj radova iz oblasti hemostaze, perkutane koronarne intervencije, akutnog koronarnog sindroma i transplantacije matičnih ćelija u kardiologiji. U periodu 2009–2011. bio je predsednik radne grupe za trombozu i hemostazu Udruženja kardiologa Srbije. Trenutno je član nekoliko savetodavnih tela za primenu novih antikoagulantnih i antiagregacionih lekova u Srbiji. Član je uredništva VSP-a od 2010. godine.

O izvanrednoj stvaralačkoj energiji prof. dr Slobodana Obradovića govori i podatak da on, uprkos veoma zahtevnom stručnom i naučnoistraživačkom radu, nalazi vremena i za pisanje, ali ne samo naučnih članaka i recenzija, već kratkih priča i pesama u kojima često obrađuje teme iz svakodnevne lekarske prakse.

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

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Another emerging pathogen – Zika virus

Zika virus – još jedan novoiskrsli patogen

Srdjan Lazić

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Zika virus (ZIKV) disease is caused by an emerging mosquito-borne virus from the *Flavivirus* genus, Flaviviridae family, from the Spondweni group. It was first isolated in 1947 in rhesus monkeys through a monitoring network of sylvatic yellow fever, in the Zika forest Uganda, then in mosquitoes (*Aedes africanus*) in the same forest in 1948, and in a human in Nigeria in 1952. There are two ZIKV lineages: the African lineage and the Asian lineage which has recently emerged in the Pacific and the Americas [1–4].

For many years only sporadic human cases were detected in Africa and Southern Asia. Serological surveys in Africa and Asia indicate a most likely silent ZIKV circulation with detection of specific antibodies in various animal species (large mammals such as orangutans, zebra, elephants, water buffaloes) and rodents [4, 5]. The knowledge of geographical distribution of ZIKV is based on the results of serosurveys and viral isolation in mosquitoes and humans, and with reports on travel-associated cases and very few published outbreaks. Before 2007, the areas with reported ZIKV circulation included tropical Africa and Southeast Asia. In 2007, the outbreak of Zika virus disease occurred in the Pacific. This was the first outbreak of ZIKV identified outside of Africa and Asia [6]. Between 2013 and 2015, several significant outbreaks were notified on the islands and archipelagos from the Pacific region including a large outbreak in French Polynesia. In 2015, ZIKV emerged in South America with widespread outbreaks reported in Brazil and Columbia [1–3]. Given the expansion of environments where mosquitoes can live and breed, facilitated by urbanisation and globalisation, there is a potential for major urban epidemics of Zika virus disease to occur globally.

The incubation period of disease is not clear, but is likely to be three to 12 days after the bite of an infected mosquito. Most of the infections remain asymptomatic (between 60% to 80%). The symptoms are similar to other arbovirus infections such as dengue. Signs and simptoms are usually mild and the disease is usually characterised by a short-lasting self-limiting febrile illness of 4–7 days duration without severe complications, with no associated fatalities and a low hospitalisation rate. The main symptoms are macular or papular rash, fever, arthralgia, non-purulent conjunctivitis/conjunctival hyperaemia, myalgia and headache. The maculopapular rash often starts on the face and then spreads throughout the body. Less frequently, retro-orbital pain and gastrointestinal signs are present [3].

Autoimmune, neurological and neurodevelopmental conditions such as Guillain-Barré syndrome and microcephaly in fetuses and newborns from mothers possibly exposed to ZIKV in the two first trimesters of the pregnancy were notified during recent Zika disease outbreaks in French Polynesia and Brazil, in 2013 and 2015, respectively. Further evidence is needed to establish a causal link between these neurological/neurodevelopmental impairments and infections with ZIKV [1, 2, 7].

Zika virus is transmitted to people through the bite of an infected mosquito from the *Aedes* genus, mainly *Aedes aegypti* in tropical regions. This is the same mosquito that transmits dengue, chikungunya and yellow fever. Other *Aedes* mosquito species (notably *Ae. africanus*, *Ae. albopictus*, *Ae. polynesiensis*, *Ae. unilineatus*, *Ae. vittatus* and *Ae. hensilli*) are considered as potential vectors of ZIKV. These species bite during the day (especially in mid-morning and between late afternoon and twilight) [8]. Till now in Serbia, we have not confirmed the presence of Zika vectors, but some underlying field investigations could show different picture.

Additional modes of transmission also have been identified. Perinatal transmission can occur most probably by transplacental transmission or during delivery when the mother is infected. Sexual transmission was reported in two case reports. There is a potential risk of ZIKV transfusion-derived transmission [9, 10].

The ZIKV disease diagnostics is primarily based on detection of viral RNA from clinical specimens in acutely ill patients. The viraemic period appears to be short, allowing for direct virus detection during the first 3–5 days after the onset of symptoms. ZIKV RNA has been detected in urine

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up to 10 days after onset of the disease. From the day five post-onset of fever, serological investigations can be conducted by detection of Zika-specific IgM antibodies and confirmation by neutralisation, seroconversion or four-fold antibody titer increase of Zika specific antibodies in paired serum samples. Diagnosis by serology can be difficult as the virus can cross-react with other flaviviruses, therefore serological results should be interpreted according to the vaccination status and previous exposure to other flaviviral infections [3].

Mosquitoes and their breeding sites pose a significant risk factor for this infection. Prevention and control relies on reducing mosquitoes through source reduction (removal and modification of breeding sites) and reducing contact between mosquitoes and people.

This can be done by using insect repellent; wearing clothes (preferably light-coloured) that cover as much of the body as possible; using physical barriers such as screens, closed doors and windows; and sleeping under mosquito nets. It is also important to empty, clean or cover containers that can hold water such as buckets, flower pots or tyres, so that places where mosquitoes can breed are removed. Special attention and help should be given to those who may not be able to protect themselves adequately, such as young children, the sick or elderly.

Residents and travellers visiting affected areas (Table 1) [3], particularly pregnant women, must take individual protective measures to prevent mosquito bites all day round as Zika virus disease, chikungunya and dengue are transmitted by a

Table 1

Countries and territories with recent local Zika virus transmission (EECD)

Country/Territory	Affected in the past 2 months	Affected in the past 9 months
American Samoa	Increasing or widespread transmission	Yes
Aruba	Sporadic transmission following recent introduction	Yes
Barbados	Increasing or widespread transmission	Yes
Bolivia	Sporadic transmission following recent introduction	Yes
Brazil	Increasing or widespread transmission	Yes
Bonaire	Sporadic transmission following recent introduction	Yes
Cape Verde	Increasing or widespread transmission	Yes
Colombia	Increasing or widespread transmission	Yes
Costa Rica	Sporadic transmission following recent introduction	Yes
Curaçao	Increasing or widespread transmission	Yes
Dominican Republic	Increasing or widespread transmission	Yes
Ecuador	Increasing or widespread transmission	Yes
El salvador	Increasing or widespread transmission	Yes
Fiji	No	Yes
French Guiana	Increasing or widespread transmission	Yes
Guadeloupe	Increasing or widespread transmission	Yes
Guatemala	Increasing or widespread transmission	Yes
Guyana	Sporadic transmission following recent introduction	Yes
Haiti	Increasing or widespread transmission	Yes
Honduras	Increasing or widespread transmission	Yes
Jamaica	Sporadic transmission following recent introduction	Yes
Maldives	No	Yes
Marshall Islands	Sporadic transmission following recent introduction	Yes
Martinique	Increasing or widespread transmission	Yes
Mexico	Increasing or widespread transmission	Yes
New Caledonia	No	Yes
Nicaragua	Increasing or widespread transmission	Yes
Panama	Increasing or widespread transmission	Yes
Paraguay	Increasing or widespread transmission	Yes
Puerto Rico	Increasing or widespread transmission	Yes
Saint Martin	Sporadic transmission following recent introduction	Yes
Samoa	Increasing or widespread transmission	Yes
Solomon Islands	No	Yes
Suriname	Increasing or widespread transmission	Yes
Thailand	Sporadic transmission following recent introduction	Yes
Tonga	Increasing or widespread transmission	Yes
Trinidad and Tobago	Sporadic transmission following recent introduction	Yes
Vanuatu	No	Yes
Venezuela	Increasing or widespread transmission	Yes
US Virgin Islands	Sporadic transmission following recent introduction	Yes

Based on data reported by European Centre for Disease Prevention and Control, 19 February, 2016 (http://ecdc.europa.eu/en/publications/Publications/communicable-disease-threats-report--20-feb-2016.pdf)
This table contains information on countries and territories that have recently experienced or are currently experiencing local Zika virus transmission. The classification of countries above is based on: 1) the number of reported autochthonous confirmed cases; 2) the number of affected areas in the country; 3) duration of the circulation.

daytime-biting mosquito. Consequently, protective measures should be taken, especially during the day.

Travellers that are pregnant, have immune disorders or severe chronic illnesses, or are accompanied by young children should consult their doctor or seek advice from a local public health institute before travelling in order to receive recommendations on the use of repellents and other preventive measures. Travellers showing symptoms compatible with dengue, chikungunya or Zika virus disease within three weeks after returning from an affected area should contact their healthcare provider. Pregnant women who have travelled to areas with Zika virus transmission should mention their travel during antenatal visits in order to be assessed and monitored appropriately.

Blood safety authorities should consider the deferral of donors with a relevant travel history to areas with active Zika virus transmission, in line with measures defined for dengue virus [3, 11].

Zika virus disease is usually relatively mild and requires no specific treatment. The differential clinical diagnostic should be considered as well as co-infection with other mosquito-borne diseases such as dengue fever, chikungunya and malaria. The treatment is symptomatic and mainly based on pain relief, fever reduction and antihistamines for pruritic rash. If symptoms worsen, they should seek medical care and advice. There is currently no vaccine available. Treatment with acetylsalicylic acid and non-steroidal anti-inflammatory drugs was discouraged because of a potential increased risk of haemorrhagic syndrome reported with other flaviviruses as well as the risk of Reye's syndrome after viral infection in children and teenagers.

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C-reactive protein in drainage fluid as a predictor of anastomotic leakage after elective colorectal resection

C-reaktivni protein u drenažnoj tečnosti kao pokazatelj dehiscencije anastomoze nakon resekcije kolona i rektuma

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Abstract

Background/Aim. C-reactive protein (CRP) is considered to be an indicator of postoperative complications in abdominal surgery. The aim of this study was to determine the significance of serial measurement of CRP in drainage fluid in the detection of anastomotic leakage (AL) in patients with colorectal resection. **Methods.** CRP values in serum and drainage fluid, respectively, were measured on the first, third, fifth, and seventh postoperative day (POD) in 150 patients with colorectal resection and primary anastomosis. The values obtained were compared between the groups of patient without complications of surgical treatment and those with AL. **Results.** Clinically evident AL was observed in 15 patients – in two (4.2%) patients with left colonic surgery, and 13 (12.6%) patients with colorectal anastomosis. Mean values of CRP were higher in the patients with AL than in the patients without complications, both in serum and drainage fluid, with the most significant differences recorded on the PODs 5 and 7 ($p < 0.001$). Correlation analysis showed a positive correlation between serum and drainage fluid CRP levels in both groups of patients. Serum and drainage fluid CRP values on the PODs 5 and 7 are most important in the detection of AL. In 80% of patients with CRP values in the drainage fluid of 53 mg/L for the POD 5 and 42 mg/L for the POD 7 AL was observed. The method specificity was 77% for the POD 5, and 83% for the POD 7. All the patients with CRP values in drainage fluid above 108 mg/L on the POD 5 and 93 mg/L on the POD 7 had AL. **Conclusion.** Serial measurement of CRP in drainage fluid can reliably be used in the detection of AL in patients with colorectal resection. The most significant values obtained on the PODs 5 and 7 were positively correlated with the values registered in serum.

Key words:
c-reactive protein; prognosis; surgical wound dehiscence; surgical procedures, operative; colorectal neoplasms.

Apstrakt

Uvod/Cilj. Smatra se da je C-reaktivni protein (CRP) indikator postoperativnih komplikacija u abdominalnoj hirurgiji. Cilj rada bio je da se utvrdi značaj serijskog merenja vrednosti CRP u drenažnoj tečnosti u detekciji dehiscencije anastomoze kod bolesnika sa resekcijom kolona i rektuma. **Metode.** Vrednosti CRP u serumu i drenažnoj tečnosti kod 150 bolesnika sa kolorektalnom resekcijom i primarnom anastomozom određivane su prvog, trećeg, petog i sedmog postoperativnog dana. Vrednosti CRP u serumu i drenažnoj tečnosti upoređivane su između grupa bolesnika bez komplikacija operativnog lečenja i sa dehiscencijom anastomoze. **Rezultati.** Klinički manifestnu dehiscenciju anastomoze imalo je 15 bolesnika, dva (4,2%) sa kolokoloničnom i 13 (12,6%) sa kolorektalnom anastomozom. Srednje vrednosti CRP su bile veće kod bolesnika sa dehiscencijom anastomoze nego kod bolesnika bez komplikacija i u serumu i u drenažnoj tečnosti, sa statistički najznačajnijim razlikama petog i sedmog postoperativnog dana ($p < 0,001$). Korelaciona analiza pokazala je postojanje pozitivne korelacije između vrednosti CRP u serumu i drenažnoj tečnosti kod obe grupe bolesnika. Najznačajnije vrednosti CRP u detekciji dehiscencije anastomoze dobijene su petog i sedmog postoperativnog dana i za serum i za drenažnu tečnost. Kod 80% bolesnika sa dehiscencijom anastomoze CRP u drenažnoj tečnosti bio je iznad 53 mg/L petog i 42 mg/L sedmog postoperativnog dana. Specifičnost metode bila je 77% za peti i 83% za sedmi postoperativni dan. Kod svih bolesnika sa CRP iznad 108 mg/L petog i 93 mg/L sedmog postoperativnog dana registrovana je dehiscencija anastomoze. **Zaključak.** Serijsko merenje vrednosti CRP u drenažnoj tečnosti pouzdano je u detekciji dehiscencije anastomoze kod obolelih sa kolorektalnom resekcijom. Najznačajnije vrednosti dobijene su petog i sedmog postoperativnog dana i u pozitivnoj su korelaciji sa vrednostima registrovanim u serumu.

Ključne reči:
c-reaktivni protein; prognoza; rana, hirurška, dehiscencija; hirurgija, operativne procedure; kolorektalne neoplazme.

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Introduction

Anastomotic leakage (AL) is the life-threatening commonest major complication after colorectal cancer surgery. Breakdown of anastomosis results in increased morbidity and mortality [1, 2] and adversely affects quality of life [3], duration of hospital stay, cost [1, 4] and cancer recurrence [5–7]. The reported leak rate varies between 3% and 19% depending on the definition [1, 2, 8–11].

Some studies including our one [12] have shown that elevated serum C-reactive protein (CRP) concentrations in the postoperative period may predict an increased chance of postoperative infection [13] and AL [14]. The fact that CRP begins to increase before the occurrence of postoperative infectious complications, in contrast to clinical signs such as fever, tachycardia, and pain [13], makes it an ideal predictor of postoperative infectious complications. Since this is a nonselective marker of inflammation, before searching for specific, it is necessary to exclude the other infectious complications [13, 14].

According to our knowledge there are no data on the determination of CRP in drainage fluid in the detection of AL in patients with colorectal resection. Peritoneal fluid in its composition is a filtrate of plasma and it is in equilibrium with serum. Our assumption is that the values of CRP in drainage fluid reflect values obtained in serum.

The aim of this study was to determine the sensitivity and specificity of CRP in drainage fluid in the detection of AL in patients with elective colorectal resection.

Methods

Our prospective study enrolled 150 patients with cancer of the left colon and rectum surgically treated at the Clinic for General Surgery, Military Medical Academy, Belgrade in the period from April, 2011 to November, 2012. Characteristics of these patients were given in our previous article [12].

The patients with clinical signs of infection or some other inflammatory condition present preoperatively were excluded from the study. The analysis involved only the patients in whom conventional elective, radical, or palliative surgical intervention was done, with colocolonic or colorectal anasomosis, handsewn or stapled. All the operations were doune by the surgeouns performing at least 30 similar surgical procedures *per* year. Mechanical preoperative large bowel preparation was done only in those with rectal cancers. The patients surgically treated for tumor recurrence were excluded from the study. The creation of diverting ileostomy or transversocolostomy depended on the individual assessment of surgeons. Before the closure of laparotomy incision, abdominal cavity of the patients was routinely drained with at least one drain placed in the area of the pouch of Douglas or in the presacral area, in the region of colorectal anastomosis.

In the immediate postoperative course, within a month of surgery, all remote (pneumonia, urinary infection, infections caused by central venous line) and surgical site (wound infection, AL, intraabdominal abscess collections) infectious complications were registered. Redness, edema, and purulent secretion at the site of laparotomy wound were the clinical criteria establishing the presence of infection in the surgical incision site [16]. Clinical parameters of AL were defined by the presence of purulent or fecal content at the drain site, pelvic abscess, peritonitis, rectovaginal fistula, or the appearance of purulent content from the rectum (*per recti*) [17]. Routine, contrast-enhanced x-ray control of the anastomosis was not implemented, since the patients with asymptomatic leakage, were not relevant for the study. In patients with low colorectal anastomosis, digital rectal examination was an integral part of the examination to detect possible AL. Intraabdominal abscesses were detected by way of the presence of purulent secretion after surgical or percutaneous ultrasound-guided drainage of these collections [16]. Appropriate clinical presentation with a positive x-ray finding in pneumonia, urinary sediment and urine culture in urinary infection, and positive blood culture in infections caused by central venous line, defined the presence of individual remote infections.

On the first, third, fifth, and seventh postoperative day (POD), serum CRP levels were measured, utilizing the method immunonephelometry on a SIEMENS autoanalyzer (DADE Behring BN II). Drain fluid was collected from the intraperitoneal drains placed in the pelvis and CRP levels were measured by the same method at the Institute of Medical Biochemistry, Military Medical Academy, Belgrade.

The usual descriptive statistic parameters were used in statistical analysis of the obtained results (mean value, standard deviation, range, 95% confidence interval, frequency of individual characteristics). Depending on the normality of distribution of the observed parameters and the number of groups among which the statistical significance was sought for, non-parametric tests, the Mann-Whitney *U*-test and Pearson´s correlation test were used. The sensitivity and specificity of relevant biochemical markers were analyzed using the receiver operating characteristic curve (ROC).

Commercially available statistical software package SPSS version 17 (USA) was used for statistical analysis.

Results

We analyzed 150 patients in total, 94 (66.7%) men and 56 (33.3%) women (male-to-female ratio, 1.7 : 1). The youngest operated patient was 33, and the oldest 87 years of age. The average age of the patients was 65 ± 11 years.

The overall morbidity rate associated with surgical treatment was 34%, and mortality rate 4%. Surgical site infections were observed in 41 (27.3%) and remote infections in 10 (6.7%) patients. In 99 surgically treated patients postoperative course was without any complications.

Clinically evident AL was observed in 15 patients – in 2 (4.2%) patients with left colonic surgery, and 13 (12.6%) patients with colorectal anastomosis. The postoperative mortality rate associated with AL was 13%, and out of 6 fatal outcomes, leakage was the immediate cause of death in two of the patients.

Table 1 shows a comparison of the mean values of CRP in serum and drainage fluid for the observed PODs between the group of patients without complications and those with

AL. In all the patients, there was an increase of CRP, with maximum values reported for the POD 3, except for the CRP values in drainage fluid in the group of patients with AL. In those without complications, there was a descending tendency in serum and drainage fluid CRP values, significantly more evident compared to descending CRP values in those with AL. On the POD 1, there was no statistically significant differences in CRP values in the group of patients without complications and those with AL. On the PODs 5 and 7, there was a statistically most significant difference in the values of CRP in serum and drainage fluid between the group of patients without complications and with AL ($p < 0.001$).

Table 2 shows the results of correlation analysis for CRP values in serum and drainage fluid in the group of patients without complications and those with AL for the observed days. Statistically highly significant positive correlation ($p < 0.001$) between the value of CRP in serum and drainage fluid is present for all the observed days in patients without complications, while the highest correlation in the group with AL was observed on the POD 7 (the highest value of the correlation coefficient).

Sensitivity and specificity of serum and drainage fluid CRP measurement in the detection of AL were analyzed using the ROC curve and were shown in Table 3 and Figures 1 and 2.

Table 1

Mean values of C-reactive protein (CRP) in serum and drainage fluid in the patients without complications and those with anastomotic leakage (AL) by postoperative days (POD 1, 3, 5, 7)

Sample	CRP values (mg/L), $\bar{x} \pm SD$			
	Group without complications (n = 99)	AL group (n = 15)		*p*
Serum				
POD 1	95.15 ± 37.97	102.11 ± 39.65	0.532	0.595
POD 3	113.47 ± 40.72	197.25 ± 75.76	3.449	0.001
POD 5	57.10 ± 28.15	175.93 ± 72.51	5.336	< 0.001
POD 7	49.71 ± 29.95	155.61 ± 77.49	5.508	< 0.001
Drainage fluid				
POD 1	21.49 ± 15.61	27.18 ± 23.85	0.666	0.505
POD 3	58.56 ± 17.89	84.20 ± 33.50	3.106	0.002
POD 5	41.87 ± 16.20	84.79 ± 44.01	4.698	< 0.001
POD 7	31.07 ± 15.42	77.75 ± 56.75	4.028	< 0.001

***Mann-Whitney test; $\bar{x}$ – mean; SD – standard deviaton.**

Table 2

Correlation of C-reactive protein (CRP) values in serum and drainage fluid in the patients without complications and those with anastomotic leakage (AL), by postoperative days (POD 1, 3, 5, 7)

POD	CRP (mg/L), $\bar{x} \pm SD$			
	Serum	Drainage fluid	*r*	*p*
Group without complications (n = 99)				
POD 1	95.15 ± 37.97	21.49 ± 15.61	0.4504	< 0.001
POD 3	113.47 ±40.72	58.56 ± 17.89	0.7275	< 0.001
POD 5	57.10 ± 28.15	41.87 ± 16.20	0.7199	< 0.001
POD 7	49.71 ± 29.95	31.07 ± 15.42	0.6808	< 0.001
AL (n = 15)				
POD 1	102.11 ± 39.65	27.18 ± 23.85	0.6150	0.019
POD 3	197.25 ± 75.76	84.20 ± 33.50	0.5700	0.026
POD 5	175.93 ± 72.51	84.79 ± 44.01	0.5041	0.055
POD 7	155.61 ± 77.49	77.75 ± 56.75	0.9053	< 0.001

***Pearson's test, *r* – correlation coefficient; $\bar{x}$ – mean; SD – standard deviaton.**

Table 3

Sensitivity and specificity of serum and drainage fluid C-reactive protein (CRP) values in the patients with anastomotic leakage by postoperative days (POD 1, 3, 5, 7), expressed as an area under the ROC curve

POD	Cutoff value for CRP (mg/L)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	*p*
Serum (n = 15)					
POD 1	111	54	71	0.538 (0.348–0.729)	0.65
POD 3	140	69	79	0.748 (0.567–0.929)	0.004
POD 5	77	85	77	0.920 (0.849–0.991)	< 0.001
POD 7	90	92	89	0.960 (0.925–0.994)	< 0.001
Drainage fluid (n = 15)					
POD 1	21	53	63	0.552 (0.386–0.719)	0.515
POD 3	77	67	84	0.754 (0.602–0.907)	0.002
POD 5	53	80	77	0.879 (0.770–0.987)	< 0.001
POD 7	42	80	83	0.824 (0.684–0.964)	< 0.001

ROC – receiver operating characteristic; AUC – area under the curve; CI – confidence interval.

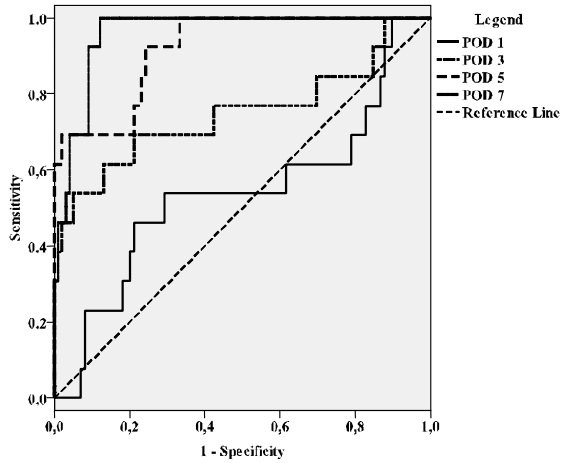

Fig. 1 – Diagnostic accuracy of serum C-reactive protein values in the detection of anastomotic leakage, expressed as a receiver operating characteristic curve; POD – postoperative day

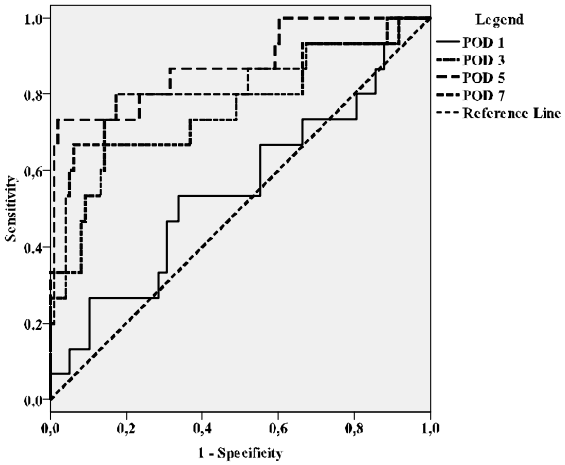

Fig. 2 – Diagnostic accuracy of C-reactive protein values in drainage fluid in the detection of anastomotic leakage expressed as a receiver operating characteristic curve; POD –postoperative day.

Discussion

Despite many advances in surgery, the quest for uneventful healing of the intestinal anastomosis remains a challenge after colon and rectal resections. AL is the most serious complication of surgical treatment and a significant obstacle to the successful treatment of patients with colorectal resection [18]. It is more common after rectal surgery, between 8% and 14% [2, 10, 19], compared to the colon, ranging from 3% to 7% [20, 21].

Differences in the frequency of, among other things, are due to different criteria used for the diagnosis of leakage. In order to better define, a new grading system was proposed by the International Study Group of Rectal Cancer (ISGRC) [22]. In analysis of our patients, we used exclusively the clinical criteria to establish AL [22, 23], and it was reported in 15 patients, in two (4.2%) patients with left colonic surgery, and 13 (12.6%) patients with colorectal anastomosis. The rates of morbidity and mortality significantly increase after AL, with mortality reported between 12% and 27% [1, 24, 25]. Postoperative mortality rate with AL in our study was 13%, and out of six deaths, leakage was the immediate cause of death in two. Early detection of this, potentially most dangerous complication, in the absence of clear clinical manifestations, would make possible an early introduction of appropriate therapeutic measures intended to alleviate or eliminate adverse effects.

CRP is the most popular and most widely available marker of the acute inflammatory response [26]. The production of CRP occurs almost exclusively in the liver by hepatocytes as part of the acute-phase response upon stimulation by interleukin (IL)-6, tumor necrosis factor-α, and IL-1-β originating at the site of inflammation. It is a pentameric protein with various molecular functions including complement activation and opsonization [27]. Within 6 h after stimulation, CRP serum levels exceed normal values and peak after about 48 h. CRP has a nearly constant serum half-life of about 19 hours. Therefore, the CRP serum concentration is determined by its synthesis rate and reflects the intensity of the stimulus for acute inflammatory responses [28]. In numerous studies, the significance of the serial measurement of CRP in serum in detecting infectious complications of surgical treatment and/or AL was shown [29–32].

According to the available literature, we found no data on whether someone is determined CRP levels in drainage fluid. While some advocates [8], and others do not find justification [33], a routine approach in our institution involves mandatory drainage of the abdominal cavity after colorectal resection by placing at least one drain that runs through a separate incision in the skin. A few years ago another option proved to be interesting in patients with drainage done and refers to the ability to analyze the drainage of fluid in the purpose of early detection of AL [34]. Some biomarkers have been proposed as objective diagnostic parameters [35] and are collected from the fluid through the drain located near the site of interest. Potential biomarkers include immune (IL-1, IL-6 and IL-10, tumor necrosis factor alpha) parameters, tissue repair (matrix metalloproteinase-1, 2, 9 and 13) parameters, parameters of ischemia (glucose, lactate, glycerol), and microbiological (lipopolysaccharides, pH, pCO2, and pO2) parameters [35].

In aim to verify the results obtained in drainage fluid in our patients, we compared them with the serum CRP values, between the group of patients without complications of surgical treatment and those with AL. The mean values of CRP in drainage fluid were lower compared with the values the serum for both groups of observed patients (without complications and with AL). The highest CRP levels were registered on the POD 3, except for the values in drainage fluid in the group of patients with AL with the highest CRP registered on the POD 5. In patients without complications, after an initial rise of CRP values, there was a gradual decline of CRP on days to follow. In those with AL, high CRP values persisted on the PODs 5 and 7 (Table 1). Similar trends in changes of CRP in serum is found in the papers of other authors [13, 29, 30]. On the POD 1, there was no statistically significant difference in the values of CRP in serum and drainage

fluid between patients without complications and with AL. Statistically, the most significant difference occurred on the fifth and seventh POD (Table 1).

Correlation analysis showed a highly statistically significant positive correlation between the measured values of CRP in serum between the groups of patients without complications and those with AL for all the observed days. In the group of patients with AL statistically significant positive correlation was found on the PODs 1, 3 and 7, while on the POD 5 positive correlation was at the border of statistical significance ($p = 0.055$) (Table 2). We can conclude that changes in serum CRP values were accompanied by appropriate changes in the values of CRP in drainage fluid.

Serum and drainage fluid CRP values on the PODs 5 and 7 are most important in the detection of AL. The area under the ROC curve on the POD 5 was 0.920 and 0.960 on the POD 7 for serum CRP, and 0.879 and 0.824 for CRP values in drainage fluid. AL was observed in 85% of the patients on the POD 5 and in 92% on the POD 7, with serum CRP values of 77 mg/L and 90 mg/L. The method specificity was 77% for the POD 5, and 88% for the POD 7. All the patients with AL had serum CRP values above 139 mg/L on the POD 5 and 150 mg/L on the POD 7. In 80% of patients with CRP values in the drainage fluid of 53 mg/L for the POD 5 and 42 mg/L for the POD 7 AL was observed. The method specificity was 77% for the POD 5, and 83% for the POD 7. All the patients with CRP values in drainage fluid above 108 mg/L on the POD 5 and 93 mg/L on the POD 7 had AL.

However, as a nonselective marker of inflammation and is not a completely reliable indicator of infection, CRP could be taken into consideration only within the clinical presentation context [36].

Conclusion

Our results indicate that measurement of CRP in drainage fluid can be reliably used in early detection of anastomotic leakage as the most serious infectious complication after colorectal resection. These values were positively correlated with CRP in serum, especially on the fifth and seventh day, when the risk from the appearance of this complication is greatest. Whether measurement of C-reactive protein in common with other biomarkers, in drainage fluid, contributes to more accurate early detection of patients at risk of developing anastomotic leakage after colorectal resection remains to be confirmed in future studies. Anastomotic leakage is inevitable companion of colorectal resection in a certain number of patients, and hence, its detection is of crucial importance because it allows early implementation of therapeutic measures to reduce the adverse effects.

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Benzodiazepine poisoning in elderly

Akutna trovanja benzodiazepinima kod starijih bolesnika

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Abstract

Background/Aim. Benzodiazepines are among the most frequently ingested drugs in self-poisonings. Elderly may be at greater risk compared with younger individuals due to impaired metabolism and increased sensitivity to benzodiazepines. The aim of this study was to assess toxicity of benzodiazepines in elderly attempted suicide. **Methods.** A retrospective study of consecutive presentations to hospital after self-poisoning with benzodiazepines was done. Collected data consisted of patient's characteristics (age, gender), benzodiazepine ingested with its blood concentrations at admission, clinical findings including vital signs and Glasgow coma score, routine blood chemistry, complications of poisoning, details of management, length of hospital stay and outcome. According the age, patients are classified as young (15-40-year old), middle aged (41–65-year old) and elderly (older than 65). **Results.** During a 2-year observational period 387 patients were admitted because of pure benzodiazepine poisoning. The most frequently ingested drug was bromazepam, the second was diazepam. The incidence of coma was significantly higher, and the length of hospital stay significantly longer in elderly. Respiratory failure and aspiration pneumonia occurred more frequently in old age. Also, flumazenil was more frequently required in the group of elderly patients. **Conclusion.** Massive benzodiazepines overdose in elderly may be associated with a significant morbidity, including deep coma with aspiration pneumonia, respiratory failure, and even death. Flumazenil is indicated more often to reduce CNS depression and prevent complications of prolonged unconsciousness, but supportive treatment and proper airway management of comatose patients is the mainstay of the treatment of acute benzodiazepine poisoning.

Key words:
poisoning; benzodiazepines; overdose; flumazenil; aged.

Apstrakt

Uvod/Cilj. Benzodiazepini su najčešće korišćeni lekovi u slučaju akutnih samotrovanja. Zbog izmenjenog metabolizma i povećane osetljivosti na benzodiazepine, starije osobe su pod većim rizikom u odnosu na mlađe. Cilj ove studije bio je procena toksičnosti benzodiazepina u slučaju suicidalnih trovanja starijih osoba. **Metode.** Retrospektivno su analizirani podaci bolesnika primljenih na hospitalno lečenje zbog samotrovanja benzodiazepinima. Prikupljeni podaci obuhvatili su karakteristike bolesnika (pol, starost), vrstu ingestiranog benzodiazepina i koncentracije u krvi na prijemu, klinički nalaz uključujući vitalne parametre i Glazgov koma skor, osnovne laboratorijske analize, komplikacije trovanja, dužinu hospitalizacije i ishod. U odnosu na starost, bolesnici su grupisani u tri grupe: mlađe osobe (15–40 godina), osobe srednje dobi (41–65 godina) i starije osobe (stariji od 65). **Rezultati.** U posmatranom 2-godišnjem periodu zbog samotrovanja benzodiazepinima lečeno je 387 bolesnika. Najčešći uzročnik trovanja bio je bromazepam, a drugi po učestalosti bio je diazepam. U poređenju sa mlađim osobama, kod starijih osoba incidencija kome bila je statistički značajno veća, a trajanje hospitalizacije duže. Respiratorna insuficijencija i aspiraciona bronhopneumonija bile su češće kod starijih osoba. Takođe, starijim bolesnicima češće je bio potreban flumazenil. **Zaključak.** Akutna intoksikacija benzodiazepinima kod starijih osoba može biti udružena sa značajnim morbiditetom, dubokom komom, aspiracionom pneumonijom, respiratornom insuficijencijom, pa čak i smrtnim ishodom. Flumazenil je indikovan zbog reverzije depresije centralnog nervnog sistema i prevencije poremećaja nastalih kao posledica produženog poremećaja svesti, ali suportivna terapija i zaštita disajnog puta komatoznog bolesnika predstavljaju osnov terapije akutnog trovanja benzodiazepinima.

Ključne reči:
trovanje; benzodiazepini; predoziranost; flumazenil; stare osobe.

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Introduction

Benzodiazepines are widely used drugs, primarily as anxiolytics and hypnotics. Often prescribed on a long-term basis for the treatment of anxiety and insomnia in elderly patients [1], benzodiazepines are readily available for deliberate drug overdose, which is the commonest type of suicidal behaviour in the old age [2]. Benzodiazepines are generally thought to be among the safest psychoactive drugs in overdose [3]. Clinical picture of poisoning basically includes central nervous system (CNS) depression, usually manifested as mild to moderate sedation. Deep coma, with respiratory or circulatory failure is rare [4]. Aspiration, as well as certain level of respiratory depression may be a cause of death in benzodiazepine overdoses [5]. Though even a large overdoses taken alone rarely cause death, there is a concern about benzodiazepines adverse effects because of additive effects with other CNS depressants and alcohol. Also, elderly may be at greater risk compared with younger individuals, since the safety of therapeutic doses of benzodiazepines may be reduced due to impaired metabolism and increased sensitivity of this population [6]. Studies on benzodiazepine pharmacokinetics in old age imply some alterations, especially in distribution and elimination of certain compounds [7]. Though benzodiazepines are among the most frequently ingested drugs in self-poisonings, studies on benzodiazepines effects due to significant overdose in elderly are rare [8].

The aim of this study was to assess toxicity of benzodiazepines in elderly attempted suicide in comparison with younger patients.

Methods

This was a retrospective study of consecutive presentations to hospital after self poisoning with benzodiazepines during a 2-year period (2010–2012). Patients with co-ingestion of other drugs or alcohol were excluded from the study. Collected data consisted of patient's characteristics (age, gender), benzodiazepine ingested with its blood concentrations at admission, clinical findings including vital signs and Glasgow coma score (GCS), routine blood chemistry, complications of poisoning, details of management (mechanical ventilation and flumazenil administration), length of hospital stay (LOS) and outcome. According the age, patients are classified as young (15–40-year old), middle aged (41–65-year old) and elderly (older than 65).

Results

During the observational period 387 patients (284 females and 103 males) were admitted to our hospital because of pure benzodiazepine poisoning. Patients' age ranged from 15 to 93 (mean 45 ± 17.7 years for females and 42 ±1 5.5 for males).

Ingestion of single drug was recorded in 349 (90%) patients, while 38 patients (10%) ingested two or three different benzodiazepines. The most frequently ingested benzodiazepine was bromazepam. About 50% of the patients in all the age groups ingested this drug. There was no poisoning with clonazepam in the group of elderly patients. In this group short acting hypnotic midazolam was more frequently ingested than in younger patients. Types of benzodiazepine ingested in different age groups are listed in Table 1.

Table 1

Ingested benzodiazepines

Benzodiazepine	Patients' age (year)			Total (n)
	15–40 n (%)	41–65 n (%)	> 65 n (%)	
Bromazepam	102 (51)	79 (39.5)	19 (9.5)	200
Diazepam	33 (44)	32 8 (42.7)	10 (13.3)	75
Lorazepam	19 (40.4)	21 (44.7)	7 (14.9)	47
Clonazepam	22 (51.2)	21 (48.8)	0 (0.0)	43
Alprazolam	17 (50)	16 (47)	1 (3.0)	34
Midazolam	10 (38.5)	12 (46.1)	4 (15.4)	26

Level of sedation, according to GCS in different age groups is shown in Table 2. Among the patients with GCS of 13–15 there were 5 patients (1.29%) with episodes of paradoxal excitation. All of them were younger than 65. The incidence of coma (GCS of 3–8) was 12.4% (48/387 patients). Statistical analysis (χ^2 test) revealed significant differences in the level of sedation between the age groups, with more pronounced sedation with the increase in patients age.

Respiratory failure developed in 11 (2.8%) patients, 4 of them were older than 65. The incidence of respiratory failure in elderly patients was higher than in younger ones (10.5% *vs* 2%), but a statistically significant difference could not be proved because of a smal number of patients with this effect. Mechanical

Table 2

Level of sedation aceording to the Glasgow coma score (GCS)

Level of sedation (GCS)	Patients' age (years)			Total number of patients
	Group I (15–40) n (%)	Group II (41–65) n (%)	Group III (> 65) n (%)	
Mild (somnolence) GCS = 13–15	171 (92.43)	112 (68.29)	10 (26.32)	293
Moderate (sopor) GCS = 9–12	6 (3.25)	27 (16.47)	13 (34.21)	46
Severe (coma) GCS = 3–8	8 (4.32)	25 (15.24)	15 (39.47)	48
Total number of patients	185	164	38	387

Comparation:
Group I *vs* Group II: $\chi^2 = 33.27$; $p < 0.001$;
Group I *vs* Group III: $\chi^2 = 90.22$; $p < 0.001$;
Group II *vs* Group III: $\chi^2 = 23.05$; $p < 0.001$.

ventilation was necesary in three patients, two of them belonged into the group of elderly. About 1% of patients younger than 65 and 5% of elderly patients needed mechanical ventilation.

Hypotension with systolic blood pressure lower than 80 mmHg was recorded in 32 (8.26%) patients. There was no difference in the incidence of hypotension between elderly and younger patients (7.9% *vs* 8.3% respectively).

Biochemistry analyses included complete blood count (CBC), blood concentrations of glucose, urea nitrogen, creatinine, transaminases (AST, ALT) and creatine kinase (CK). Except for a single patient in the group II with chronic renal failure, there were no patients whose biochemistry indicated renal or liver disorders. Transient leukopenia with minimal white blood cells count of 1.78×10^9/L was revealed in a single patient with bromazepam overdose. Leucocytosis was registered in 34 patients. In 25 of them pneumonia was proved on chest radiography.

Major sedation was complicated with aspiration pneumonia and rhabdomyolysis. Elevated activity of CK accompanied with slight elevation of AST and ALT activities was noted in 21(5.42%) patients, probably due to rhabdomyolysis caused by pressure on muscles during severe sedation. Maximal value of CK in elderly was 8.464 U/L, and in patients under 65 was 13,000 U/L. The incidence of rhabdomyoliysis in comatose patients was 21.4%.

Aspiration pneumonia was noted in more than a half of patients in coma (52%). The incidence of pneumonia in comatose elderly, middle aged and young patients was 75%, 52%, and 25%, respectively.

Toxicological analyses were obtained for 376 patients. In 340 (90.42%) patients, concentrations of benzodiazepine in blood were in the range of toxic [9], and in the rest 36 (9.57%) were in therapeutic range. In the group of elderly, 4 of 5 patients with therapeutic concentration of benzodiazepines in blood had marked sedation (GCS score was even in the range of 3–8 in 2 patients). In groups of younger patients there were no comatose patients with therapeutic blood concentration of ingested drugs.

Maximal revealed concentrations for the two most frequently ingested drugs, bromazepam and diazepam were 8.52 mg/L and 11.84 mg/L, respectively. For diazepam, concentrations of its active metabolite, temazepam, are also obtained. Details on patients with maximal blood concentrations of these drugs are shown in Table 3.

Differences in severity of poisoning among the age groups may be the consequence of simply greater dose ingested. Therefore, to check this possibility, concentrations of drugs on admission were compared between the groups. Statistical analysis was possible for the most frequently ingested benzodiazepine, bromazepam, as blood concentrations were obtained for 192 patients poisoned only with this drug. Though the mean concentration of bromazepam was higher in elderly, the difference between the age groups was not significant (Table 4). Furthermore, there was no significant difference between the concentration of drugs among the groups with moderate and severe level of sedation (Table 5).

All the patients received intravenous solutions as supportive treatment. Gastric lavage was performed in 128 (33%) patients, admitted up to 2 hours after ingestion. Specific antidote, flumazenil was administered for diagnostic and/or therapeutic purposes to 64 patients. This drug was used as diagnostic tool given in single bolus dose to 38 (10%) patients with severe sedation. Flumazenil was given as an antidote in the cases of deep coma, with respiratory failure and hypotension, or in order to avoid intubation and prevent complications of prolonged unconsciousness. According the physician's estimation, flumazenil

Table 3

Characteristics of poisonings with the maximal concentration of bromazepam or diazepam

Parameters	Patients under 65		Elderly > 65	
Benzodiazepine	Bromazepam	Diazepam + Temazepam	Bromazepam	Diazepam + Temazepam
Blood concentration (mg/L)	8.52	5.22 + 3.46	7.22	11.84 + 7.46
Age (years), and sex	25, female	47, female	86, female	83, female
Level of sedation (GCS)	13	9	3	3
Complications	No	No	Pneumonia Respiratory failure	Pneumonia
Lenght of hospital stay (days)	3	5	42	14

GCS – Glasgow coma score.

Table 4

Comparation between the mean bromazepam concentrations on admission in relation to the age of patients

Age (year)	Bromazepam concentration (mg/L) mean ± SD	Kruskal-Wallis test
Group I (15 – 40)	0.87 ± 1.03	Group I *vs* Group II: n.s
Group II (41 – 65)	1.07 ± 1.04	Group II *vs* Group III: n.s
Group III (> 65)	1.28 ± 1.53	Group I *vs* Group III: n.s

Table 5

Comparation between mean bromazepam concentrations on admission in relation to the level of sedation

Level of sedation	Bromazepam concentration (mg/L) mean ± SD	Kruskal-Wallis test
Mild (somnolence): GCS = 13–15	0.86 ± 0.03	Mild *vs* Moderate: n.s
Moderate (sopor): GCS = 9–12	1.46 ± 1.82	Moderate *vs* Severe: n.s
Severe (coma): GCS = 3–8	1.45 ± 1.46	Mild *vs* Severe: n.s

GCS – Glasgow coma score.

was administered in repeated boluses or in continued intravenous infusion in 24 (6.26 %) patients. All the patients older than 65 years needed flumazenil for some of these reasons.

With the increase in age, the patents needed longer hospital treatment.

Hospital stay in the elderly was significantly longer than in the young and middle aged patients. There was also a significant difference between young and middle aged patients (Figure 1).

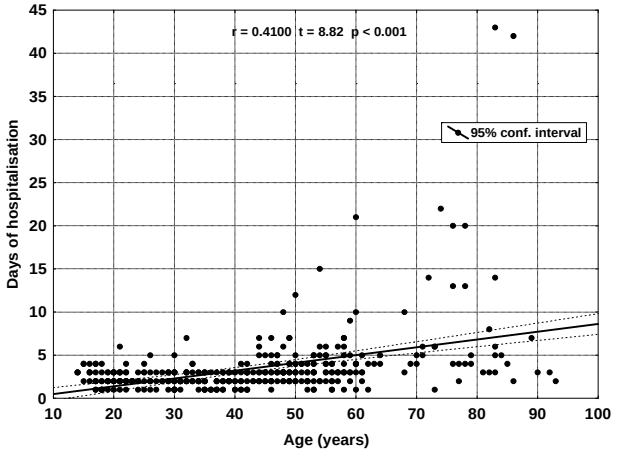

Fig. 1 – Correlation between age of the patients and the duration of hospital treatment.

In two female patients there was fatal outcome (0.5% of the total number); one belonged to the group of elderly (2.6%), and the other to the group of middle aged (0.6%). Both were without apparent biochemical findings suggesting liver or renal impairment on admission. However, the first was sick with ischemic heart and brain disease and the second had disseminated carcinoma.

Discussion

Our study suggests that clinical picture of benzodiazepine poisoning in elderly is more severe than in younger patients. It is primarily manifested as significantly greater level of CNS depression, with more frequent occurrence of coma. Consequently, the incidence of related complications is higher and the length of hospital stay is longer. We recorded more cases of respiratory failure among elderly patients, but because of overall infrequent occurrence of this disorder, there was no statistically significant difference between the groups.

Differences in severity of poisoning between elderly and younger patients may be caused by several reasons. Elderly patients may simply be more resolute to commit suicide taking higher doses of drugs. Reliable comparable data on ingested doses were not available in our patients. The most frequently ingested benzodiazepine was bromazepam. Bromazepam was often used for acute self-administered drug overdoses in other countries, for instance it was also ranked first in France [10], and second after diazepam in some Brazil states [11]. The obtained blood concentrations on admission in our 192 patients with bromazepam ingestion allowed comparison among the age groups. The mean concentration was over the therapeutic level of 0.08–0.2 mg/L [9] in all the age groups (Table 4), and was slightly, but not significantly higher in older patients. Study of bromazepam pharmacokinetics in volunteers divided into young (aged 21 to 29 years) and elderly (aged 60 to 81 years) groups who received single 6 mg oral doses revealed that elderly compared with young subjects had significantly higher peak serum bromazepam concentrations (132 *vs* 82 ng/mL respectively), smaller volume of distribution (0.88 *vs* 1.44 L/kg respectively), lower oral clearance (0.41 *vs* 0.76 mL/min/kg respectively), and increased serum free fraction (34.8% *vs* 28.8% unbound respectively) [12]. Accordingly, the difference in the obtained concentrations between the groups in our study may rather be age-related due to the changes in the pharmacokinetics than dose-related. In general, elderly have reduced muscle and fat mass, which may influence the volume of distribution of the drugs leading to higher plasma concentrations [13]. Other reason for higher susceptibility of elderly to benzodiazepines may be impaired metabolism due to subclinical deterioration in liver and renal function. In the case of benzodiazepines with active metabolites like diazepam, increases in the elimination half-life of both parent compound and metabolites may contribute to the more pronounced sedation [14].

Some pharmacodynamics changes in the elderly are commonly ascribed to alteration in the sensitivity to drugs, especially to those affecting central nervous system, irrespective of changes in drug disposition [15, 16]. Thus, the doses of midazolam needed to reach sedation in the elderly were 50% of those required in young subjects, though there were no differences in pharmacokinetics between the groups [17]. Comorbidity and possible interactions with drugs for treatment of these illnesses may also contribute to the vulnerability of elderly.

In our study the majority of cases could be managed with supportive care including adequate management of airway. Specific antidote flumazenil was administered in the selected cases. Coma in benzodiazepine poisoning is not commonly accompanied with respiratory depression, so ventilatory failure was rarely the indication for flumazenil administration. Decision to intubate or not was primarily based on physicians assessment of risk of aspiration and the majority of our patients received flumazenil to avoid intubation. Because sedation was more severe and prolonged in elderly flumazenil is used more often in this group of patients. Other studies also report on more frequent use of flumazenil with the increased age, severe poisoning and respiratory failure [18]. However, a high incidence of aspiration pneumonia in comatose patients suggests the obligation of proper airway management and intubation, despite the use of flumazenil.

Conclusion

Our study indicates that benzodiazepines massive overdose in elderly may be associated with significant morbidity, including deep coma with aspiration pneumonia, respiratory failure, and even death. Flumazenil is indicated more often to reduce central nervous system depression and prevent complications of prolonged unconsciousness. However, supportive treatment and proper airway management of comatose patients is the mainstay of treatment in these patients.

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Diagnostic value of breast ultrasound in mammography BI-RADS 0 and clinically indeterminate or suspicious of malignancy breast lesions

Dijagnostička vrednost ultrazvučnog pregleda dojke kod žena sa mamografskom kategorijom BI-RADS 0 i lezijom koja je klinički nejasna ili sumnjiva na malignitet

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Abstract

Background/Aim. Not only that ultrasound makes the difference between cystic and solid changes in breast tissue, as it was the case at the beginning of its use, but it also makes the differential diagnosis in terms of benign-malignant. The aim of this study was to assess the role of sonography in the diagnosis of palpable breast masses according to the American College of Radiology Ultrasonographic Breast Imaging Reporting and Data System (BI-RADS) and to correlate the BI-RADS 4 and BI-RADS 5 category with pathohistological findings. **Methods.** A retrospective study was conducted with the breast sonograms of 30 women presented with palpable breast masses found to be mammography category BI-RADS 0 and ultrasonographic BI-RADS categories 4 and 5. The sonographic categories were correlated with pathohistological findings. **Results.** Surgical biopsy in 30 masses revealed: malignancy (56.7%), fibroadenoma (26.7%), fibrocystic dysplasia with/without atypia (10%), lipoma (3.3%) and intramammary lymph node (3.3%). Correlation between BI-RADS categories and pathohistological findings was found ($p < 0.05$). All BI-RADS 5 masses were malignant, while in BI-RADS 4A category fibroadenomas dominated. A total of 53.8% of all benign lesions were found in women 49 years of age or younger as compared with 35.3% of all malignancies in this group ($p < 0.05$). **Conclusion.** Ultrasonography BI-RADS improved classification of breast masses. The ultrasound BI-RADS 4 (A, B, C) and BI-RADS 5 lesions should be worked-up with biopsy.

Key words:
breast neoplasms; diagnosis, differential; mammography; ultrasonography, doppler, color; predictive value of tests; risk assessment.

Apstrakt

Uvod/Cilj. Ultrazvučnim pregledom može ne samo da se napravi razlika između cističnih i solidnih promena u tkivu dojke, kao što je bilo na početku njegove primene, već i da se napravi diferencijalna dijagnoza u smislu razlikovanja benignih od malignih promena. Cilj rada bio je procena uloge ultrazvuka u dijagnostici palpabilnih promena u dojci u skladu sa terminologijom *Breast Imaging Reporting and Data Systems* (BI-RADS) i korelacija kategorija BI-RADS 4 i BI-RADS 5 sa patohistološkim nalazom. **Metode**. Retrospektivna studija sprovedena je u grupi od 30 žena sa palpabilnim promenama u dojci, sa mamografskom kategorijom BI-RADS 0 i ultrazvučnim kategorijama BI-RADS 4 i 5. Sonografske kategorije su korelisane sa patohistološkim nalazom. **Rezultati**. Eksciziona biopsija 30 promena je pokazala: malignitet (56,7%), fibroadenom (26,7%), fibrocističnu displaziju sa ili bez atipije (10%), lipom (3,3%) i intramamarni limfni nodus (3,3%). Korelacija između BI-RADS kategorija i patohistološkog nalaza je dokazana ($p < 0,05$). Sve BI-RADS 5 promene bile su maligne, dok je u BI-RADS 4A kategoriji dominirao fibroadenom. Ukupno 53,8% svih benignih promena pronađene su kod žena starosti 49 godina ili mlađih u poređenju sa 35,3% malignih promena u ovoj grupi ($p < 0,05$). **Zaključak.** Upotreba ultrazvučne BI-RADS nomenklature poboljšala je klasifikaciju promena u dojci. U slučaju ultrazvučnih kategorija BI-RADS 4 (A, B, C) i BI-RADS 5 trebalo bi raditi biopsiju.

Ključne reči:
dojka, neoplazme; dijagnoza, diferencijalna; mamografija; ultrasonografija, dopler, kolor; testovi, prognostička vrednost; rizik, procena.

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Introduction

Breast cancer is a leading disease by mortality in Serbian women regardless age and it is a leading cause of early death in women between 25 and 44 years of age in Serbia [1]. Mammography is still the "gold standard" for breast examination. Screening mammography has certain limitations as well. First of all, about 20% of cancers present during mammography examination may be overlooked. This happens more often in young women due to density of their breast parenchyma [2]. Then, if we take into account the amount of radiation in a ten-year time period starting at the age of 40, cancer induced by radiation will be a cause of death at most in 8 women in 100,000 performed mammography examinations [3]. Ultrasonography is an additional diagnostic method. Not only that ultrasound can make the difference between cystic and solid changes, as it was a case at the beginning of its use, but it can also make the differential diagnosis in terms of benign-malignant. The main objection of ultrasonographic examination in early detection of cancer is inability to recognize microcalcifications. According to the latest papers, microcalcifications may be viewed in 70%, and in case of cancer itself in 90% of cases by new ultrasonographic devices. The Breast Imaging Reporting and Data System (BI-RADS) was developed by American College of Radiology (ACR) in 1993, in order to standardize mammography reports and to enable easier communication between clinical practitioners dealing with this issue. BI-RADS classification has been applied in mammography only, and it appeared for two breast imaging modalities in the fourth revision of BI-RADS atlas: breast ultrasonography and magnetic resonance imaging (MRI). BI-RADS system is aimed to assess the risk, whether a viewed change is malignant, *ie* the following could be advised: biopsy, frequent radiology follow-ups or regular preventive examinations. The essence of BI-RADS nomenclature is a final radiology report with a clearly numerically indicated conclusion. A motive for this paper was the fact that ultrasonography BI-RADS lexicon has had short history and that there have been fewer data on its use as compared to mammography [4].

Criteria for assessment of pathological changes in the breast, diagnosed by ultrasonography examination are as follows: shape (round, oval or irregular), orientation (horizontal or vertical), contours (very well-defined, poorly defined, angular, micro-lobular and spiculated), transition zone (well-defined or echogenic halo), echogenicity (non-echogenic, hyperechogenic, complex lesion, hypoechogenic and isoechogenic), posterior phenomena (without posterior phenomena, acoustic amplification, acoustic attenuation, alternating posterior phenomena), tissue around lesion (ductis, direction of Cooper's ligaments, parenchymal edema, skin and impaired architectonic of tissue), calcifications, special examples (grouped cysts, complicated cysts), flow (without color signals, with color signals and with color signals around the change) (Table 1).

There are seven BI-RADS categories. BI-RADS 0 category implies need for further evaluation with the other available methods except for ultrasonography, *ie* definite ultrasonographic evaluation is not possible. BI-RADS 1 category implies negative finding. BI-RADS 2 category is a benign finding. BI-RADS 3 category implies possibly benign finding, and a follow-up in a short period is recommended. The probability of this lesion being malignant is < 2%. Some papers report that this kind of changes may be observed periodically not longer than 2 years, and after that time it can be considered as absolutely benign. BI-RADS 4 category is a finding suspect to a malignant change and biopsy is recommended. A risk that it is about a malignant change is between 3% and 94% of patients. BI-RADS 5 category is a highly suspicious finding with a high risk for malignant disease being more than 95%. BI-RADS 6 category is a pathohistologically proved malignant disease [1].

Table 1

Characteristics of tumor changes depending on BI-RADS category

Characteristics	BI-RADS 4 (A, B, C)	BI-RADS 5
Tumor change		
shape	Lobular/oval	Irregular
orientation	Horizontal/vertical	Vertical
contours	Well-defined or poorly defined	Poorly defined
transition zone	Well-defined or echogenic halo	Echogenic halo
echogenicity of change	Hypoechogenic, isoechogenic, complex lesion	
posterior phenomena	Acoustic amplification, acoustic attenuation, alternating posterior phenomena, without posterior phenomena	Acoustic attenuation, alternating posterior phenomena
tumor change surroundings	Change of ductus direction and/or echogenic content, thickening and/or withdrawal of Cooper's ligaments, parenchymal edema, altered breast echostructure	
Calcifications		
within tumor change or in surrounding tissue	Observed only in correlation with mammography, for targeted detection by ultrasonography, and for evaluation of nature of change, and which can be achieved only by mammography	
Flow (vascularization)		
within tumor change or in surrounding tissue	With no color signals, with color signals within a change, with color signals around it (marginally, diffusively)	With color signals around it (marginally, diffusively)

BI-RADS – the Breast Imaging Reporting and Data System.

BI-RADS classification in breast diseases defines whether a change detected in a breast carries a risk of malignancy and whether biopsy of that change is indicated. For BI-RADS 4 (A, B, C) and BI-RADS 5 categories of ultrasonographic finding, pathohistological (PH) verification is necessary, which yields an appropriate indicator of BI-RADS classification accuracy. Therefore, the aim of this study was to categorize breast ultrasonographic finding into BI-RADS 4 (A, B, C) and BI-RADS 5 categories and correlation between BI-RADS 4 (A, B, C) and BI-RADS 5 categories with PH finding of a breast change.

Methods

From the Registry of Cancer from the Institute for Oncology and Radiology of Serbia, and from radiologycal reports of ultrasonographic breast examinations performed in the Clinic for Radiotherapy and Radiology Diagnostics in the same institution, a group of 30 women was created, having clinical, mammographic and ultrasound breast examination followed by surgical biopsy with PH verification of a breast change in a period between November 1 2008 and March 31 2009. Criteria for patient selection were clinical, mammographic and ultrasonographic. Clinical criteria implied: premenopausal and postmenopausal patients; breast cancer diameter up to 3 cm or resistance with the third dimension regardless dimensions, but without engagement of the skin [T1 and T2 category as *per* tumor-nodus-metastasis (TNM) classification], status of regional lymph nodes N0, without distant metastasis (M0), mammographic criteria: a change scored as BI-RADS 0 based on standard mammography in two directions, *ie* a change that is not completely defined and which requires additional diagnostics; and ultrasonographic criteria: additional diagnostic procedures, additional ultrasonography examination, clinically and mammographically, detected a change scored as BI-RADS 4A (slightly suspicious of malignancy), BI-RADS 4B (moderately suspicious of malignancy), BI-RADS 4C (medium suspicious of malignancy) and BI-RADS 5 (highly suspicious of malignancy) according to BI-RADS classification. Ultrasonographic examination of breasts was performed with a 7.5 MHz probe (Sonoview, Acuson device). Before ultrasonographic examination of breasts, the oncologists clinically examined breasts of every patient, and mammography in two directions was performed and analyzed by the radiologist (analogue mammography Lorad M-4, Hologic and digital mammography Selenia, Hologic). Ultrasonographic examination of breasts was performed with the standard approach, with a patient being in supination and lateral decubitus position, with examination of regional lymph nodes and by using power Doppler color signalization. PH analysis implied *ex tempore* evaluation of preparations obtained by surgical biopsy, and than a standard analysis of preparations colored with hematoxylin eosin (HE). The results are presented in Tables and as graphs. Evaluation of normal distribution as *per* age of patients revealed that data are homogenous and that parameter methods (χ^2-test, level of significance $p < 0.05$) can be used for further comparisons.

Results

Sonographic morphology of cancer in BI-RADS 5 category is of the stellate type: irregular tumor change, with hypoechogenic, heteroechogenic centre, hyperechogenic border, acoustic posterior attenuation, interruption of ligaments and fascia, with removing hypoechogenicity of subcutaneous fat tissue and thick skin (Figure 1).

Figures 1 and 2 depict ultrasound features of breast masses categorized as BI-RADS 5 and BI-RADS 4A, respectively.

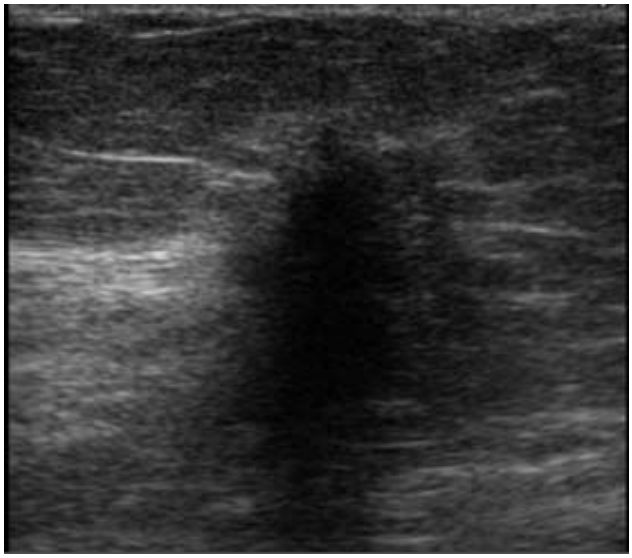

Fig. 1 – Ultrasound image of breast masses categorized as Breast Imaging Reporting and Data System (BI-RADS) 5.

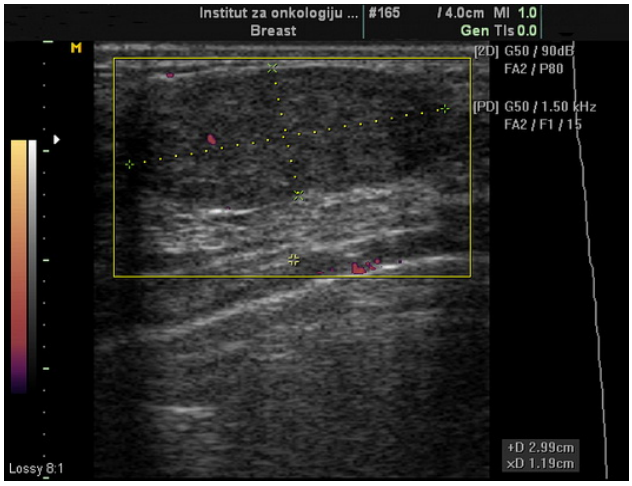

Fig. 2 – Ultrasound image of breast masses categorized as Breast Imaging Reporting and Data System (BI-RADS) 4A.

Analysis of a total number of BI-RADS categories in the group of 30 patients (Table 2) revealed a highly statistically significant difference ($\chi^2 = 18.174$; df = 1; $p < 0.01$), resulting from the much greater presence of BI-RADS 4 (86.7%) as compared to BI-RADS 5 (13.3%). Practically, the ratio was 6 : 1. The analysis of a total number of PH findings as compared to BI-RADS in the group of 30 patients (Table 2) also revealed a statistically significant difference (F = 7.338; df = 8; $p < 0.05$), resulting ftom the greater presence

Table 2

Pathohistological (PH) finding of tumors in relation to BI-RADS 4 and BI-RADS 5 categories

PH finding	BI-RADS 5 (n)	BI-RADS 4 (n)	Total (n)
Carcinoma			
ductale invasivum	1	6	7
lobulare invasivum	2	5	7
tubulare	0	1	1
in situ	1	1	2
Fibroadenoma	0	8	8
Lipoma	0	1	1
Nodus lymphaticus intramammarius	0	1	1
Dysplasia atypica	0	2	2
Dysplasia fibrocystica	0	1	1
Total	4	26	30

BI-RADS – Breast Imaging Reporting and Data System.

of fibroadenoma in BI-RADS 4 while each finding in patients with BI-RADS 5 was cancer.

Analysis of a total number of PH findings in the group of 30 patients (Table 3) also revealed a highly statistically significant difference ($F = 11.278$; $df = 5$; $p < 0.01$), resulting from the greater presence of cancer (56.7%) and fibroadenoma (26.7%) as compared to other types of findings, and particularly relatively rare lipomas, fibrocystic dysplasia and lymph nodes in breasts (by 3.3%).

In 6 patients with BI-RADS 4C category, PH findings were as follows: invasive ductal cancer in three patients, invasive lobular cancer in two, and intramammary lymph node hyperplasia in one patient.

In 9 patients with BI-RADS 4B category, PH findings were as follows: invasive lobular cancer in three patients, ductal cancer in two, tubular cancer (one patient), atypical lobular hyperplasia (one patient), fibroadenoma (one patient) and fibrocystic dysplasia (one patient).

In 11 patients with BI-RADS 4A category, PH findings were as follows: fibroadenoma in seven patients, lipoma i one patient, invasive ductal cancer one patient, ductal cancer *in situ* in one patient, and ductal hyperplasia with atypia in one patient.

If PH findings assigned into two categories (benign and malignant) are compared with BI-RADS categories (Table 4) we get a statistically significant difference ($F = 6.188$; $df = 6$; $p < 0.05$), resulting from the fact that BI-RADS 4B and C (64.7%) prevail in malignant tumors, while BI-RADS 4A (69.2%) being a rather rare finding in malignant tumors (11.8%) prevails in benign tumors. It could be said that BI-RADS 4A finding is almost always related to benign changes, and all the other with malignant ones (sensitivity for BI-RADS 5 is 100.0%, for BI-RADS 4C 83.3%, and for BI-RADS 4B 67%).

Table 3

Pathohistological (PH) finding of tumors in relation to BI-RADS 4A, B, C and BI-RADS 5 categories

PH finding	BI-RADS 5 (n)	BI-RADS 4C (n)	BI-RADS 4B, (n)	BI-RADS 4A, (n)	Total n (%)
Carcinoma mammae	4	5	6	2	17 (56.8)
Fibroadenoma	0	0	1	7	8 (26.6)
Lipoma	0	0	0	1	1 (3.3)
Nodus lymphaticus intramammaris	0	1	0	0	1 (3.3)
Dysplasia atypical	0	0	1	1	2 (6.7)
Dysplasia fibrocystica	0	0	1	0	1 (3.3)
Total	4	6	9	11	30 (100)

BI-RADS – Breast Imaging Reporting and Data System.

Table 4

Benign and malignant changes in relation to BI-RADS 4A, B, C and BI-RADS 5

BI-RADS types	Pathological finding (n)		
	benign	malignant	Total
BI-RADS 4 (n = 26)			
A	9	2	11
B	3	6	9
C	1	5	6
BI-RADS 5 (n = 4)	0	4	4
Total	13	17	30

BI-RADS – Breast Imaging Reporting and Data System.

If PH findings are compared with BI-RADS findings assigned into only two gradations (Table 4) we get a highly statistically significant difference ($p = 0.002$; $p < 0.01$), resulting from fact that BI-RADS 5 (100.0%) prevails in patients with malignant tumors, while BI-RADS 4 is present in an equal number of benign and malignant tumors.

When we consider age of the patients in relation to BI-RADS findings assigned into only two gradations (Table 5) we get a highly statistically significant difference ($p = 0.002$; $p < 0.01$), resulting from the fact that patients from BI-RADS 5 group were older than 50 years of age, while only 50% of patients from BI-RADS 4 group were older than 50.

If age of the patients is compared with PH findings assigned into two gradations (benign/malignant) (Table 6) we get a highly statistically significant difference ($p = 0.032$; $p < 0.05$), resulting from the fact that 53.8% of patients from the group with benign tumors were younger than 50 years of age, while this part was only 35.3% of patients with malignant findings; accordingly they were much older.

Table 5

Age of the patients in relation to BI-RADS 4 and BI-RADS 5 categories

Age (years)	BI-RADS 5 (n)	BI-RADS 4 (n)	Total (n)
30–49	0	13	13
50–79	4	13	17
Total	4	26	30

BI-RADS – Breast Imaging Reporting and Data System.

Table 6

Age of the patients in relation to pathohistological (PH) finding of either benign or malignant changes

Age (years)	Benign (n)	Malignant (n)	Total (n)
30–49	7	6	13
50–79	6	11	17
Total	13	17	30

Discussion

Ultrasonographic evaluation of breast changes, as a method complemented to clinical examination and to either diagnostic ("symptomatic" breast) or screening mammography ("asymptomatic" breast) can identify malignancy in some cases, which would otherwise be unidentified, which particularly relates to the glandular structure of breasts because sensitivity of mammography reduces as density of glandular breast tissue becomes higher [5, 6]. When it is about ultrasound descriptors according to data in the literature, the highest objectivity and concordance in the assessment among various physicians performing examination, is for a criterion of tumor change orientation (horizontal, *ie* in parallel with skin, characteristic for benign changes, or vertical, typical for malignant changes). The least accordance is found in the assessment of change contour (very well-defined contours usually in benign changes, poorly defined usually in malignant changes) and its echogenicity (nonechogenic and hyperechogenic are benign changes, while hypoechogenic, isoechogenic changes and complex lesions can be seen in both types of changes, but they are more suspicious of malignant nature) while shape, surrounding tissue and posterior phenomena are between these extremes [7]. According to pathohistological findings in our study, breast cancer was identified in 17 out of 30 women (56.6%), invasive ductal carcinoma in 7 women, invasive lobular carcinoma also in 7 women, tubular carcinoma in one woman and ductal carcinoma *in situ* in 2 women. Fibroadenoma was identified in 8 women (26.6%), dysplasia with atypia in two women (6.7%), while lipoma, intramammary lymph node or fibrocystic dysplasia was pathohistological finding in other patients (by 3.3%). If patients are assigned into two groups: the first one of 49-year-old patients and younger, and the second one of 50-year-old patients and older in relation to PH finding of benign/malignant change, we will get a statistically significant difference because 53.8% of patients from the group with benign changes are younger than 49 years, while only 35.3% of patients have malignant finding. This information is consistent with data in the literature stating that cancer rate suddenly increases after 40 years of age and with data for Central Serbia stating that in less than one fourth of the total number of women breast cancer was diagnosed before their 50 years of age [8]. BI-RADS 4 changes were six times more common reason for biopsy than BI-RADS 5 ($p < 0.01$) which is consistent with data from the literature. BI-RADS 4 category is a change with ultrasonographic characteristics having small (A), moderate (B) or medium (C) risk of malignancy. A supposed risk of cancer is 3–94% in this category. BI-RADS 5 is a change of ultrasonographic finding with high malignancy risk (risk of cancer is higher than 95%) and biopsy is required [9].

Therefore, this paper proves the correlation between BI-RADS 4 and 5 categories and PH finding ($p < 0.05$). Every tumor in BI-RADS 5 category was cancer (4/4), while none of benign tumors belonged to BI-RADS 5 category. In BI-RADS 4 category, in malignant tumors, it was found that BI-RADS 4 B and C prevail (64.7%), while BI-RADS 4A prevail in benign tumors (69.2%), being a very rare finding in malignant tumors (11.8%). Therefore, it could be said that a BI-RADS 4A finding is almost always related to benign changes, primarily to fibroadenoma, and all other with malignant (specificity for BI-RADS 5 is 100.0%, for BI-RADS 4C 83.3%, and for BI-RADS 4B 67%).

A PH finding was invasive ductal cancer in one patient, then ductal cancer *in situ* in one, and invasive lobular cancer in two out of four patients with BI-RADS 5 category.

According to the literature, this is a typical ultrasonographic view of malignant tumors, with specificity for cancer up to 98%. Pathohistologically, it corresponds to cancers with pronounced desmoplastic reaction and it most often confirms invasive ductal, tubular or lobular cancer.

Ultrasonographic findings correspond to nodular tumor type: irregular, circular or oval tumor change, microlobular contours, hyperechogenic border, vertical orientation, with possible areas of microcystic degeneration (by the type of complex lesion – solid change with nonechogenic zones), without posterior phenomena or with posterior amplification of the acoustic beam. According to the literature, an ultra-

sonographic finding being nodular cancer type corresponds to dominant cellularity tumors and it is mostly found in invasive lobular cancer and invasive ductal cancer [3].

Moreover, according to a new, molecular classification of breast cancers, triple-negative breast cancers [estrogen-receptor negative, progesterone-receptor negative and human epidermal receptor (HER)2 negative cancer], have mostly nodular type of expression in ultrasonographic finding with no necessary elements for a benign change [10]. Triple-negative breast cancer are aggressive tumors with poor prognosis, and their frequency is higher in women younger than 50 years of age [11].

Intramammary lymph node certainly belongs to a benign finding (BI-RADS 2). The basis of certainly benign change is visible fat tissue within lymph node hilus [12]. However, in case of lymph node hyperplasia, when it crosses longitudinal diameter of 1 cm, then in atypical localization (inner quadrants) or unfavorable contrast in relation to basic structure of breasts, and accordingly its poor visualization, intramammary lymph node may simulate a malignant change [13, 14].

The BI-RADS 4B group presents heterogeneity of pathohistological and sonomorphological characteristics by four types: nodular form (previously mentioned), well-bordered tumor with pseudocapsule, cystic tumor type and diffuse infiltrative growth tumors.

The pseudobenign aspect of well-defined tumor with pseudocapsule implies: circular or oval shape, well-defined contours, non-homogeneous echotexture and pseudocapsule. According to the literature, this type is particularly characteristic for medullary carcinoma, when they look like cysts with thick content and acoustic amplification. The probability of malignancy in this sonomorphological type is 1–4% at more than 40 years of age [3].

Structurally changed zone, without defined border, angular and dilated ductus, hypoechogenic zones with acoustic shadow are viewed in ultrasonographic finding of diffuse infiltrative growth tumor. This way of sonographic expression is most common in lobular cancer, confirmed in three patients in our group with BI-RADS 4B category. During tumor growth, three-dimensional tumor change does not develop initially, but most often focal nodularity or parenchymal asymmetry, like dysplastic changes, and therefore tumor is diagnosed in its late stage [14].

Cystic cancer is cystic with intracystic proliferation (complex – semisolid, semicystic lesion) by type. According to the literature, it is a lesion with low frequency of occurrence (less than 1%) [3]. In our group, one patient had pathohistological finding *Dysplasia polycystica proliferativa mammae.* Four types of cystic lesions are listed in BI-RADS atlas: simple cyst, grouped microcysts, complicated cyst and complex lesion. Simple cyst belongs to BI-RADS 2 category. Grouped microcysts belong to BI-RADS 3 if they are non-palpable, while palpable cysts are subjected to fine needle aspiration. Complicated cysts (not necessarily indicating inflamed cysts) refer to hypoechogenic lesion with other characteristics of a benign change. Nonpalpable cysts belong to BI-RADS 3 category, and palpable are subjected to fine needle aspiration. Complex lesion refers to the presence of solid and cystic component and it can belong to: intracystic papilloma, cancer, cystic degeneration of either malignant or benign tumors and it is classified into BI-RADS 4 category.

The aforementioned overlaps of sonomorphological types seen in this type of cancer with benign changes are the reason for biopsy and pathohistological evaluation, but false negative findings are possible, as well.

Sonomorphological pseudobenign tumor type with capsule prevailed in the group with BI-RADS 4A category, while the most common PH finding was fibroadenoma.

Overlapping of clinical, mammographic and sonographic findings of fibroadenoma with malignant changes is possible. Therefore, a question remains on the algorithm of diagnostics in palpable change of clinically benign aspect, which is mammographically a type of circular, oval or lobular and well-defined shadow. In this case, ultrasonographic diagnostics is a necessary additional modality of examination, after clinical examination and mammography. If a change is of the solid type, not cystic, a finding is defined as solid tumor, with sonomorphologically benign characteristics, with low risk of malignancy (BI-RADS 4A category) and core needle biopsy of change is indicated (Figure 2). The probability of malignancy is excluded only if fibroadenoma is pathohistologically proved. Therefore, fibroadenoma is significantly present in BI-RADS 4A in this study. A common incidental sonographic finding of non-palpable solid tumors with benign aspect, with size less than 1 cm, has defined another algorithm over time, and it differs from the abovementioned algorithm so far as it is related to nonpalpable change. Namely, a retrospective analysis revealed that risk of malignancy for nonpalpable changes being sonographically a solid type and benign morphology is less than 1–2%. Therefore, the approach is sonographic monitoring in 2 years' time every 6 months, as BI-RADS 3 category, and in case of a stationary finding, it should be translated into BI-RADS 2 category (certainly benign change) after two years of monitoring, without indications for biopsy [15].

Ductal carcinoma *in situ* is normally nonpalpable, subclinical change, detected by mammography during preventive examinations, primarily through microcalcifications typical for malignant changes. However, it was found in two patients with palpable change in our group of patients, as BI-RADS 5 and BI-RADS 4A ultrasound category. Ductal carcinoma *in situ* may sometimes clinically manifest as palpable resistance or nodularity. Non-specific cystic or solid lesion, not well bordered hypoechogenic change, microlobular tumor, ductus dilatation or calcifications, are present in the ultrasonographic finding. It is hard to differentiate ductal carcinoma *in situ* without typical radiologic suspicious calcifications on mammography from benign lesions only by ultrasonography, so further cytological or pathohistological evaluation is necessary [16].

Conclusion

Changes poorly defined by mammography, belonging to either BI-RADS 5 or BI-RADS 4 (A, B, C) have the following characteristics according to our results: BI-RADS 4 category is

six times more common cause for biopsy than BI-RADS 5 category. BI-RADS 5 and BI-RADS 4 categories are the following pathohistological types of lesions: breast cancer (56.7%), fibroadenoma (26.7%), dysplasia with atypia (6.7%) and lipoma, intramammary lymph node or fibrocystic dysplasia by 3.3%. There is a correlation between BI-RADS 4 and 5 categories and pathohistological findings: BI-RADS 4A finding is almost always related to benign changes, primarily to fibroadenoma, and all other with malignant (specificity for BI-RADS 5 is 100.0%, for BI-RADS 4C 83.3%, and for BI-RADS 4B 67%). BI-RADS 4 and 5 categories and age of women are related to pathohistological finding: benign changes are more present (53.8% of all benign changes) than malignant (35.3% of all malignant changes) in 49 year-old women and younger. Heterogeneity of pathohistological findings in BI-RADS 4 category with the domination of malignant changes in BI-RADS 4B and BI-RADS 4C groups, as well as only malignant changes in BI-RADS 5 category confirm necessity of pathohistological verification of lesions from these categories, particularly in women older than 50 years of age.

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Effectiveness of various surgical methods in treatment of Hirschsprung's disease in children

Efikasnost različitih hirurških procedura u lečenju Hiršprungove bolesti kod dece

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Abstract

Background/Aim. Hirschsprung's disease is the most common identifiable developmental disorder of the enteric nervous system, characterized by a failure of its formation in a variable segment of distal bowel. Currently available surgical therapies for Hirschsprung's disease, although lifesaving, are associated with numerous complications. The aim of our study was to evaluate the effectiveness of different surgical methods and the incidence of serious complications after radical surgery of rectosigmoid Hirschsprung's disease. **Methods.** A retrospective analysis, from June 1997 until May 2012 was carried out on 84 patients operated for Hirschsprung's disease of rectosigmoid colon. Transanal endorectal pull-through was performed in 30 (35.7%) patients (group I), while 54 (64.3%) patients were operated by other (Soave, Duhamel or Swenson) procedures (group II). The age at operation, the incidence and severity of postoperative complications, the need for previous colostomy and the number of reoperations are countered in order to evaluate the efficacy of surgical procedures. **Results.** In the group I, the mean age at operation was 9.41 ± 6.37 months and in the group II the mean age at operation was 16.8 ± 13.9 months which was significantly higher ($p < 0.01$). In the group I there were only 3 (10%) patients with complications, one (3%) of them was prone to only one redo procedure (1.00 ± 0.00) and there was no need for previous colostomy in all patients (100%). In the group II there were 16 (30%) patients with significantly frequent complications ($p < 0.05$), about 2 reoperations on the average (1.94 ± 1.84) in 4 of them (25%) and 22 (41%) redo procedures, which was, in total, significantly higher than in the group I ($p < 0.01$). Only Soave's procedure was performed without previous colostomy in 20 (37%) patients. **Conclusion.** Transanal endorectal pull-through in surgical treatment of patients with Hirschsprung's disease is more effective than other procedures concerning earlier onset, low incidence and less severe complications, which would require further operations, and no scars.

Key words:
hirschsprung disease; surgical procedures, operative; postoperative complications; reoperation; child.

Apstrakt

Uvod/Cilj. Hiršprungova bolest je jedan od najbolje izučenih urođenih poremećaja crevnog motiliteta kod dece. Različite hirurške metode za lečenje Hiršprungove bolesti razvijene su da bi se sa što manje rizika i više efektivnosti postigao normalan motilitet creva. Značajan napredak učinjen je smanjivanjem opsežnih hirurških procedura, udruženih sa povećanim morbiditetom i mortalitetom, kao i uvođenjem minimalno invazivnih operativnih tehnika u ranom, neonatalnom uzrastu. Cilj naše studije bio je da se proceni efektivnost različitih hirurških metoda i incidencija komplikacija posle opsežnih operacija Hiršprungove bolesti rekto-sigmoidne lokalizacije. **Metode.** Izvršena je retrospektivna analiza (od juna 1997. do maja 2012. godine) grupe od 84 bolesnika sa Hiršprungovom bolešću. Svi su imali aganglionaran rekto-sigmoidni segment, potvrđen preoperativnom irigografijom i histološki. Operacija metodom endorektalnog provlačenja izvedena je kod 30 (35,7%) bolesnika (grupa I), dok je 54 (64,3%) bolesnika operisano procedurama po Soavi, Duhamelu ili Swensonu (grupa II). Parametri praćenja bili su starost, incidencija i težina postoperativnih komplikacija, neophodnost preoperativne kolostome kao i broj reoperacija. **Rezultati.** U vreme operacije u grupi I prosečna starost dece iznosila je 9,41 ± 6,37 meseci, a u grupi II 16,8 ± 13,9 meseci, što je bilo statistički značajno više u odnosu na grupu I ($p < 0.01$). Postoperativne komplikacije imalo je 3/30

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(10%) bolesnika u grupi I kod kojih je urađena operacija endorektalnog provlačenja i 16/54 (29,6%) bolesnika kod kojih su preduzete druge procedure. Pripadnici grupe II imali su značajno češće komplikacije ($p < 0,05$). Bolesnici grupe I imali su samo jednu (1,00 ± 0,00), dok su oni iz grupe II imali oko dve reoperacije u proseku (1,94 ± 1,84). Više od jedne reoperacije urađeno je kod 6/16 (37,5%) bolesnika iz grupe II. Pripadnici grupe II imali su značajno više reoperacija od onih iz grupe I ($p < 0,05$). Operativna procedura kod svih bolesnika (100%) grupe I i samo procedura po Soavi kod 20 (37%) bolesnika grupe II, preduzete su bez prethodne kolostome. **Zaključak.** Rezultati naše studije pokazuju da je operacija metodom endorektalnog provlačenja u lečenju Hiršprungove bolesti mnogo efikasnija nego ostale procedure, posebno u odnosu na njeno rano preduzimanje, kao i manju incidenciju ozbiljnih komplikacija koje zahtevaju ponovno hirurško lečenje.

Ključne reči:
hiršprungova bolest; hirurgija, operativne procedure; postoperativne komplikacije; reoperacija; deca.

Introduction

Hirschsprung's disease (HD) is the most common identifiable developmental disorder of the enteric nervous system (ENS), characterized by failure of its formation in variable segments of distal bowel. Traditionally, surgical therapy for HD considered preliminary proximal defunctioning colostomy, followed months later by the definitive reconstructive pull-through procedure, mostly by Swenson, Duhamel or Soave's technique. These surgical therapies for HD, although lifesaving, were associated with a significant incidence of disturbances of bowel function. The most frequent postoperative complications include enterocolitis after the Swenson's procedure, constipation following Duhamel's repair, and diarrhea and incontinence after the Soave's pull-through procedure. Even reports on long-term outcomes after definitive repair for HD are conflicted highlighting the need for newer curative therapies [1, 2].

In the beggining, the routine use of colostomy have been abandoned in favor of one-stage pull-through, with multiple studies suggesting this approach as safe and efficacious. Over the past few decades, the popularity of minimally invasive surgical techniques has led to a number of modifications to the standard one-stage procedure and solely transanal approach, which has been adopted by many surgeons and associated with the same advantages, without the need for extensive intraabdominal dissection. Several other creative approaches have been described, including a modification of the transanal approach with laparoscopic assistance [3, 4]. Current regenerative strategies are under investigation to restore function in aganglionic intestine. Stem cell transplantation to regenerate the ENS is a subject of many recent experimental series. Though auspicious, these discoveries warrant further study to translate cell-based therapies into clinical practice [5].

The aim of our study was to evaluate the effectiveness of different surgical methods and the incidence of serious complications after radical operation of rectosigmoid HD.

Methods

We identified cases by retrospective review of HD admissions to the University Children's Hospital in Belgrade, Serbia, over a 15-year-period, from June 1997 to May 2012. During this period there were 84 patients with recto-sigmoid aganglionosis treated surgically. They were divided into two groups: the group I included 30 (35.7%) patients operated on by single-stage transanal endorectal pull-through (TEPT) and the group II of 54 (64.3%) patients operated on by Soave, Duhamel or Swenson's open technique. They were also observed concerning the presence of colostomy: 50 (59.5%) patients operated on by single-stage TEPT and Soave technique, had no colostomy, while other 34 (40.5%) patients were operated on by three-stage Duhamel and Swenson's procedures. They had previous colostomy, which was subsequently closed, about 4 weeks after the radical procedure.

The study was performed according to the principles of Good Clinical Practice and the Declaration of Helsinki, after the Institutional Ethical Committee approval and signing the form of parents consent.

Prior to surgery, all the patients underwent water-soluble contrast or barium enema that helped to identify a transition zone between a narrowed aganglionic and dilated, otherwise normally innervated segments. Histological findings including the absence of ganglion cells and hypertrophic extrinsic nerve fibers in submucosal and myenteric layers of rectal biopsy specimen, confirmed the diagnosis of HD. Antimicrobial prophylaxis considered monotherapy of ertapenem, in a course of 3 days in the group I, or triple antibiotic treatment of ampicillin, gentamycin and metronidazole, in a course of 5 days in the group II. Colonic lavage, consisting of mechanical irrigation with a large-bore rectal tube and large volumes of irrigant, had been required preoperatively. The surgery was done under general anesthesia.

The age at operation, the incidence and severity of postoperative complications, the need for previous colostomy and the number of reoperations were countered in order to evaluate the efficacy of surgical procedures.

The complication frequency was presented as a whole number and percents. χ^2 test was performed to establish a statistical significance between the study groups of patients, regarding complication frequency. The average value of redo operations was presented as a mean value (x) with the standard deviation (SD). Fisher's test was used to evaluate complication frequencies among different surgical procedures in the groups of patients with and without colostomy. Mann-Whitney U-test was used to evaluate statistical significance of mean values of reoperations between the study groups. Statistical significance was set at $p < 0.05$.

Results

From the total number of 84 patients, 67 (79.8%) were males and 17 (20.2%) females, so the ratio was 4 : 1. In the group I,

the mean age at operation was 9.41 ± 6.37 months, which was significantly lower comparing to the group II ($p < 0.01$); there were only 3 (10%) patients with complications, one (3.3%) of them was prone to only one redo procedure (1.00 ± 0.00) (Table 1) and there was no need for previous colostomy in all the patients (100%) (Table 2).

In the group II, the mean age at operation was 16.8 ± 13.9 months. There were 16 (30%) patients with significantly frequent complications ($p < 0.05$), about 2 reoperations on the average (1.94 ± 1.84) in 4 (25%) of them (Table 1) and 22 (41%) redo procedures, which was, in total, significantly higher than in the group I ($p < 0.01$) (Table 3). In this group, only Soave's procedure was performed without previous colostomy in 20 (37%) patients. The patients operated by single-stage TEPT and Soave's procedure without colostomy had far less complications and reoperations than the others with colostomy (Table 2).

Within these very groups with and without colostomy, there was no significant difference in distribution of complications and reoperations among different surgical procedures ($p > 0.05$) (Table 2).

The group I had significantly lower number of complications ($p < 0.05$). Anastomotic stricture was the only (100%) complication in the group I and the most frequent with Soave's operation (67%), while enterocolitis was the most frequent complication with Duhamel's (45%) and Swenson's (100%) procedures. Almost every third patient with colostomy had stoma complications (38%) with the similar incidence of prolapse (46%) or stoma stenosis (54%) (Table 3).

Table 1

Evaluated parameters regarding surgical procedures

Evaluated parameters	Group I†	Group II†	*p*-values
Number of patients, n (%)	30 (35.7)	54 (64.3)	
Complications, n (%)	3 (10.0)	16 (29.6)	< 0.05*
Reoperation, $\bar{x} \pm SD$	1.00 ± 0.00	1.94 ± 1.84	< 0.05**
Age (months), $\bar{x} \pm SD$	9.41 ± 6.37	16.81 ± 13.95	< 0.01**

Group I – group with transanal endorectal pull-through procedure; Group II – group with Soave, Duhamel and Swenson's procedures.*χ^2 test; **Mann-Whitney U-test.

Table 2

Evaluated parameters regarding the presence of colostomy

Evaluated parameters	n (%)	Complications n (%)	*p**	Reoperation $\bar{x} \pm SD$	*p***
Without colostomy					
TEPT	30 (100)	3 (10)		1.00 ± 0.00	
Soave	20 (37)	3 (15)	> 0.05	1.00 ± 0.00	> 0.05
With colostomy					
Duhamel	29 (54)	11 (38)		2.03 ± 1.97	
Swenson	5 (9)	2 (40)	> 0.05	1.00 ± 0.00	> 0.05
Stoma complications		13 (38)			

***Fisher test; **Mann-Whitney U test; TEPT – transanal endorectal pull–through procedure.**

Table 3

Distribution of complications and reoperations in evaluated groups

Type of surgical procedure	n (%)	Complications associated with surgical procedures	n (%)	*p*
TEPT	3 (10)	Anastomotic stricture	3 (100)	
Soave	3 (15)	Anastomotic stricture	2 (67)	
		Anastomotic dehiscence	1 (33)	
Duhamel		Residual aganglionosis	2 (18)	< 0.05*
	11 (38)	Anastomotic dehiscence	2 (18)	
		Obstructed pouch	2 (18)	
		Enterocolitis	5 (45)	
Swenson	2 (40)	Enterocolitis	2 (100)	
Stoma	13 (38)	Prolapse	6 (46)	
		Stenosis	7 (54)	> 0.05*
Reoperations	19 (23)			
one			15 (79)	
more than one			4 (21)	< 0.01**

***χ2-test; **Fisher test, TEPT – transanal endorectal pull-through procedure.**

In the entire group of patients who required reoperation, one redo was performed predominantly in 79% of cases ($p < 0.01$). In the group 1, there was only one (3%) redo operation, because of omitting the posterior myectomy of the cuff, by mistake in the first case of TEPT. In the group II, one redo was done in 14 (26%) patients and 2 on the average in each of 4 (15%) patients which was in total significantly higher, 22 (41%), redo procedures ($p < 0.01$) (Figure 1). More than one redo were done because of overlooked residual aganglionosis in 2 cases and residual pouch obstruction with incomplete resection of the common colorectal wall in Duhamel's technique in another 2 cases.

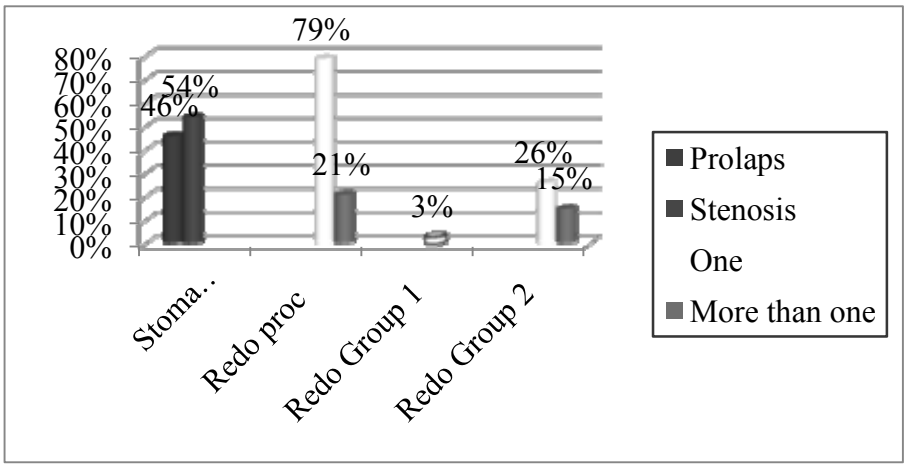

Fig. 1 – Distribution of stoma complications and redo procedures in the evaluated groups.
Group I – group with transanal endorectal pull-through procedure;
Group II – group with Soave, Duhamel and Swenson's procedures.

Discussion

There are several surgical techniques that are used for the treatment of patients with HD [6]. However, despite advances in medicine and surgery, there are still children with functional problems even after surgical treatment [7]. It is important to stress out that the main goal of surgery in these patients is to remove aganglionic segment and to enable bowel function management. Previous studies notice that TEPT is better surgical choice, mainly due to infrequent complications and better functional outcome [8, 9]. The main indications that require reoperation refer to the anatomical problems (*eg* strictures, twisted pull-through, obstructions), pathological problems (*eg* residual aganglionosis) and others (*eg* enterocolitis, fistulas) [10].

Our results clearly demonstrate that TEPT is most effective surgical option in the treatment of patients with HD. The advantage of such method is that TEPT is minimally invasive procedure with no need for colostomy and could be performed in neonatal period. Currently, approximately 90% of patients with Hirschsprung's disease are diagnosed and also could be cured in the newborn period. In spite the fact that TEPT procedure is associated with common complications including constipation, enterocolitis and strictures, we show that the presence of complications and particular need for reoperation are far less frequent than with other techniques [11]. The enterocolitis that is associated with HD in children still presents a significant cause of morbidity and mortality [12]. The occurrence of postoperative enterocolitis in our study is confirmed in almost every tenth patient (8.3%) that is similar with previous reports in the literature [13, 14]. In our study the enterocolitis is predominantly associated with surgical procedures requiring colostomy (Duhamel and Swenson) and also with strictures as complication. The outcomes of TEPT procedure have been similar to open single-stage approaches, and analgesia requirements and hospital stays are decreased. Recent studies also report lower rates of postoperative incontinence and shorter operating times among transanal pull-through procedures [15]. We showed that even Soave's surgical technique did not require colostomy; it had slightly higher frequency of complications comparing to TEPT. It was noticed as well that both Duhamel and Swenson's procedures had highest rates of complications, but the need for more than one reoperation was significantly higher in patients who underwent Duhamel's procedure. It is consistent with previous reports which stated that pouch in Duhamel's procedure is mainly responsible for serious complications like impaction, overflow incontinence and enterocolitis [16]. The overall morbidity and mortality in staged procedures are increased by complications associated with procedures requiring colostomy [17, 18]. Therefore, a single-stage TEPT procedure could be of a great benefit in further surgical practice for patients with HD, since it reduces complications, shorten hospitalization time and hospital costs, possibilities of acquiring infection and finally reduces further necessity for reoperations [18, 19]. Such a technique is more favorable due to less severe postoperative pain and excellent cosmetic result with no scar [19]. The study of Zhang et al. [20] it was noticed that functional outcome (stooling patterns and colonic motility) is satisfactory in patients who undergo TEPT procedure. Although, the limitation of our study refers to the lack of these long-term functional predictors after TEPT, we consider that regarding the surgical procedure, choosing the appropriate time and technique and

including opting for a less invasive approach are the key factors for a good long-term functional outcome.

Conclusion

The results of our study show that the TEPT procedure in surgical treatment of patients with HD is more effective than other procedures concerning early onset, low incidence and less severe complications that would require further operations and also, excellent cosmetic result with no scar.

Conflict of interest

No one of the authors has an affiliation or financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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In-hospital mortality analysis in patients with proximal femoral fracture operatively treated by hip arthroplasty procedure

Analiza bolničke smrtnosti kod bolesnika sa prelomom proksimalnog dela femura operativno lečenog metodama artroplastike kuka

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Abstract

Background/Aim. Hip fracture remains the leading cause of death in trauma among elderly population and is a great burden to national health services. In-patient death analysis is important to evaluate risk factors, make appropriate selection and perform adequate treatment of infections for patients to be operated. The aim of this study was to analyze in-hospital mortality in proximal femoral fracture patients operatively treated with hip arthroplasty procedure. **Methods.** We followed 622 consecutive patients, and collected data about age, gender, the presence of infection preoperatively and postoperatively, American Society of Anesthesiologists (ASA) score, diabetes mellitus and the type of surgical procedure. Postoperative infections included pneumonia, urinary tract infections, surgical site infections and sepsis. **Results.** We found a statistically significant influence of preoperative and postoperative infection presence for in-patient mortality with relative risk for lethal outcome of 4.53 (95% CI: 1.44–14.22) for patients with preoperative infection and 7.5 (95% CI: 1.90–29.48) for patients with postoperative infection. We did not confirm a statistically significant influence of age, gender, ASA score, diabetes mellitus or the type of surgical procedure for increased mortality rate. **Conclusion.** Adequate preoperative selection, risk evaluation and adequate treatment of infections are of the key importance for lowering the risk of death in patients operated due to proximal femoral fracture and treated by hip arthroplasty procedures. Special attention is to be paid for the presence of preoperative and postoperative infections in patients operatively treated due to the risk for increased in-hospital mortality.

Key words:
femoral fractures; aged; arthroplasty; cross infection; mortality; risk factors.

Apstrakt

Uvod/Cilj. Fraktura proksimalnog dela femura je vodeći uzrok smrti od povreda kod starijih bolesnika, kao i veliko opterećenje za nacionalne zdravstvene službe. Analiza bolničke smrtnosti je važna da bi se ustanovili faktori rizika, napravila adekvatna selekcija bolesnika, i sprovelo adekvatno lečenje pre- i postoperativnih infekcija. Cilj ove studije bio je analiza bolničke smrtnosti kod bolesnika operativno lečenih artroplastikom kuka zbog preloma proksimalnog femura. **Metode.** Pratili smo 622 uzastopna bolesnika sa prelomom proksimalnog dela femura i analizirali starost, pol, prisustvo preoperativne i postoperativne infekcije, skor Američkog udruženja anesteziologa (ASA), prisustvu dijabetes melitusa i vrstu operativne metode. Postoperativne infekcije uključivale su pneumoniju, urinarnu infekciju, infekciju operativnog mesta (IOM) i sepsu. **Rezultati.** Našli smo statistički značajan uticaj preoperativnog i postoperativnog postojanja infekcije na hospitalnu smrtnost sa relativnim rizikom od smrtnog ishoda 4,53 (95% CI: 1,44–14,22) kod bolesnika sa preoperativnom infekcijom i 7,5 (95% CI: 1,90–29,48) kod bolesnika sa postoperativnom infekcijom. Nije registrovan statistički značajan uticaj starosti, pola, ASA skora, dijabetesa melitusa ili vrste operativne metode na povećanje mortaliteta u toku hospitalizacije. **Zaključak.** Adekvatna preoperativna selekcija, procena rizika i preduzimanje adekvatnih terapijskih mera u cilju lečenja infekcija od ključnog su značaja za smanjenje rizika smrtnog ishoda u toku hospitalizacije kod bolesnika operisanih zbog preloma proksimalnog femura metodama artroplastike kuka. Posebnu pažnju potrebno je obratiti na prisustvo preoperativne i postoperativne infekcije kod operisanih zbog povećanog rizika od smrtnog ishoda u toku hospitalizacije.

Ključne reči:
femur, prelomi; stare osobe; artroplastika; infekcija, intrahospitalna; mortalitet; faktori rizika.

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Introduction

Hip is defined as the region of proximal femur from articular cartilage proximal, to the extent of 5 cm below the lesser trochanter. Hip fracture remains the leading cause of death in trauma among elderly population and is a great burden to national health services. There is about 300 000 hip fractures in the USA yearly [1]. The fact that the population mostly exposed to this injury is elderly, suggests that comorbidities and complications are common. In-patient death rate varies from 2.3% [2] to the range from 5% to even 50% according to various studies [3]. In-patient death analysis is important to evaluate risk factors, make appropriate selection and perform adequate treatment of infections for patients to be operated on.

Methods

The population of 622 consecutive patients, with proximal femoral fracture (PFF), treated with hip arthroplasty procedure were included in this study. Hemiarthroplasty was performed in 393 patients (group 1) and total hip arthroplasty in 229 cases (group 2). Data was collected about age, gender, American Society of Anesthesiologists (ASA) score, the presence of diabetes mellitus (DM), the presence of infection preoperatively and postoperatively and the type of surgical procedure. Postoperative infections included pneumonia, urinary tract infections, surgical site infections (SSI) and sepsis. Regarding postoperative infection we used the criteria established by the USA Center for Disease Control and Prevention [4].

This cohort prospective study was designed to analyze risk factors for in-hospital death for the study population.

Complete statistical analysis of data was done with the statistical software package, SPSS Statistics 17 (Chicago, IL, USA). Most of the variables were presented as the frequency of certain categories, while statistical significance of differences was tested with the χ^2 test.

In case of continuous data, the variables were presented as the mean value ± standard deviation (SD) and the statistical significance of differences was tested by *t*-test.

Calculations of odds ratios and their 95% confidence intervals (CI) were done to determine the association between risk factors and outcomes (survival). For that purpose, the most promising independent variables as a single risk factor were incorporated into binary logistic regression analyses. All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

During the period January 1, 2006 till December 31, 2010 a total number of 622 patients were operatively treated by hip arthroplasty procedure due to the PFF. The overall death rate was 27 patients that was 4.3% of the total study population (Table1).

Hemiarthroplasty was performed in 393 and total hip arthroplasty in 229 cases. A total of 16 (4.1%) patients in the hemiarthroplasty group and 11 (4.8%) in the group treated by total arthroplasty died. There was no significant statistical difference in mortality rate between the groups ($p = 0.819$) (Table1).

Table 1
Most important basic characteristics of the patients

Parameters	Value
Age of patients (years), $\bar{x} \pm SD$	75.79 ± 10.36
Sex, n (%)	
male	202 (32.5)
female	420 (67.5)
Total	622 (100.0)
Type of arthroplasty (%)	
total	229 (36.8)
partial	393 (63.2)
Diabetes, n (%)	
yes	107 (17.2)
no	515 (82.8)
BMI (kg/m^2), $\bar{x} \pm SD$	25.22 ± 3.75
ASA score, $\bar{x} \pm SD$	2.60 ± 0.62
Clinical outcome, n (%)	
non-survivors	27 (4.3)
survivors	595 (95.7)
Total	622 (100.0)

BMI – body mass index; ASA – American Sociaty of Anesthesiologists.

The patients were grouped into 3 categories according to age: up to 65 (93 patients), from 65.1 to 75 years (142 patients) and older than 75 (387 patients). A total number of 4 (4.3%) patients in the category 1, 4 (2.8%) in the category 2 and 19 (4.9%) in the category 3 died. There was no significant statistical difference in the mortality among the categories ($p = 0.578$) (Tables 1 and 2).

According to the ASA score (physical status classification system) the patients were classified as ASA-1 (11 patients had zero mortality), in ASA-2 group (n = 265 patients) 9 (3.4%) died, in ASA-3 group (n = 310 patients) 14 (4.5%) died and in ASA-4 group (n = 36 patients) 4 (11.1%) patients died.

Table 2
Distribution of in-hospital deaths according to the age group

Age category (years)	Outcome, n (%)		Total
	non-survivors	survivors	
≤ 65	4 (4.3)	89 (95.7)	93 (100.0)
66–75	4 (2.8)	138 (97.2)	142 (100.0)
> 75	19 (4.9)	368 (95.1)	387 (100.0)
Total	27 (4.3)	595 (95.7)	622 (100.0)
χ^2 test	$\chi^2 = 1.09$; $p = 0.578$		

Comparing mortality for all ASA classes there was no significant difference in the mortality ($p = 0.167$) (Tables 1 and 3).

Considering the patients with diabetes mellitus there were 107 patients out of which 7 (6.5%) died, that was without statistical significance ($p = 0.33$) (Table1).

The presence of preoperative and postoperative infection showed a statistically important influence on in-patient mortality ($p = 0.02$ and $p = 0.007$ respectively). Regarding preoperative infection 4 (15.4%) out of 26 patients died (Table 4). Regarding postoperative infection 3 (25%) out of 12 patients died (Table 5). By logistic regression we calculated the relative risk of 4.53 for patients with preoperative infection for lethal outcome and 7.5 for patients with postoperative infection (Tables 2, 4 and 6).

Discussion

Age is a very important risk factor concerning orthopedic surgical treatment of PFF by hip arthroplasties and final outcome. Generaly, elderly population sustains these kinds of injury mostly, according to available data. Older patients with PFF operatively treated by orthopaedic surgery methods, commonly have comorbidities that can increase the risk of postoperative complications, including mortality [1, 2, 5]. The influence of age on in-hospital mortality of these patients varies from study to study. In our study, concerning the age we confirmed no statistical difference. Our results are similar to some other ones [6], but opposite to findings in some other studies [7, 8].

In Italian study, there is no evidence of significant reduction in mortality rates after hip fractures in elderly, in the last 20 years [6]. The question is if there is any possibility to reduce mortality after PFF and optimize the outcome of operative treatment of these injuries. Pioli et al. [6] have found an unexpected results in their study with no statisticaly significant correlation between age and mortality after hip fractures. The possible explanation for this is exclusion of patients under 70 and only 1-year follow-up period. There is the possibility that rate of mortality after hip fractures may not be influenced only by age but also by increased degree of frailty in this population of patients [6]. According to Diaman-

Table 3

Distribution of in-hospital deaths according to the American Society of Anesthesiologists (ASA) score

ASA score	Outcome, n (%)		Total
	non-survivors	survivors	
1	0 (0.0)	11 (100.0)	11 (100.0)
2	9 (3.4)	256 (96.6)	265 (100.0)
3	14 (4.5)	296 (95.5)	310 (100.0)
4	4 (11.1)	32 (88.9)	36 (100.0)
Total	27 (4.3)	595 (95.7)	622 (100.0)
χ^2 test	$\chi^2 = 5.06$ $p = 0.167$		

Table 4

Distribution of in-hospital deaths according to preoperative infections

Preoperative infections	Outcome, n (%)		Total
	non-survivors	survivors	
Yes	4 (15.4)	22 (84.6)	26 (100.0)
No	23 (3.9)	573 (96.1)	596 (100.0)
Total	27 (4.3)	595 (95.7)	622 (100.0)
χ^2 test	$\chi^2 = 5.43$ $p = 0.02$		

Table 5

Distribution of in-hospital deaths according to postoperative infections

Postoperative infections	Outcome, n (%)		Total
	non-survivors	survivors	
Yes	3 (25.0)	9 (75.0)	12 (100.0)
No	24 (4.3)	540 (95.7)	564 (100.0)
Total	27 (4.7)	549 (95.3)	576 (100.0)
χ^2 test	$\chi^2 = 7.15$ $p = 0.007$		

Table 6

Unadjusted risk factors (OR) that predict lethal outcome

Infections	Odds ratio (OR)	95% confidence interval		Probability
		lower	upper	
Preoperative				
yes	4.53	1.44	14.22	$p = 0.01$
no	1			$p = 1.00$
Postoperative				
yes	7.50	1.90	29.48	$p = 0.004$
no	1			$p = 1.00$

topoulos et al. [7] older age is an independent risk factor of increasing mortality after hip fracture in males and females (OR – 5.74 : 6.95, respectively).

Clague et al. [8] in their research found older age as one of the factors predicting in-hospital mortality in patients sustained hip fractures. According to the authors, increased age also has a significant effect on increasing the length of total hospital stay.

Relatively low mortality was found in our study among the oldest patient category over the age of 75 (4.9%), comparing the youngest patient category with the age ≤ 65 (4.3%). Similar mortality between the youngest and the oldest categories suggests that fracture itself has the risk of lethal outcome [9].

Although increased ASA score among elderly should suggest greater risk [10] that was not confirmed in our study. Kapicioglu et al. [10] researched risk factors for postoperative complications and mortality in extremely old patients (> 90 years) following hip fracture surgery. The authors have found significantly high incidence of postoperative complications and mortality in the group with ASA score 3 comparing to ASA score 1 ($p = 0.041$; $p = 0.022$). There was no significant correlation between gender and mortality ($p = 0.11$). Similar results can be found in a study by Vidan et al. [11]. The influence of high ASA score values on increasing in-hospital mortality explained that medical comorbidities were associated with surgical delay in patients with hip fracture. "Late surgery" in those cases is an important cause of increased in-hospital mortality ($p = 0.002$).

Regarding the ASA score most patients in our study were within the ASA 3 group (62%) but the mortality was 4.5% that is lower than in the study of Giversen [12]. The reason for lower mortality is probably due to the fact that we counted only in-patient mortality and that the study took into consideration in-patient mortality within 30 days. We find the ASA score as general, not precisely defining the nature of comorbidities [13]. Our findings of the similar mortality between the genders differs from those in some other studies [12, 14, 15] in which increased mortality was found with male predomination. Kapicioglu et al. [10] showed no difference in mortality rates between males and females ($p = 0.11$), similarly to our results. According to a research by Dorotka et al. [16] diabetic patients belong to the group of patients with higher risk of mortality and morbidity, following operative treatment of hip fractures. Medical condition in these patients can be optimized through adequate preoperative and postoperative measures under control of endocrinologist. Considering our results, we confirmed no significant correlation between DM and a higher mortality incidence.

The overall in-hospital mortality in our study (4.3%) is within the range of many studies and is closer to the lower boundaries [17]. Adequate preoperative evaluation, optimizing health status and active surveillance are of the key importance to survival rate improvement [4]. It means that predominantly cardiological and anesthesiological adequate preoperative estimation was effective in lowering the mortality risk. Preoperative and postoperative infection was the key risk factor in our study. Other papers also showed higher mortality rate due to postoperative chest infection in limb and hip trauma. Postoperative infection is a significant contributor to mortality [18–20].

Conclusion

Adequate preoperative selection, risk evaluation and adequate treatment of infections are of the key importance for lowering the risk of death for those operated on due to proximal femoral fracture treated by hip arthroplasty procedures. Special attention should be paid to the presence of preoperative and postoperative infections in patients operatively treated due to the risk for increased in-hospital mortality.

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Prevalence of Panton-Valentine leukocidin genes in community-associated methicillin-resistant *Staphylococcus aureus* in the District of Pomoravlje

Prevalencija Panton-Valentin leukocidin gena u vanbolničkim meticilin-rezistentnim *Staphylococcus aureus* u Pomoravskom okrugu

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Abstract

Background/Aim. Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains appear to have rapidly disseminated among population in the community without established risk factors for MRSA worldwide. Panton–Valentine leukocidin (PVL) is a cytolytic toxin, encoded by the *lukF-PV* and *lukF-PV* genes. PVL may be the key toxin responsible for enhanced virulence of CA-MRSA. The aim of this study was to detect the genes encoding PVL in CA-MRSA isolates from healthy people from the District of Pomoravlje. **Methods.** We took throat and nose swabs from healthy, employed persons with mandatory sanitary examinations and analyzed the presence of MRSA, between January 2011 and December 2012 in the District of Pomoravlje. Susceptibility of isolated strains to cefoxitin was investigated by using disc diffusion according to the recommendation of CLSI (Clinical Laboratory Standard Institute), and by E test. The presence of penicillin–binding protein 2a (PBP2a) in *Staphylococci* was detected using latex agglutination Slidex®MRSA Detection test. The gold standard, polymerase chain reaction (PCR) assay, was used for detection of *mecA* gene and PVL gene, and typing of SCC*mec* region. **Results.** Our investigation showed that staphylococcal carrier state was present in 2.58% of 52,910 throat and nasal swabs, and in 50 of them (3.67%) MRSA was isolated. Among these MRSA, 2 (4%) isolates were PVL-positive. **Conclusion.** The prevalence of CA-MRSA and the presence of PVL gene among healthy, employed population in the District of Pomoravlje were low. The values obtained in this study show that, our region is not significantly different from the other parts of our country, nor from the other European countries.

Key words:
methicillin resistance; staphylococcus aureus; community acquired infections; polymerase chain reaction; panton-valentine leukocidin; serbia.

Apstrakt

Uvod/Cilj. Vanbolnički (*community-associated* – CA) meticilin rezistentni *Staphylococcus aureus* (CA-MRSA) se brzo širi u opštoj populaciji, pa i među onima koji nisu bili izloženi riziku boravka u bolnici. Panton Valentin leukocidin (PVL), kodiran *lukF-PV* i *lukF-PV* genima, može biti ključni toksin odgovoran za virulenciju vanbolničkog MRSA. Cilj rada bio je detektovati gene koji kodiraju PVL u izolatima vanbolničkog MRSA u Pomoravskom okrugu. **Metode.** Prisustvo *S. aureus* rezistentnog na meticilin testirano je tokom dvogodišnjeg perioda, na 52 910 briseva grla i nosa poreklom od zdravih, radno sposobnih ljudi koji podležu sanitarnom nadzoru. Brisevi su zasejavani na krvni agar i inkubirani 24 h. Rezistencija na meticilin detektovana je disk difuzionim testom sa diskom cefoksitina i E-testom, a test aglutinacije primenjen je za dokazivanje penicillin-vezujućih proteina 2a (PVP2a). Za detekciju *mecA* gena PVL gena i tipizaciju SCCmec regiona primenjena je *polymerase chain reaction* (PCR) metoda. **Rezultati.** Stafilokokno kliconoštvo bilo je prisutno kod 1 363 (2,58%) ispitanih, a MRSA je potvrđen kod 50 izolata *S. aureus* (3,67%). PVL gen je otkriven u dva (4%) CA-MRSA izolata. Jedan od PVL-pozitivnih izolata sadržao je SCCmec region tipa IV, a drugi tipa V. **Zaključak.** Prevalenca MRSA kod zdravih kliconoša, kao i zastupljenost PVL gena bili su niski. Vrednosti dobijene u ovoj studiji, pokazuju da se naš region ne razlikuje značajno od drugih delova naše zemlje i drugih evropskih zemalja.

Ključne reči:
meticilin, rezistencija; staphylococcus aureus; infekcije, vanbolničke; polimeraza, reakcija stvaranja lanaca; panton-valentin leukocidin; srbija.

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Introduction

Until recently, methicillin-resistant *Staphylococcus aureus* (MRSA) was considered as the prototype of a nosocomial pathogen [1]. Since the mid-1990s [2], this pathogen has emerged as a cause of infection in young, previously healthy people in general community, and the term community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was established. From their health care-associated MRSA (HA-MRSA) counterparts, these isolates differ clinically, in the virulence factors, epidemiology and frequency of occurrence [3, 4].

Methicillin resistance is conferred by the *mecA* gene, which is part of a mobile genetic element called the "staphylococcal cassette chromosome (SCC) *mec*". CA-MRSA and HA-MRSA can been distinguished by molecular methods, based on the differences of SCC*mec* region. HA-MRSA isolates carry a relatively large SCC*mec* belonging to type I, II, or III. Beside methicillin, they are often resistant to many classes of non-β-lactam antimicrobials. In contrast, CA-MRSA isolates harbor smaller SCC*mec* elements, type IV or V [5], having a size up to 30 kb, and are presumable more mobile. They are resistant to fewer non-β-lactam classes of antimicrobials.

MRSA, like other *S. aureus* strains, has numerous mechanisms to produce disease and to evade host defense. In establishing an infection, numerous surface proteins mediate adherence to host tissues or prosthetic materials. After adhesion, it is able to grow and persist in various ways: it can form biofilms, invade and survive inside epithelial cells, including endothelial cells; form small-colony variants which may contribute to persistent and recurrent infection, produce antiphagocytic microcapsule that help it evade the host immune system or produce leukocidins that cause leukocyte destruction [6].

Panton-Valentine leukocidin (PVL) is a two-component *S. aureus* protein encoded by the *lukF-PV* and *lukS-PV* genes. Its ability to lyse leukocytes was first described in 1894 by Van de Vald [7]. Panton and Valentine in 1932 linked the presence of leucotoxin with skin and soft tissue infections (SSTI) [8]. Some authors indicate that infections with PVL-positive strains are more severe: pneumonia caused by PVL-positive MRSA or methicillin-sensitive *Staphyloccocus aureus* (MSSA) strains is accompanied by high fever, sepsis, hemoptysis, pleural effusion, and even death [9]. PVL is commonly observed in CA-MRSA strains, and the frequency of PVL in the United States is increasing along with the spread of CA-MRSA clones [10, 11]. Subsequently, there have been reports of PVL-positive clones emerging in the hospital [12]. While some authors proposed PVL as a genetic marker of CA-MRSA [13], a group of authors from Australia did not find a significant association between CA-MRSA-SCC*mec* type IV and PVL gene [14].

The objective of this study was to establish the prevalence of PVL in MRSA isolates associated with community.

Methods

Bacterial isolates

During 2011 and 2012 we analysed 52,910 throat and nose swab samples taken from adult, healthy population from 16 to 60 years of age, from the District of Pomoravlje.

The swabs were cultured on blood agar (Bio-Merieux, France) and then incubated for 24 h aerobically at 37°C. All isolates were stored frozen in dextrose broth at -20ºC, and recultivated on blood agar prior to each experiment. The isolates of *S. aureus* were identifided by tube coagulase test with rabbit plasma (Torlak, Belgrade) after incubation for 4 h and 24 h. Test negative after 4 h had to be reexamined after 24 h.

Antibiotic susceptibility test

The sensitivity of S. *aureus* to methicillin and other groups of antibiotics was tested by the disk diffusion (DD) method according to the recommendation of Clinical Laboratory Standard Institute (CLSI) [15]. Mueller-Hinton agar (MHA) (Bio-Merieux, France) was inoculated with suspension of 24-hour culture of staphylococci, density of 0.5 McFarland. After 15 min antibiogram disks were placed: cefoxitin (30 μg), gentamicin (30 μg), amikacin (30 μg), tetracycline (30 μg), ofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), trimethoprim-sulfamethoxazole − SXT (1.25 + 23.75 μg), fusidic acid (30 μg), vancomycin (30 μg) (BD, England), and incubated for 18−24 h at 35−37°C.

Methicillin resistance was also determined by agglutination test "Slidex MRSA Detection" to prove PBP2a (Bio-Merieux, France). The "Slidex MRSA Detection" test is a rapid slide agglutination assay designed to detect the presence of PBP2a in *S. aureus*. Test was performed as recommended by the manufacturer.

MIC of cefoxitin was determined by E test (Bio-Merieux, France). The test conditions recommended by the manufacturer are based and providing results comparable with CLSI methods and include incubation of inoculum whose density is equivalent to 0.5 McFarland standards, on MH agar with 2% NaCl, for 24 h at 35−37 °C.

The isolates were considered CA-MRSA according to criteria established by Centers for Disease Control and Prevention (CDC) [16]: they were derived from healthy people that had not been hospitalized within the preceding year.

PCR detection of the mecA gene and PVL genes and typing of SCCmec region

For PCR amplification, bacterial DNA was prepared by the use of kit for DNA isolation (B-DNA Sorb, Sacace, Italy). The resistance to methicillin was confirmed by amplifying a 162 bp fragment of *mecA* gene by primers and conditions described previously Oliveira et al. [17]. The primers used to amplify a 433 bp region of *lukF-PV* genes and PCR conditions were previously described by Lina et al. [18]. Typing of SCC*mec* region was performed using the primers and protocols described by Milheiriço et al. [19].

Results

A total of 52,910 throat and nose swabs were analysed, and in 1,363 (2.58%) *S. aureus* was isolated. By the use of antibiogram disks with cefoxitin, E test for cefoxitin, and agglutination test for MRSA detection, and according to the

criteria established by CDC, among these *S. aureus* isolates 50 (3.67%) of them belonged to CA-MRSA. In PCR amplification with primers specific for *mecA* gene, all 50 isolates were positive and proven MRSA.

Beside cefoxitin, CA-MRSA isolates were tested for sensitivity to other groups of antibiotics: fusidic acid, SXT, quinolones, aminoglycosides, macrolides and tetracyclines. The least number of isolates was resistant to fusidic acid, only 4 (8%), to SXT 8 (16%) isolates, and to amikacin 9 (18%) isolates. Resistance to ciprofloxacin was detected in 15 (30%) of isolates, to gentamicin and clindamycin in 26 (52%) of isolates each, to erythromycin in 27 (54%), and to tetracyclin in 28 (56%) of isolates (Table 1). Our CA-MRSA isolates showed multiple drug resistance (MDR) patterns: 24 (48%) of the isolates were resistant to three or more antibiotics, 9 (18%) were resistant to two, 7 (14%) showed resistance to one antibiotic, but 10 isolates (20%) were susceptible to non-β-lactam antibiotics such as fusidic acid, SXT, quinolones, aminoglycosides, macrolides and tetracyclines.

Table 1

Resistance to non-β-lactam antibiotics of community-associated methicillin-resistant *Staphylococcas aureus* (CA-MRSA) isolates (n = 50)

Antibiotic	Resistant n (%)
Tetracyclin	28 (56)
Erythromycin	27 (54)
Clindamycin	26 (52)
Gentamicin	26 (52)
Ciprofloxacin	15 (30)
Amikacin	9 (18)
SXT	8 (16)
Fusidic acid	4 (8)

SXT − sulfamethoxazole/ trimethoprim.

PCR amplification with primers specific for genes encoding PVL detected these genes in only two CA-MRSA isolates (Figure 1), so the prevalence of the PVL-positive isolates was 4%. Molecular typing of two PVL-positive isolates reveal that one of them contained type IV SCC*mec* region, specific for CA-MRSA. Another PVL-positive isolate contained type V SCC*mec* region, that is also specific for CA-MRSA.

One of the PVL positive isolates was resistant to erythromycin, clindamycin gentamicin and tetracyclin, and other, except resistance to erythromycin and gentamicin, showed inducible clindamycin resistance.

Discussion

The anterior nares are the most frequent site of colonization for *S. aureus.* It is estimated that in some individuals (about 20%) this site is persistantly colonized with *S. aureus,* while in others (about 30%) colonization is only periodical [6]. Colonized individuals represent the main reservoir of *S. aureus,* and they contribute to the spreading of this bacteria in hospitals and community. Beside that, colonized strains are increasing the rate of infection especially in the case of host defence weakening, when they can easily be introduced.

The results of prevalence examination of *S. aureus* nasal carriage vary, depending on the studied population and study design. In this study *S. aureus* was isolated from 2.58% throat and nose swabs from healthy and employed population from the the District of Pomoravlje. In another study on healthy population in Belgrade, similar results were obtained [20].

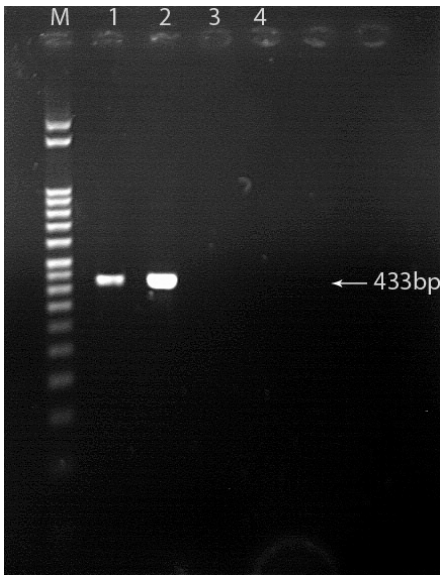

Fig. 1 – Polymerase chain reaction (PCR) detection of Panton-Valentine Leucocidin (PVL) genes. Line M: 50bp DNA ladder; lines 1, 2: PVL-positive community-associated methicillin resistant (CA-MRSA) strains; line 3: American Type Culture Collection (ATCC) 33591; line 4: ATCC 25932.

The prevalence of commensal *S. aureus* nasal colonization differed significantly in European countries, and the differences could not be explained by differences in age, gender or general practitioner (GP) practice, according to a recent research published in The Lancet Infectious Diseases [21]. A total of 32,206 nasal swabs from patients in nine countries were analysed in the study, and *S. aureus* was isolated from 6,956 (21.6%). The most extreme prevalence was in Hungary (12.1%) and in Sweden (29.4%).

In the study of von Eiff et al. [22], conducted at a single institution, 1,640 *S. aureus* strains were isolated from nasal swabs, and 5.8% of them were methicillin resistant. Among 1,363 *S. aureus* isolates in our study, 50 (3.67%) were methicillin resistant. The nasal carriage rate of MRSA in the population of medical students in Belgrade was low: 0.37% [23]. The discordant rates of colonization, probably, were driven by changes in the ecology and epidemiology of MRSA.

The strains of CA-MRSA carry SCC*mec* IV or SCC*mec* V, which are the smallest of the SCCs. In contrast to the multidrug-resistant nosocomial MRSA strains that carry larger SCC*mec* types, CA-MRSA strains are generally susceptible to several non-β-lactam antibiotics. But for some CA-MRSA strains, like epidemic clone USA300, it was noted that are becoming resistant to several non-β-lactam antibiotics [24].

The same situation is in the District of Pomoravlje. Compared to a similar research in 2009 [25], percentage of macrolide-resistant and aminoglycoside-resistant CA-MRSA isolates is higher in this study: 54% CA-MRSA isolates resistant to erythromycin *versus* 42.4% in 2009, and 52% isolates resistant to gentamicin *versus* 30.3% in 2009. "Older" antibiotics, such as fusidic acid and SXT, have retained their activity against CA-MRSA.

The basis for the apparent increased virulence of CA-MRSA strains is incompletely understood. Because these strains usually contain PVL, which is usually absent in HA-MRSA strains, this protein is postulated by some researchers to be responsible for that [6]. Highly virulent CA-MRSA strains USA400 and USA300 both harbor PVL operon and SCC*mec* IV [6]. In our CA-MRSA genes encoding PVL were present in only two (4%) isolates. Typing of SCC*mec* region conformed on molecular level that these isolats belonged to CA-MRSA. In Austria, the percentage ranges from 3% to 7% [26], and in Portugal, among healthy children colonized with MRSA, PVL gene was detected in only 1% of isolates [27]. In Canada, PVL positive CA-MRSA strains were detected in less than 5% of isolates [28]. In contrast, in the study of Vandenesch et al. [9], methicillin resistance was conferred in all CA-MRSA isolates by the truncated SCC*mec* type IV element, and all the isolates contained the PVL locus.

In our country, the first finding of PVL-positive MRSA was reported in 2013 [29]. The presence of PVL genes was demonstrated in 2.5% (4 of 162) MRSA isolates from 26 hospitals in Serbia. The three of these isolates carried SCC*mec* type V element, and one carried SCC*mec* IV element.

Conclusion

The prevalence of MRSA among carriers in the District of Pomoravlje is 3.67%. Also, only 4% of CA-MRSA isolates are PVL-positive. Because of a low percentage, the presence of PVL gene cannot be used as a marker of PVL-MRSA.

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Drug-related problems in patients with osteoporosis

Problemi u vezi sa lekovima kod bolesnika sa osteoporozom

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Abstract

Background/Aim. Drug-related problems are especially frequent among patients suffering from non-communicable diseases, like osteoporosis, leading to suboptimal treatment response. The aim of this study was to identify drug-related problems in patients with osteoporosis. **Methods.** This cross-sectional prospective study was conducted in January 2014 on outpatients with osteoporosis from three health facilities in Belgrade, Serbia. The patients included in the study were older than 50 years, and they were offered an anonymous questionnaire with open-ended questions. **Results.** There were 355 study participants, 329 (92.7%) females and 26 (7.3%) males. The patients who experienced at least one osteoporotic fracture (n = 208) were significantly less adherent to the therapy, less engaged in sports and regular physical activities, and more prone to nutrition with inadequate intake of calcium and vitamin D than patients without fractures (n = 147). **Conclusion.** The effectiveness of osteoporosis treatment is decreased by several drug-related problems encountered by both physicians and patients. However, the majority of the drug-related problems could be greatly influenced by appropriate educational programs.

Key words:
osteoporosis; risk factors; medication errors; preventive health services; questionnaires.

Apstrakt

Uvod/Cilj. Problemi zbog lekova posebno su česti kod bolesnika nezaraznim bolestima, kao što su osteoporoza, što je dovelo do suboptimalnog doziranja u lečenju. Cilj ove studije bio je da identifikuje probleme izazvane lekovima kod bolesnika sa osteoporozom. **Metode.** Ova prospektivna studija sprovedena je u januaru 2014 na bolesnicima obolelim od osteoporoze u tri zdravstvene ustanove u Beogradu, Srbija. Bolesnici uključeni u studiju bili su stariji od 50 godina i oni su popunjavali anonimne upitnike sa otvorenim pitanjima. **Rezultati.** Od 355 učesnika, 329 (92,7%) bile su osobe ženskog pola, a 26 (7,3%) muškog. Bolesnici koji su imali makar jedan prelom zbog osteoporoze (n = 208) statistički značajno manje su pristalice terapije, manje su se bavili sportom i redovnim fizičkim aktivnostima, a više su bili skloni ishrani sa neadekvatnim unosom kalcijuma i vitamina D, nego bolesnici bez preloma (n = 147). **Zaključak.** Efikasnost lečenja osteoporoze snižena je zbog nekoliko problema u vezi sa lekovima sa kojima se susreću kako lekari, tako i bolesnici. Međutim, na većinu problema u vezi sa lekovima moglo bi se u velikoj meri uticati odgovarajućim obrazovnim programima.

Ključne reči:
osteoporoza; faktori rizika; lečenje, greške; preventivno-medicinska zaštita; upitnici.

Introduction

Drug-related problems include adverse drug reactions and errors in prescribing, dispensing or administering drugs, regardless of who made the error, health worker or patient [1]. Such problems are especially frequent among patients suffering from non-communicable diseases [2]. Among the non-communicable diseases that affect European population, osteoporosis is one of the most prevalent, creating heavy economical burden for all societies. About 6% of men and 21% of women aged 50–84 years have osteoporosis in European Union; osteoporotic fractures, which are the main clinical consequence of osteoporosis, will be experienced during the remaining lifetime by 22% of men and 46% of women who are older than 50 years [3]. Serbian health system is facing similar burden, since only one of the osteoporotic fractures, hip fracture, has annual incidence rate of 143.6 *per* 100,000 inhabitants older than 50 years [4].

Drug-related problems in patients with osteoporosis are frequent, but under-investigated [5]. Under-prescribing of vitamin D supplementation was noted in 54.2% of patients at high risk of osteoporosis [6], and even 32.3% of patients taking

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oral bisphosphonates experience adverse effects [7]. A special problem is the persistence with bisphosphonates therapy, which was as low as 80% after 2 years in a group of patients from Malaysia [7]. The persistence with prescribed therapy is higher among patients with more severe forms of osteoporosis, and improves with the duration of treatment course [8, 9]. Among the elderly patients with osteoporosis, almost 16% is not being prescribed drug therapy, and this type of drug-related problem is more frequent in patients who live in their homes than in residents of nursing homes [10]. Yet, we know very little about factors that are associated with drug-related problems in patients with osteoporosis, and therefore our ability to undertake some preventive measures is limited.

The aim of this study was to identify drug-related problems in patients with osteoporosis and reveal characteristics of the patients and/or prescribed treatments which are associated with the drug-related problems.

Methods

This cross-sectional prospective study was conducted in January 2014 on outpatients with osteoporosis from three health facilities in Belgrade, Serbia: Osteoporosis Center of Orthopedic Institute "Banjica", Osteoporosis Department of Clinical Ward for Endocrinology, Diabetes and Metabolic Diseases, Clinical Hospital Center "Zvezdara" and Osteoporosis Counseling Unit of Primary Health Center "Savski Venac". Patients older than 60 years with diagnosis of osteoporosis who visited these three facilities during the study period were included in the study; only patients with cognitive disturbances or psychiatric diseases were excluded. The patients were offered an anonymous questionnaire with open-ended questions, specially designed for this study, after they completed their encounter with a rheumatologist.

The anonymous questionnaire had the following questions: about socio-demographic characteristics (age, sex, weight, occupation, place of residence, etc.); about clinical course of osteoporosis (duration of illness, treatment duration, number of previous fractures, etc.); about medication for osteoporosis (names of drugs that the patients used for treatment of osteoporosis, daily doses of these drugs, duration of use of these drugs, whether the patient had adverse effects); about treatment and therapy of chronic diseases unrelated to osteoporosis (the existence of other chronic diseases unrelated to osteoporosis and their treatment regimens, previous hospitalizations, etc.); about patients' nutrition (intake of foods rich in calcium and vitamin D, alcohol consumption, consuming more than 4 cups of coffee *per* day, frequent intake of carbonated soft drinks, etc.).

The approval for this study was obtained from the Ethics Committee of Faculty of Medical Sciences, University of Kragujevac.

The data collected by the questionnaire were at first described using measures of central tendency (mean and median) and variability (standard deviation) for continuous variables, and percentages for categorical variables. The significance of differences in continuous variables between the patients who experienced an osteoporotic fracture and those who did not was tested by Student's *t*-test for large independent samples. The differences in categorical variables were tested by χ^2 test. The differences were considered significant if the probability of null hypothesis was lower than 0.05. All calculations were performed by statistical software SPSS, version 18.

Results

The study included 355 patients, 329 (92.7%) females and 26 (7.3%) males; 70 (19.7%) of them were between 60 and 65 years, 245 (69.0%) were between 70 and 75 years, and 40 (11.3%) patients were 75 to 80 years old. There were 208 (58.6%) patients who experienced osteoporotic fractures [196 (94.2%) females and 12 (5.8%) males] and 147 (41.4%) patients with no fractures [133 (90.5%) females and 14 [(9.5%) males; (χ^2 = 1.789; df = 1; $p > 0.05$); (p = 0.181)]. There were 120 (33.8%) patients with vertebral fractures, 85 (23.9%) patients with hip fractures, 116 (32.7%) patients with fractures of radius, 58 of them (16.3%) with fractures of humerus and 37 (10.4%) patients with pelvic fractures; 107 (30.1%) experienced one osteoporotic fracture, 56 (15.8%) two, and 45 (12.7%) had more than two fractures. Characteristics of the study participants according to experience with osteoporotic fractures are presented in Table 1.

A significant number of patients was not adherent to drug therapy for osteoporosis. The main reasons for non-adherence offered by the patients were: experience with adverse effects (48.5%), belief that anti-osteoporotic drugs create more harm than benefit (68.9%), and underestimating health consequences of osteoporosis (57.3%). The effects of adherence to signs and symptoms experienced by the patients are shown in Table 2.

Discussion

Recently, treatment options for osteoporosis have increased significantly. Although bisphposphonates are still the most utilized drugs for this disorder worldwide [11], new molecular entities with specific mechanism of action are now available, like teriparatide (stimulator of osteoblasts) or denosumab (inactivator of osteoclasts). Regardless of the type of anti-osteoporosis therapy, adherence to prescribed regimen remains unsolved, since only half of the patients with osteoporosis is actually taking their drug therapy as prescribed [9]. The patients in our study were also largely non-adherent, which was related to increased osteoporosis signs and symptoms.

Previously published studies showed that some of the reasons for non-adherence of patients with osteoporosis to drug therapy are experience with unpleasant gastrointestinal adverse effects of bisphosphonates [12], forgetfulness and preoccupation with other daily routines [13]. The non-adherent patients in our study also had prejudices about both osteoporosis and drug treatment. In the first place they underestimated health consequences of osteoporosis, and then they believed that drugs for osteoporosis are ineffective and unsafe. Such attitude is at least in some part the consequence of poor knowledge of patients about their disease and medications.

Table 1

Characteristics of the patients in regard to their experience with osteoporotic fractures

Variable	The patients who experienced osteoporotic fracture (n = 208)	The patients with no osteoporotic fracture (n = 147)	*p*
Occupation, n (%)			
clarks	83 (39.9)	37 (25.2)	< 0.001
workers	55 (26.4)	73 (49.7)	
teachers	8 (3.8)	1 (0.7)	
health workers	23 (11.1)	12 (8.2)	
unemployed	39 (18.8)	24 (16.3)	
Place of living, n (%)			
city	51 (24.5)	54 (36.7)	< 0.001
suburbs	51 (24.5)	52 (35.4)	
village	106 (51.0)	41 (27.9)	
Previous engagement in sport activities, n (%)	32 (15.4)	39 (26.5)	0.010
The patients adherent to bisphosphonates, n (%)	104 (50.0)	98 (66.7)	0.002
The patients adherent to teriparatide, n (%)	32 (15.4)	146 (99.3)	< 0.001
Experienced any adverse effect of drugs for osteoporosis, n (%)	158 (76.0)	111 (75.5)	> 0.05
Regular use of food rich with vitamin D and/or calcium	20 (9.6)	122 (80.3)	0.039
Everyday use of sweet, gassed beverages	130 (62.5)	25 (17.0)	< 0.001

Table 2

Signs and symptoms of osteoporosis in regard to the patients' adherence to the prescribed drug therapy

Sign or symptom	The patients adherent to drug therapy (n = 252)	The patients not adherent to drug therapy (n = 103)	*p*
Loss of body height, n (%)	27 (10.7)	19 (18.4)	0.049
Decreased mobility, n (%)	96 (38.1)	57 (55.3)	0.003
Pain in the extremities, n (%)	177 (70.2)	85 (82.5)	0.017

In the study by La et al. [13] only 42.9% of patients knew exact names of drugs they were taking. The patients should be much more informed about their disease and treatment options, and all of their fears and concerns should be addressed. Not only prescribers, but also clinical and community pharmacists should be involved in this process of communication with patients suffering from osteoporosis, since it was already shown that pharmaceutical care increases medication adherence among patients with osteoporosis. In the study of Stuurman et al. [14] individualized counselling sessions and continuous monitoring of medication use performed by community pharmacists led to decrease of medication non-adherence from 32.8% to 19% in patients with osteoporosis.

The consequences of treatment non-adherence in patients with osteoporosis are grave and manifest themselves as increased fracture risk. In a systematic review by Imaz et al. [15] it was shown that non-adherence to prescribed therapy increases fracture risk for 46% in relation to the adherent patients.

Fractures experienced by the study patients were considered to be the consequence of osteoporosis if they happened spontaneously or were caused by minor trauma which would not cause fracture in otherwise healthy person. In patients observed in this study, the fractures were caused by bending the body, lifting light loads, by sudden movements, and sometimes without any obvious reason.

The patients with osteoporotic fractures from our study were much more non-adherent to bisphosphonates or teriparatide than the patients without fractures The number and percentage of patients with or without fractures, depended among other things on the profession and place of residence of the respondents. The distribution of the respondents without fractures according to their profession was as the following: workers, clarks, unemployed, health workers and teachers. Such result was expected, since physical activity delays progression of osteoporosis. Our analysis also show that the majority of patients who did not have fractures caused by osteoporosis came from urban areas, while the minority came from rural areas. The reason for this difference could be found in lower access of rural population to healthcare service, and therefore lower treatment rate and lower treatment adherence.

The increase in fracture rate is associated with great increases in mortality and healthcare costs, since treatment of fractures requires hospitalization and surgical interventions [16]. In our study, the largest percentage of respondents had vertebral fractures, which are difficult to treat and bear high treatment costs.

General signs and symptoms of osteoporosis are also more pronounced in non-adherent patients: in our study loss of body height and decreased mobility were more frequent in

the patients who did not take their therapy as prescribed. Increased occurrence of osteoporosis symptoms is associated with lower quality of life, as already shown in the literature [17].

Regular and moderate physical activity has protective effect against osteoporosis and occurrence of fractures. This is the consequence of both direct beneficial effect on bone metabolism and on improved balance, coordination and muscle strength, contributing to prevention of falls [18, 19]. Fractures were less frequent in the groups of our patients that were engaged in sport activities and employed as physical workers, probably because prolonged physical activity in the past improved their coordination and muscle strength, making them less prone to falls. However, since beneficial effects of physical activity on osteoporosis are not age-limited, even elderly patients with overt osteoporosis should be enrolled in tailored physical activity programs. It was recently shown that fracture-preventing programs in patients with osteoporosis which include physical activity are the most cost/effective [20].

Besides regular physical activity and osteoporosis medication, appropriate nutrition providing for sufficient intake of vitamin D and calcium is also important for prevention of osteoporosis and its complications. It was shown previously that bone mineral density in postmenopausal women is positively correlated with intake of dairy products rich in calcium and vitamin D [21]. This was noted also in our study, since patients with fractures far less regularly ate food rich in calcium and vitamin D, and far more frequently used sweet and gassed beverages whose nutritional value is very low. A lot of studies demonstrated effectiveness of educational interventions on improvement of nutrition in patients with osteoporosis [22], and education of patients about adequate nutrition should be implemented in everyday clinical practice.

Conclusion

The effectiveness of osteoporosis treatment is decreased by several drug-related problems encountered by both physicians and patients: adverse drug reactions, non-adherence to the treatment, prejudices of patients towards drug efficacy and safety, and non-adherence to appropriate diet and regular physical activity. Much should be done on education of patients with osteoporosis, since the majority of the drug-related problems could be greatly influenced by appropriate educational programs.

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Urinary KIM-1 and AQP-1 in patients with clear renal cell carcinoma: Potential noninvasive biomarkers

Molekul oštećenja bubrega-1 (KIM-1) i akvaporin-1 (AQP-1) u urinu kod bolesnika sa karcinomom svetlih ćelija bubrega: potencijalni neiznvazivni biomarkeri

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Abstract

Background/Aim. Kidney injury molecule-1 (KIM-1) and aquaporin-1 (AQP-1) are potential early urinary biomarkers of clear renal cell carcinoma (cRCC). The aim of this study was to ascertain relationship between the urine concentrations KIM-1 and AQP-1 with tumor size, grade, pT stage and type of operation (radical or partial nephrectomy) in patients with cRCC. **Methods.** Urinary concentrations of urinary KIM-1 (uKIM-1) and urinary AQP-1 (uAQP-1) were determined by commercially available ELISA kits. The analysis included 40 patients undergoing partial or radical nephrectomy for cRCC and 40 age- and sex-matched healthy adult volunteers. **Results.** The median preoperative concentrations of KIM-1 in the cRCC group [0.724 ± 1.120 ng/mg urinary creatinine (Ucr)] were significantly greater compared with controls (healthy volunteers) (0.210 ± 0.082 ng/mgUcr) ($p = 0.0227$). Postoperatively, uKIM-1 concentration decreased significantly to control values (0.177 ± 0.099 ng/mgUcr *vs* 0.210 ± 0.082 ng/mgUcr, respectively). The size, grade and stage of tumor were correlated positively with preoperative uKIM-1 concentrations. Contrary to these results, concentrations of uAQP-1 in the cRCC group were significantly lower (0.111 ± 0.092 ng/mgUcr) compared with the control group (0.202 ± 0.078 ng/mgUcr) ($p = 0.0014$). Postoperatively, the concentrations of uAQP-1 increased progressively up to control values, approximately. We find no significant correlation between preoperative uAQP-1 concentrations and tumor size, grade and stage. **Conclusion.** uKIM-1 was found to be a reliable diagnostic marker of cRCC, based on its significantly increased values before and decreased values after the nephrectomy.

Key words:
kidney neoplasms; diagnosis; biological markers; urine; nephrectomy.

Apstrakt

Uvod/Cilj. Molekul oštećenja bubrega-1 (KIM-1) i akvaporin-1 (AQP-1) su potencijalni rani biomarkeri karcinoma svetlih ćelija (cRCC). Cilj ove studije bio je da se utvrdi povezanost između koncentracija KIM-1 i AQP-1 u urinu i veličine, gradusa, stadijuma i vrste operacije (radikalna ili parcijalna nefrektomija) kod bolesnika sa cRCC. **Metode.** Urinarne koncentracije KIM-1 i AQP-1 određene su primenom komercijalnih ELISA kitova. Analizom je bilo obuhvaćeno 40 bolesnika koji su bili podvrgnuti parcijalnoj ili radikalnoj nefrektomiji zbog tumora bubrega i 40 zdravih odraslih ispitanika. Grupe su bile komparabilne po polu i godinama života. **Rezultati.** Srednja preoperativna koncentracija urinarnog KIM-1 (uKIM-1) u cRCC grupi [0,724 ± 1,120 ng/mg kreatinia u urinu (Ucr)] bila je statistički značajno viša u poređenju sa koncentracijom u kontrolnoj grupi (0,210 ± 0,082 ng/mgUcr) ($p = 0,0227$). Postoperativno, koncentracija uKIM-1 značajno je padala i približavala se vrednosti u kontrolnoj grupi (0,177 ± 0,099 ng/mgUcr

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nasuprot 0,210 ± 0,082 ng/mgUcr). Veličina, gradus i stadijum tumora bili su u pozitivnoj korelaciji sa preoperativnim koncentracijama uKIM-1. Nasuprot ovim rezultatima, koncentracija urinarnog AQP-1 (uAQP-1) u cRCC grupi bila je značajno niža (0,111 ± 0,092 ng/mgUcr) u poređenju sa kontrolnom grupom zdravih osoba (0,202 ± 0,078 ng/mgUcr) ($p = 0,0014$). Postoperativno, koncentracija uAQP-1 progresivno se povećavala, približno do vrednosti u kontrolnoj grupi. Nismo našli značajnu korelaciju između preoperativnih koncentracija uAQP-1 i veličine, gradusa i stadijuma tumora. **Zaključak.** uKIM-1 bi mogao biti dodatni pouzdani dijagnostički marker za cRRC na osnovu njegove značajno više preoperativne koncentracije i sniženja vrednosti nakon nefrektomije.

Ključne reči:
bubreg, neoplazme; dijagnoza; biološki pokazatelji; mokraća; nefrektomija.

Introduction

Renal cell carcinoma (RCC) represents approximately 3.8% of all malignancies in adults, increasing permanently over the past 30 years by 2−4% *per* year [1]. The most common and also the most aggressive form of RCC is clear renal cell carcinoma (cRCC), often characterized by the lack of early symptoms, signs and laboratory abnormalities. For these reasons cRCC is most often diagnosed accidentally during abdominal imaging performed for unrelated diagnostic reasons. Consequently, at the time of diagnosis, one-third of patients are already at metastatic stage of the disease [2]. Thus, reliable marker for cRCC should be powerful tool in screening patients for this particular type of the tumor and additionally, it could be used for monitoring of response to therapy and for the post-treatment surveillance, as well.

Recently, elevated urine concentrations of kidney injury molecule-1 (uKIM-1) and aquaporin-1 (uAQP-1) in patients with cRCC were recognized as specific and sensitive early markers of the disease [3]. KIM-1 is a type I transmembrane protein, whose ectodomain is secreted into urine in a response to the damage of the proximal tubule [4]. It has been shown that up regulation of KIM-1 in tumor cells is caused by de-differentiation of cells of the proximal tubules, such as cRCC and papillar renal cell carcinoma (pRCC). Patients with histologically confirmed cRCC have significantly higher concentration of uKIM-1 in pre-surgery phase, as compared with healthy individuals [5]. Current studies are focused on potential usage of levels of uKIM-1 for the early diagnosis of cRCC and possibility of preoperative determination of the RCC type [6, 7]. Aquaporin-1 (AQP-1) is a water channel expressed in many epithelial tissues and endothelium, including the proximal tubule of the kidney [8]. More recently, the diagnostic and prognostic usefulness of AQP-1 has been tested in cRCC tissues by using microarray techniques showing a reduction of AQP-1 expression in cRCC, as compared to normal renal tissue. A general decrease of AQP-1 protein can be related to the loss of differentiation, which is more pronounced in higher grade tumor [9]. Contrary to the above histopathological findings, recent studies have shown elevated uAQP-1 concentrations, determined by specific Western blot analysis in patients with cRCC [10–12].

The aim of this study was to determine if uKIM-1 and uAQP-1 concentrations in cRCC diagnosed patients undergoing partial or radical nephrectomy are related to tumor size, grade, and pT stage.

Methods

Patients

KIM-1 and AQP-1 were measured in urine samples of 40 patients submitted to radical or partial nephrectomy of renal tumor and in urine samples of 40 healthy adult volunteers. The study was conducted by our Institution between September 2012 and August 2013 and it was approved by the Ethical Committee of the Military Medical Academy, Belgrade, Serbia. Written and informed consent was obtained from each participant. All patients were preoperatively staged by thoracoabdominal multislice computed tomography (MSCT) imaging. The preoperative estimated glomerular filtration rates (GFR) − eGFR were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [13]. The patients with a GFR less than 60 mL/min/1.73 m^2 and/or the history of previous renal and malignant diseases were not included. For each patient/healthy volunteer, the following variables were obtained from the medical records: gender, age, comorbidities that could influence the baseline level of markers, baseline serum creatinine, eGFR and the type of surgery (partial or radical nephrectomy). A radical or partial nephrectomy was performed by open approach. The postoperative pathology reports provided tumor type, size according to the largest dimension, tumor-node-metastasis (TNM) stage and Fuhrman grade. The study included only patients with the diagnosis of cRCC categorized according to the American Joint Committee on Cancer 2010 TNM staging system [14].

Sample collection

Voided urine samples were aseptically collected preoperatively and on the day 7 and 30 after the operation. All fresh samples were immediately processed within four hours of collection to ensure optimal protein stability. Urine was centrifuged (1,800 g, 10 min) to remove debris, dividing into 1.5 mL aliquots and frozen at -80°C until analysis. Preoperative blood samples were also collected to measure serum creatinine. Patients' venous blood was drawn by trained, qualified flebotomists. The levels of uKIM-1 and uAQP-1 were determined by commercially available ELISA kits (ELISA, TIM-1/KIM-1/HAVCR, R&D Systems Inc, Minneapolis, MN, USA and AQP1 (Human) ELISA Kit, Abnova, Heidelberg, German), respectively. The absolute values (pg/mL) of uKIM-1 and uAQP-1 were calculated *per* norma-

lized urinary creatinine (Ucr) to avoid variability in urine flow and the results were expressed in ng/mgUcr. The minimum detectable dose for uKIM-1 and uAQP was 0.009 ng/mL and 0.04 ng/mL, respectively.

Creatinine determination

Serum and urine creatinine concentrations were measured by the modified Jaffe method using Siemense Dimension Rxl Max chemistry analyzer.

Statistics

Statistical software GraphPad Prism 5.0 was used for statistical analysis. After determining basic parameters of descriptive statistical analysis [mean value ($\bar{x}$), standard deviaton (SD), median (med), standard error of the mean (SEM)] we have analyzed Gaussian distribution of data with Kolmogorov Smirnov test. Data from multiple groups were compared with one way analysis of variance with Bonferroni multiple post-testing comparison. Nonparamteric Mann Whitney (MW) test was used to compare the differences between the two groups, where needed, while serial data from each patient in different time points were compared as paired data with Wilcoxon test.

Results

The clinical and pathological characteristics of the studied participants are shown in Table 1. In the cRCC group (n = 40) we measured uKIM-1 and uAQP-1 before surgery in all participants, in 37 (92.5%) patients on the postoperative day 7 and in 23 (57.5%) patients on the postoperative day 30. Urine samples on the day 30 after the operation were not available from 14 patients with cRRC because they did not come to follow-up visit. The control group included 40 healthy volunteers (n = 40). The cRCC group and the control group did not differ significantly according to age, sex and eGFR. The pathologic tumor size for the cRCC group was 5.50 ± 3.05 cm ($\bar{x}$ ± SD). The distribution of the pathologic stage was as follows: pT1a in 13 (32.5%), pT1b in 10 (25%), pT2a in 3 (7.5%), pT3a in 14 (35%) and there was no patients in pT4. The postoperative TNM stage was as follows: stage I in 22, stage III in 13 and only 3 and 2 patients in stage II and IV, respectively. The classification according to the Fuhrman grade was: 30 (75%) patients with grade 2; 9 (22.5%) patients with grade 3; and 1 (2.5%) patient with grade 4. None of the patients had tumor grade 1 and positive lymph nodes. Two patients had metastases in the lung preoperatively.

The median pre- and postoperative uKIM-1 concentrations are listed in Table 2.

The median preoperative concentration of uKIM-1 in the cRCC group (0.724 ± 1.120 ng/mgUcr) was significantly greater compared with that in the control group (0.210 ± 0.082 ng/mgUcr, $p = 0.0227$) (Figure 1). The postoperative uKIM-1 concentration decreased significantly on the first postoperative control visit (0.327 ± 0.225 ng/mgUcr) compared with the preoperative concentration. On the second control visit the concentration of uKIM-1 in the cRCC group was statistically indistinguishable from the control group (0.177 ± 0.099 ng/mgUcr *vs* 0.210 ± 0.082 ng/mgUcr, respectively) (Table 2).

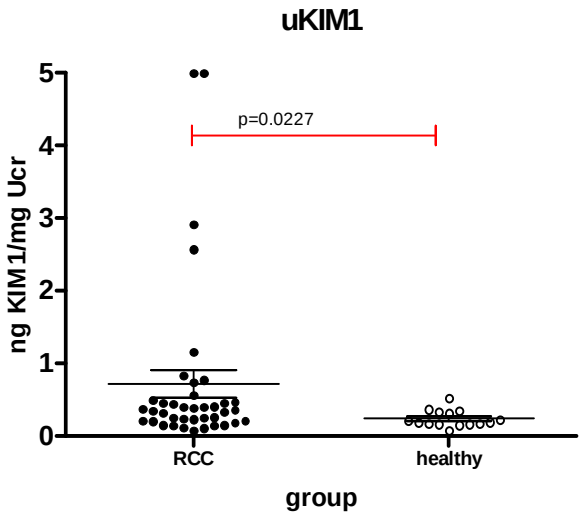

Fig. 1 – uKIM-1 in urine samples of the cRCC patients and the healthy volunteers.
For abbreviations see Table 2.

Table 1

Clinical and pathological characteristics of the examined participants

Parameter	cRCC group (n = 40)	Control group (healthy volunteers) (n = 40)
Gender, (male/female), n	25 / 15	22 / 18
Age (years), $\bar{x}$ ± SD	56.24 ± 11.73	60.45 ± 18.12
GFR (mL/min/1.73m^2), $\bar{x}$ ± SD	87.49 ± 16.46	88.82 ± 18.39
Tumor dimension (cm), $\bar{x}$ ± SD	5.50 ± 3.05	/
Grade, n	G1 = 0 G2 = 30 G3 = 9 G4 = 1	/
TNM stage, n	I = 22 II = 3 III = 13 IV = 2	/

cRCC – clear renal cll carcinoma; GFR – glomerular filtration rate; TNM – tumor-node-metastasis.

Table 2

Urinary kidney injury molecule-1 (uKIM-1) and urinary aquaporin-1 (uAQP-1) in the examined patients with clear renal cell carcinoma (cRCC)

Parameter	uAQP-1 (ng /mg creatine), x̄ ± SD			uKIM-1 (ng /mg creatine), x̄ ± SD		
	before operation	I control visit	II control visit	before operation	I control visit	II control visit
	0.111 ± 0.092	0.153 ± 0.163	0.212 ± 0.201	0.724 ± 1.120	0.327 ± 0.225	0.177 ± 0.099
Fuhrman gradus						
G1	none	none	none	none	none	none
G2	0.110 ± 0.097	0.113 ± 0.068	0.202 ± 0.172	0.409 ± 0.503	0.326 ± 0.225	0.170 ± 0.096
G3	0.113 ± 0.080	0.287 ± 0.293	0.237 ± 0.283	1.852 ± 2.146	0.339 ± 0.248	0.190 ± 0.112
Tumor size, cm						
≤ 4	0.141 ± 0.115	0.119 ± 0.072	0.267 ± 0.298	0.293 ± 0.169	0.357 ± 0.206	0.246 ± 0.124
4.1−7	0.073 ± 0.035	0.110 ± 0.069	0.205 ± 0.064	0.278 ± 0.139	0.307 ± 0.209	0.133 ± 0.033
>7	0.099 ± 0.075	0.261 ± 0.284	0.123 ± 0.105	2.092 ± 2.116	0.342 ± 0.300	0.153 ± 0.096
TNM stage						
I	0.117 ± 0.109	0.117 ± 0.072	0.210 ± 0.205	0.259 ± 0.133	0.319 ± 0.217	0.189 ± 0.113
II	0.129 ± 0.108	0.131 ± 0.058	0.000 ± 0.000	0.383 ± 0.075	0.380 ± 0.308	0.095 ± 0.029
III	0.099 ± 0.059	0.212 ± 0.248	0.224 ± 0.215	1.623 ± 1.924	0.347 ± 0.249	0.181 ± 0.100
Surgery type						
partial nephrectomy	0.126 ± 0.137	0.074 ± 0.047	0.160 ± 0.101	0.202 ± 0.118	0.327 ± 0.314	0.225 ± 0.164
radical nephrectomy	0.107 ± 0.078	0.173 ± 0.176	0.226 ± 0.222	0.917 ± 1.456	0.204 ± 0.226	0.138 ± 0.087

Note: The concentration uAQP-1 in the healthy volunteers before operation were 0.202 ± 0.079 μ/mg urinary creatinine (Ucr) and 0.210 ± 0.082 ng/mg Ucr, respectively.

TNM – tumor-node-metastasis.

There was a positive correlation between preoperative uKIM-1 concentration and tumor size, grade and stage (Table 3). The preoperative uKIM-1 concentration in the patients with cRCC correlated positively with tumor size, based on maximum tumor dimension. The mean uKIM-1 concentration in the group of patients with tumors larger than 7 cm was significantly higher (p = 0.0008) compared with the mean concentration of uKIM-1 in the patients with the tumor size of 4.1−7 cm and/or less and equal to 4 cm (p = 0.0004) (Figure 2). In addition, a positive correlation was obtained between the concentration of uKIM-1 and tumor grade (p = 0.00075).

The patients with high-grade tumors had a higher uKIM-1 level compared with those who had low-grade lesions (1.852 ± 2.146 ng/mgUcr *vs* 0.409 ± 0.503 ng/mgUcr), respectively (Table 2) and these differences were statistically significant (p = 0.0003) (Figure 3).

The preoperative mean values of uKIM-1 were significantly higher in patients with TNM stage III than with stage I (p < 0.0001, MW test) (Figure 4). The elevated baseline uKIM-1 (1.623 ± 1.924 ng/mgUcr) was significantly reduced to 0.347 ± 0.249 ng/mgUcr on the first control visit after the operation in patients with stage TNM III. In the patients with stage TNM I and TNM II, a significant reduction of uKIM-1 occurred only at the second control after the surgery.

It was shown that the type of surgery, partial or radical, had an impact on the concentration of uKIM-1, based at the first postoperative control visit. Before surgical intervention, the median uKIM-1 value was significantly higher in the group that later had radical resection compared to the partial resection group (p = 0.0044) (Figure 5). Contrary, on the first control examination uKIM-1 concentration was higher in the partial resection group than in the those who had radical resection. On the second control examination the average uKIM-1 value did not differ significantly regardless of the type of surgery.

Interestingly, analysis of uAQP-1 levels after adjustment to urinary creatinine (Ucr) concentration showed completely different dynamics compared to uKIM-1. In pati-

Table 3

Correlation analysis between uKIM-1 and uAQP-1 and tumor size, Fuhrman gradus, tumor pathological stage and TNM grade

Analyse	*vs* parameter	R coefficient	*p*
uKIM1 (0)	Tu (cm)	+0.564	0.00004
uKIM1 (0)	Tu (pT std)	+0.512	0.00085
uKIM1 (0)	G (Fuhrman)	+0.490	0.00064
uKIM1 (0)	TNM	+0.580	0.00005
uAQP-1 (0)	Tu (cm)	-0.112	> 0.05
uAQP-1 (0)	Tu (pT std)	-0.151	> 0.05
uAQP-1 (0)	G (Fuhrman)	+0.024	> 0.05
uAQP-1 (0)	TNM	+0.042	> 0.05

For abbreviations see Table 2.

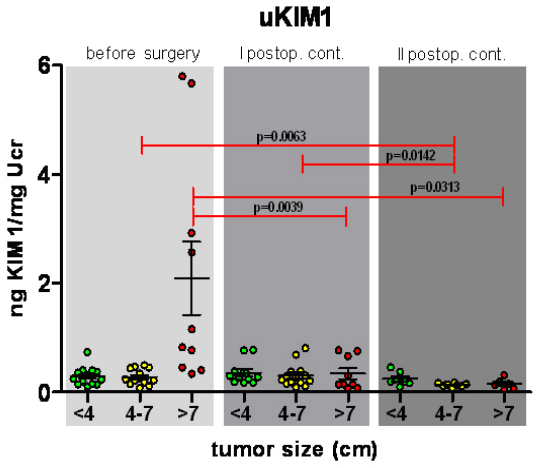

Fig. 2 – uKIM-1 according to tumor size. For abbreviations see Table 2.

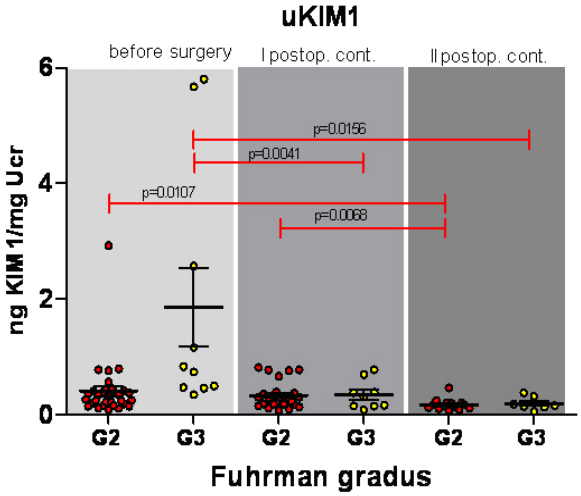

Fig. 3 – uKIM-1 according to Fuhrman grade. For abbreviations see Table 2.

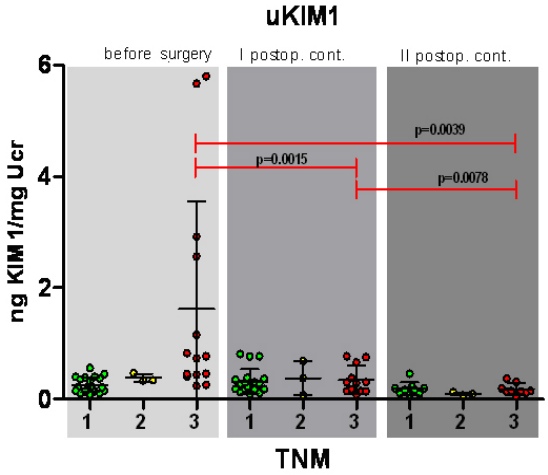

Fig. 4 – uKIM-1 according to TNM stage. For abbreviations see Table 2.

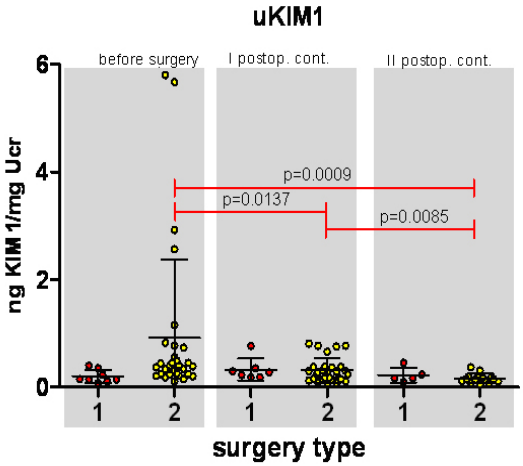

Fig. 5 – uKIM-1 according to the applied surgery type (partial nephroctomy and 2- radical nephroctomy). For abbreviations see Table 2.

ents with cRCC, uAQP-1 was significantly lower (0.111 ± 0.092 ng/mgUcr) than in the control group (0.202 ± 0.078 ng/mgUcr) ($p = 0.0014$). Postoperatively, the concentration of uAQP-1was progressively increasing and on the second control achieved approximately the same value as the control group (Table 2). We find no significant correlation between the preoperative uAQP-1 concentration and the tumor size, grade and stage (Table 3).

Discussion

The widespread use of modern radiological techniques such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) performed for unrelated diagnostic reasons has increased the diagnosis of renal tumors, especially tumors of smaller size detected incidentally [15, 16]. Although, preoperative needle biopsy of small lesion (≤ 3 cm) is followed by a relatively high sensitivity and specificity for the diagnosis of RCC, it carries a risk of false negative results and complications such as hematuria, subcapsular/perinephric hematoma and spread of malignant cells [17]. Sensitive and specific marker of the tumor needs to be able to predict certain type of cancer and also to monitor disease after treatment.

In order to determine the nature of preoperative tumor changes in the kidney and optimal treatment, we investigated urinary concentrations of KIM-1 and AQP-1 as a potential diagnostic marker for CRCC.

The results of our study showed that the preoperative concentrations of uKIM-1 in the patients with cRCC were statistically significantly higher than the value of postoperative uKIM-1 and than in the control group of healthy volun-

teers. After resection of the renal tumor using either partial or radical nephrectomy, uKIM-1 concentrations decreased to the levels essentially equivalent to that of the controls. Similar findings were reported by Han et al. [5]. Morrissey et al. [3] and Zhang et al. [7] indicating significantly higher preoperative uKIM-1 concentration in patients with cRCC compared with the patients with non-cRCC. Accordingly to Han et al. [5] and Morrissey et al. [11] we also found a positive correlation between tumor volume and uKIM-1 level. A different observation was reported by Shalabi et al. [18] who found no correlation between the concentration uKIM-1 and the size of the tumor, which might be explained by the fact that the most of the patients had tumor of small size (4.57 ± 0.37 cm). Zhang et al. [7] also found no correlation between uKIM-1 and the size of the tumor.

Tumor size is associated with malignant potential in RCC. A study of Thompson et al. [19] shows that the risk of malignancy of tumor and the percentage of high nuclear grade increases with the size of the tumor. Our study shows that the prevalence of high nuclear grade tumors increases with the tumor size. Thus, in the group with tumor ≤ 4 cm in diameter the prevalence of high grade tumor (G3 and G4) was 7.14%, in the group with tumor of 4.1−7 cm in diameter it was 17.64%, while in the group with tumor of > 7 cm in diameter the prevalence was 60%. The data we obtained showed a positive correlation between the size of the tumor and the nuclear grade.

In our study, the highest percentage of patients had G2 (75.61%) and G3 (21.95%) tumor grade, whereas only one patient had grade G4 (2.44%). None of the patients had G1 tumor grade. A similar distribution of Furman grade was found by other authors [20, 21]. In our study, a positive correlation was obtained between concentration of uKIM-1 and tumor grade. Different results were observed by Shalabi et al. [18] who found no correlation between uKIM-1 and tumor grade. Statistical analysis showed a different time course profile of uKIM-1 depending on the grade of the tumor. In the group of G3 grade operations induced a significant decrease of uKIM-1 on the first control examination, which later retained virtually the same level. In the group with G2 grade there was a significant decrease in uKIM-1 only between the first and the second control examination. A possible explanation for these results lies in the fact that the secretion of KIM-1 was independent of the tumor. Specifically, all eight patients who were submitted to a partial nephrectomy had G2 grade of the tumor. Obviously, after the surgery the recovery process resulted in elevated uKIM-1 because of renal ischemic injury during partial nephrectomy. The same result was obtained by Morrissey et al. [3] who confirmed a significantly lower reduction in postoperative uKIM-1 in patients with partial nephrectomy than in patients submitted to radical nephrectomy.

Partial nephrectomy is the optimal therapeutic modality of treatment in most patients with organ-confined RCC, achieving good disease control with preservation of renal function. Chronic Kidney Disease Prognosis Consortium Matsushita et al. [22] showed that the reduction of GFR below 60 mL/min/1.73m^2 is an independent predictor of total and cardiovascular mortality. To our knowledge, a study of Abassi et al. [23] is the only one comparing uKIM-1 before and after partial nephrectomy at different time intervals in 27 patients with RCC. A significant increase of uKIM-1 was registered from 3 to 24 hours after ischemic injury. In our study average concentrations of uKIM-1 at the first control examination on the postoperative day 7 was almost twice as high in the group of patients with partial nephrectomy compared to the group with a radical nephrectomy, but it did not reach statistical significance. This result was expected because the kidney still has been in the process of recovering at the time of the first control. Average uKIM-1 values did not differ significantly on the second control examination on the postoperative day 30, regardless of the applied intervention type, partial nephrectomy or radical nephrectomy, confirming acute ischemic renal damage. In addition, the patients subjected to radical nephrectomy had statistically significant higher uKIM-1 before the operation than the group subjected to partial nephrectomy. This result is a consequence of the larger size and higher-nuclear grade and stage of the tumor in the radical nephrectomy group. We showed that the uKIM-1 directly correlates with pathological characteristics of tumor.

Interestingly, in contrary to uKIM-1, analysis of uAQP-1 levels after adjustment to Ucr concentration showed a completely different dynamics during examination period. At first, the concentration uAQP-1 was significantly lower in the cRCC group than in the group of healthy subjects. Although a nonstatistically significant correlation was found between uAQP-1 and the size, stage or grade of the tumor, we observed slightly lower but not significant levels of this biomarker in patients with more aggressive grade (III–IV) and larger (> 7 cm) tumors.

In contrast to our results, Morrissey et al. [11] showed a direct correlation between the concentration uAQP-1 (determined by Western blot analysis) and the size and stage, but not the grade of the tumor. Similarly, Morrissey et al. [11] Sreedharan et al. [12] found a strong positive correlation between uAQP-1 and cRCC in 11 patients before operation. But, due to the small number of patients in their study correlation between the concentration uAQP-1 and pathological characteristics of the tumor was not performed [12]. Unlike previous studies in which the concentration of uAQP-1 was determined by Western blot analysis, we used commercial ELISA test to quantify the concentration uAQP-1, for the first time, to our knowledge.

Our results are complementary to the results of the histopathological study of the expression of AQP-1 in cRCC tissue. Ticozzi-Valerio et al. [24] showed a reduction of AQP-1 expression in tissue of cRCC from moderate to almost complete, depending on tumor grade. Tumor with high grade had a higher reduction of tissue AQP-1 expression compared to normal kidney tissue. Mazal et al. [25] proved a significant reduction or loss of AQP-1 expression in higher nuclear grade cRCC compared with those of lower grade, while there was no significant correlation accomplished between pT stage and expression of AQP-1. Unfortunately, we were not able to determine the expression of AQP-1 in the tumor tissue.

Conclusion

In summary, uKIM-1 but not uAQP-1 was statistically and clinically significantly increased in the patients with cRCC compared to the group of healthy subjects. Taking into the consideration the type of sample used for KIM-1 measurement (urine is a sample of choice what implies noninvasive sampling, simplicity and commitment of uKIM-1 measurements by using a commercial ELISA kit, mainly literature supported), we concluded that uKIM-1 might be used as a valuable and reliable biomarker of cRCC diagnosis and for monitoring patients with cRCC after operation, in routine clinical practice.

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Problems in diabetes managment in school setting in children and adolescents with type 1 diabetes in Serbia

Problemi u kontroli dijabetesa tokom boravka u školi dece i adolescenata s dijabetesom tipa 1 u Srbiji

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Abstract

Background/Aim. Children with type 1 diabetes typically spend one-third of the day in school and they should achieve the same level of diabetes management there as they do outside the school environment. The aim of this study was to identify problems in diabetes management in children with type 1 diabetes at school according to the perceptions reported by children and parents. **Methods.** This cross-sectional survey was carried out at nine public hospitals in Serbia with a cohort of 6–18-year old children/adolescents. The parents were personally informed about the objectives of the survey and the necessity to involve their children. The self-reporting questionnaire included demographic information as well as some questions that helped to evaluate the general situation of children with type 1 diabetes at school. **Results.** The obtained results show that not all children test blood glucose levels at school (50% of children in the 6–10-year-old age group and 67.3% in the age group over 11 years) and that not all children receive insulin at school (81.1% *vs* 18.9%, and 57.7% *vs* 42.3%, respectively). The frequency of severe hypoglycemia was 2.7% in children and 3.3% in adolescents. A high proportion of teachers did not have diabetes training. **Conclusion.** This brief report about problems in children and adolescents with type 1 diabetes at school in Serbia indicates what happens in the school setting and suggests how to improve control of this disease and facilitate the complete integration of children with diabetes at school.

Key words:
diabetes mellitus, type 1; child; serbia; schools; questionnaires; preventive health services.

Apstrakt

Uvod/Cilj. Deca sa dijabetesom melitusom tipa 1 obično provode trećinu dana u školi i trebalo bi da postignu isti nivo kontrole dijabetesa kao i izvan školskog okruženja. Cilj ovog istraživanja bio je da se uoče problemi dece sa dijabetesom tipa 1 u školi, uzimajući u obzir zapažanja i dece i roditelja. **Metode.** Ovo studija preseka sprovedena je u devet državnih bolnica u Srbiji kod dece/adolescenata uzrasta 6–18 godina. Roditelji su bili lično obavešteni o ciljevima istraživanja i potrebi da uključe svoju decu. Upitnik koji su sami popunjavali obuhvatao je demografske podatke, kao i neka pitanja koja treba da opišu opštu situaciju dece sa dijabetesom tipa 1 u školi. **Rezultati.** Dobijeni rezultati ukazuju da se u školi ne određuje nivo šećera u krvi (kod 50% dece starosti 6–10 godina i 67,3% dece starosti preko 11 godina) i da ne primaju sva deca insulin (81,1% *vs* 18,9% i 57,7% *vs* 42,3%). Učestalost ozbiljne hipoglikemije iznosila je 2,7% kod dece i 3,3% kod adolescenata. Veliki procenat nastavnika nije imao obuku za dijabetes**. Zaključak.** Ovaj kratak izveštaj o problemima školske dece i adolescenata sa dijabetesom tipa 1 u Srbiji ukazuje na stanje u školskom okruženju i na to kako bi se mogla poboljšati kontrola bolesti i olakšati njihov boravak u školi.

Ključne reči:
dijabetes melitus, insulin-zavisni; deca; srbija; škole; upitnici; preventivno-medicinska zaštita.

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Introduction

Diabetes management in children and adolescents requires multiple daily management tasks that can challenge caregivers [1]. Nevertheless, the scientifically proven long-term health benefits of optimal diabetes control mandate that best efforts be made to control diabetes at school as well as at home [1]. The American Diabetes Association (ADA) and the American Association of Diabetes Educators issued very useful guidelines regarding optimal diabetes management in schools [1–3]. Children typically spend one-third of the day in school and they should achieve the same level of diabetes management there as they do outside the school environment. This means that children should incorporate frequent glucose monitoring, meal planning and possibly insulin injections at school, but also they should be allowed to participate fully and safely in all school activities [4]. The objective of this cross-sectional study was to identify the school problems of children with type 1 diabetes at primary and secondary school taking into account the perceptions reported both by the children older than 11 and by the parents of the children aged 6–10 years.

Methods

This cross-sectional study was carried out in nine public hospitals in Serbia. The cohort of children was aged 6–18 years, which in Serbia is the age for primary (6–10, first four classes; 11–14, second four classes) and secondary school (15–18).

Recruitment took place between January 2013 and December 2013. The parents of those aged 6–18 with type 1 diabetes who attended the pediatric unit of each participating hospital were contacted by the diabetes educator and/or pediatrician. They were informed about the objectives of the study and about the need to include in the study their children/adolescents. In addition to information given by word of mouth, written information about the study was also given to each parent. The study was conducted only after the parents had given their oral informed consent. No interventions, nor treatments were given. The study did not require approval from the institutional review board at the hospitals.

A team of pediatrician endocrinologists from two tertiary care referral teaching hospitals in Belgrade, the capital city of Serbia, designed the questionnaire. Databases used were MEDLINE and PUBMED. The inclusion criterion for selecting studies was that the study focus was on special needs and general situation of children and adolescents at school [4–7]. Keywords used in the search were adolescent, child day care centers, type 1 diabetes, school. Some questions required a single answer [dischotomic (yes/no) or were on a hierarchic scale in which scores from 1 to 3 or 4 were assigned]. Other questions comprised multiple answers of precoded items. Once the questionnaire was approved by the endocrinologists of the University of Belgrade tertiary care hospitals, the project was presented to all major public hospitals in Serbia. All public hospitals agreed to participate.

Of the 346 questionnaires received, 346 (100%) were accepted, in that at least 90% of the questions were answered. The patients were divided into two age groups: 6–10 years (younger age group) and over 11 years (older age group). $\chi 2$ test and Fisher exact test were used to detect potential significant differences between the groups according to the age of children. Results were expressed as frequencies or means ± SD unless otherwise stated. Probability values of less than 0.05 were considered to be significant. SPSS version 10.1 (SPSS, Chicago, IL) was used for analysis.

Results

The 346 accepted questionnaires were completed by either the mother or the father of the child with type 1 diabetes (21.4%), or the adolescent itself (78.6%). Demographic characteristics of children with type 1 diabetes are shown in Table 1. Male and female gender were almost equally represented (53.2% and 46.8%, respectively). The median duration of diabetes (range) was 5.36 years (0.1–19.3), respectively. Most of the children and adolescents attended primary school (62.5%).

Table 1

Characteristics of the children (n = 346)

Demography	n (%)
Age (year)	
6–10	74 (21.4)
>11	272 (78.6)
$\bar{x}$ ± SD	13.9 (±3.3)
Gender	
male	184 (53.2)
female	162 (46.8)
Duration of diabetes in years	
< 3	105 (30.3)
3–6	123 (35.6)
> 6	118 (34.1)
$\bar{x}$ ± SD	5.4 (±3.8)
Type of school	
primary	216 (62.4)
secondary	130 (37.6)
Other diseases	
no	277 (80.1)
thyroid alterations	24 (6.9)
celiac disease	10 (2.9)
other (dermatitis, asthma, allergies etc.)	35 (10.1)

Table 2 summarizes some of the results stratified *per* the age group. At school, 50% of children in the 6–10-year-old group and 67.3% in the age group over 11 years not underwent glucose monitoring during the school day ($p < 0.05$), which they usually (73.0% *vs* 92.9%) performed without any assistance ($p = 0.001$). Most of the children (81.1% and 57.7% respectively) did not take insulin at school ($p = 0.001$), whereas the younger group did it with the help of a parent/relative (71.4%) and the older group did it by themselves (96.5%) ($p < 0.001$). It was rare to find other personnel such as a teacher (0.9%) who administered insulin treatment, with injection or pump.

A rate of 2 in 74 children and 9 in 272 adolescents experiencing severe hypoglycemia requiring call to the emergency services suggests that as many as 2.7/3.3% of diabetic children could experience serious hypoglycemia in a given school year ($p < 0.05$). A significantly lower percent of

Table 2

Selected questions and answers stratified by the age group

Questions	6–10 yr n (%)	> 11 yr n (%)	*p*
Does the child require glucose monitoring at school? (n = 74/272)			
yes	37 (50.0)	89 (32.7)	0.043*
no	37 (50.0)	183 (67.3)	
If necessary, who helps the child to perform glucose monitoring? (n = 37/183)			
a teacher	3 (8.1)	3 (1.6)	
a family member	7 (18.9)	4 (2.2)	0.001*
a peer	/	6 (3.3)	
nobody	27 (73.0)	170 (92.9)	
Does the child need insulin administration at school? (n = 74/272)			
yes	14 (18.9)	115 (42.3)	0.001*
no	60 (81.1)	157 (57.7)	
During school time, who is the person responsible for insulin administration? (n = 14/115)			
nobody	4 (28.6)	111 (96.5)	
a parent/relative	10 (71.4)	2 (1.7)	< 0.001*
a teacher	/	1 (0.9)	
a peer	/	1 (0.9)	
Has the child ever experienced hypoglycemic episode at school requiring treatment with rapid-acting glucose? (n = 74/272)			
yes	6 (8.1)	38 (14.0)	0.201
no	68 (91.9)	234 (86.0)	
At your school, did some-one of the school staff know to administer glucagon? (n = 74/272)			
yes	2 (2.7)	7 (2.6)	
no	40 (54.1)	140 (51.5)	0.277
i don't know	32 (43.2)	125 (45.9)	
Is glucagon available in the first-aid kit at school? (n = 74/272)			
yes	3 (4)	27 (9.9)	
no	19 (25.7)	74 (27.2)	0.239
i don't know	52 (70.3)	171 (62.9)	
Have school personnel (physical education teacher, other teachers, or staff) received diabetes training? (n = 74/272)			
yes	1 (1.3)	23 (8.5)	0.043*
no	73 (98.6)	249 (91.5)	
Is the child worried about being different from her/his peers? (n = 74/272)			
yes	26 (35.1)	103 (37.9)	0.853
no	48 (64.9)	169 (62.1)	
Do you think that more information about diabetes would improve children's integration at school? (n = 74/272)			
yes	67 (90.5)	231 (84.9)	
no	5 (6.8)	32 (11.8)	0.439
maybe	2 (2.7)	9 (3.3)	

parents (1.3% *vs* 98.6%) and adolescents (8.5% *vs* 91.5%) indicated that their school personnel have no diabetes training.

Discussion

This study shows that not all children and adolescents with type 1 diabetes in Serbian schools test their blood glucose levels and receive insulin at school, particularly the younger age group, due to reliance on parent/relative assistance.

Also, not testing blood glucose at school increases the risk of undetected hypoglycemia. The findings of about 3% occurence of severe hypoglycemia in both age groups, requiring call to emergency services, show lack of any plan for preventing and treating hypoglycemia. Because a large portion of a child's and adolescent's day might be spent at school, the management priority for them is the prevention, recognition, and treatment of hypoglycemia [2, 4, 6, 7].

It is disappointing that few participants reported diabetes training for school personnel. At the time of the study, the training of school staff in Serbia was not standard. Diabetes is a private affair involving only the families. So, it is likely that the degree of knowledge about the different aspects of diabetes still appears to be superficial for everyone other than those who are directly involved (patients/relatives), as has also been reported by other authors [7, 8].

Of interest here is an example of state intervention reported by Hellems and Clarke [9], the so-called 'Virginia (USA)

experience': in 1999, this state passed legislation to ensure that any public school having one or more students with type 1 diabetes has to have at least two members of the teaching, administrative, or other staff (including coaches and cafeteria workers) instructed in the blood glucose monitoring, administration of insulin and glucagon when no registered nurse/physician was present. This legislation further provided immunity from liability for such non-medical personnel under the 'Good Samaritan' law and exempted these individuals from the nursing and medical practice acts when performing such diabetes treatment [9]. The American Diabetes Association (ADA) has developed the Safe at School Statement of Principles, which encourages all school districts to identify individuals who will be responsible for the safe management of children and adolescents with type 1 diabetes during attendance at school [2].

Conclusion

This first brief report on problems in diabetes management in school children and adolescents with type 1 diabetes in Serbia, suggests how to improve control of the disease in school setting. It is needed to develop a plan that includes a series of interventions involving health-care providers, parents and school staff to facilitate the complete integration of these children at school.

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CASE REPORTS

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Chilaiditi's sign and syndrome: theoretical facts and a case report

Chilaiditi-jev znak i sindrom

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Abstract

Introduction. Chilaiditi's syndrome is a rare condition manifested by gastrointestinal symptoms, and radiologically verified by transposition of the large intestine loop. This radiological finding with no manifested symptoms is termed the Chilaiditi's sign. The aim of this case report was to remind the clinicians of the possibility of this rare syndrome, whose symptoms and signs may be misinterpreted and inadequately treated, with consequent diverse complications. **Case report**. We presented the theoretical facts and a patient in whom the diagnosis of Chilaiditi's syndrome was established incidentally, when hospitalized for an exacerbation of his chronic obstructive pulmonary disease. The Chilaiditi's sign was verified as an incidental finding on chest X-ray performed to evaluate the primary disease. **Conclusion**. Chilaiditi's syndrome is a benign condition which rarely requires surgery. Its clinical importance lies in adequate differential diagnostic approach and timely management of potentially serious complications.

Key words:
chilaiditi syndrome; diagnosis; diagnosis, differential; radiography; tomography, x-ray computed.

Apstrakt

Uvod. Chilaiditi-jev sindrom je retko stanje koje se manifestuje gastrointestinalnim simptomima, a radiološki se potvrđuje pozicioniranim vijugama creva u prostoru između jetre i desne hemidijafragme. Postojanje ovakvog radiološkog nalaza i odsustvo simptoma naziva se Chilaiditi-jev znak. Cilj rada bio je podsećanje kliničara na mogućnost postojanja tog retkog sindroma, koje može rezultirati pogrešnim tumačenjem simptoma i znakova bolesti, neadekvatnim lečenjem i različitim komplikacijama. **Prikaz bolesnika**. Prikazane su teorijske činjenice i bolesnik kod koga je dijagnoza Chilaiditi-jev sindrom postavljena prilikom hospitalizacije zbog pogoršanja hronične opstruktivne bolesti pluća. Chilaiditi-jev znak potvrđen je kao slučajni nalaz na radiogramu grudnog koša načinjenom u cilju procene osnovne bolesti. **Zaključak.** Chilaiditi-jev sindrom je benigno stanje koje retko zahteva hiruršku intervenciju. Klinički značaj tog sindroma ogleda se u adekvatnom diferencijalnodijagnostičkom pristupu i pravovremenom zbrinjavanju mogućih ozbiljnih komplikacija.

Ključne reči:
chilaiditi sindrom; dijagnoza; dijagnoza, diferencijalna; radiografija; tomografija, kompjuterizovana, rendgenska.

Introduction

Chilaiditi's syndrome is a very rare condition, characterized by the presence of different symptoms due to either temporary or permanent malposition of the colon loops in between the liver and the right hemidiaphragm [1–9]. This radiological finding accompanied with no symptoms is called Chilaiditi's sign [1, 3–9]. The etiology of this anatomic anomaly is still unknown, and considered multifactorial. The syndrome is manifested by isolated or concurrent symptoms – gastrointestinal, pulmonary, and/or cardiac, of different intensity and frequency [4]. The differential diagnosis includes a variety of diseases of numerous organ systems, primarily the pneumoperitoneum [4, 5]. Treatment is usually conservative, or surgical in case of complications [4–9].

Case report

A old-75-year patient, was admitted to the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia, due to exacerbation of his chronic obstructive pulmonary disease, outpatiently treated for over twenty years. Having responded poorly to the intensified ambulatory desobstruction treatment, the patient was referred to hospital. He was presented with a variety of comorbidities, including arterial hypertension, atrial fibrillation, valve defect, abdominal her-

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nia, cholelithiasis, prostatic hyperplasia, degenerative spinal disease, depression. The patient complained on the symptoms of cough, dyspnea, fatigue, heart palpitations and arrhythmia, occasional pains below the right rib arch, and dyspeptic problems. On admission, the patient was conscious, oriented, exhausted, moving with difficulties, afebrile, dyspnoic, normocardic, normotensive, with no signs of cardiac decompensation, giving the impression of a moderately severe patient. On auscultation, bronchospasm signs were registered. The abdomen was at the level of the chest, soft on palpation, with a reponible hernia of the anterior abdominal wall, and a mildly painful, sensitive epigastric region, audible peristalsis, and no signs of meteorism or ascites. The chest X-ray finding was presented with bilateral striped paracardial lesions, adhering hemidiaphragms, and the presence of an air collection below the right hemidiaphragm (Figure 1).

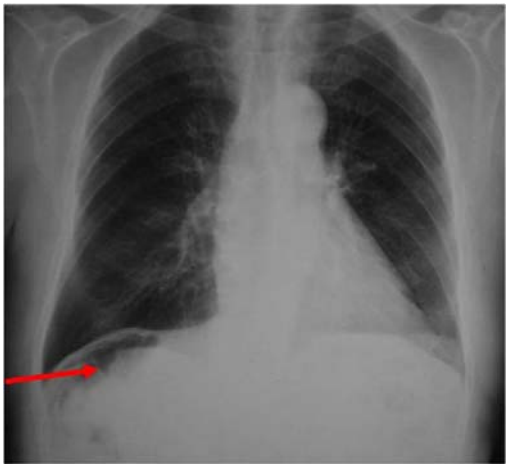

Fig. 1 – The chest x-ray reveals air collection below the right hemidiaphragm.

The pulmonary gas exchange at rest was preserved, and bronchoobstruction was verified on spirometry. Standard laboratory test findings were within normal ranges. The bacteriological sputum finding was normal as well. Computed tomography (CT) of the chest excluded the presence of infiltrative lesions in the pulmonary parenchyma, verified bilateral bronchiectases, bilateral excrescences in the basal pleura, degenerative spinal lesions, and the Chilaiditi's sign (Figure 2).

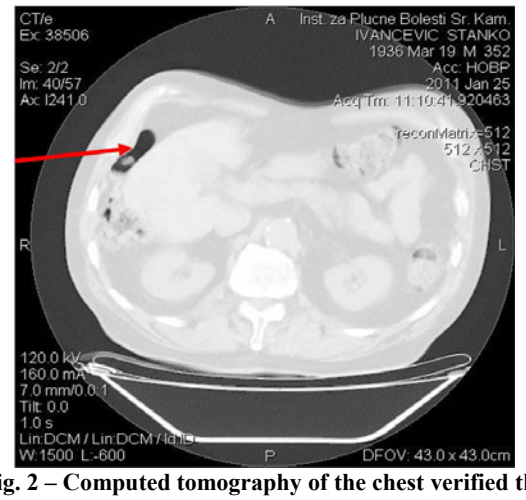

Fig. 2 – Computed tomography of the chest verified the Chilaiditi's sign (arrow).

Echocardiography verified ejection fraction of 50%, paradoxical septal movements, initial concentric hypertrophy of the left ventricle myocardium, sclerosis of aortic valves, mitral regurgitation 3+. The treatment included inhalant and parenteral desobstructive therapy, gastroprotective medication, peroral anticoagulant therapy and other formerly prescribed cardiological therapy. The gastroenterologist was consulted, who recommended conservative treatment measures and advised to consult the abdominal surgeon in case of an acute exacerbation of gastrointestinal symptoms. The applied treatment improved the patient's general and respiratory condition, and he was discharged with recommendations for further ambulatory treatment, and instructed what to do in case of exacerbated respiratory and gastrointestinal symptoms.

Discussion

Chilaiditi's sign is an incidental radiological finding, presented as a crescent lucency below the diaphragm on the right, occurring due to malpositioned loops of the colon and/or small intestine.[5] It was for the first time described by Cantini in 1865 but the first three case reports were published by the Greek radiologist Demetrius Chilaiditi in 1910, after whom this condition has been named [2, 3]. Its incidence ranges from 0.025% to 0.028% [3]. In males and the elderly, it is registered four times as frequently as in other population groups, the incidence amounting to around 1% [3, 4]. Being an asymptomatic condition, its diagnosis is established incidentally, on the occasion of different radiological examinations of the chest or abdomen (CT, standard chest X-ray, or ultrasound) [7].

Predisposing factors include all the conditions resulting in the increased right subphrenic space or intestinal hypermobility. These factors may be classified as congenital or acquired (Table 1) [1, 5, 7–9], diaphragmatic, intestinal, hepatic, and others (Table 2) [4].

Table 1
Congenital and acquired predisposing factors for Chilaiditi's sign and syndrome [1, 5, 7–9]

Predisposing factors	
Congenital	Acquired
Absence of:	Chronic obstipation
- suspensory ligament (*ligamentum suspensorium duodeni*)	Fecal impaction
	Cirrhosis with liver atrophy
	Ascites
- falciform ligament (*ligamentum falciforme hepatis*)	Aerophagia
	Obesity
	Right hemidiaphragm paralysis
Malposition of abdominal organs	COPD
Dolichocolon	Multiple pregnancies
Right hemidiaphragm paralysis	Colonoscopy
	Pregnancy
Laxitas (weak connective tissue)	

COPD – chronic obstructive pulmonary disease.

The diagnosis is established on the basis of the chest X-ray finding presented with the following three features [5, 7]: elevation of the right hemidiaphragm; a crescent lucency be-

low the diaphragm on the right (the air-distended intestine – "pseudoperitoneum"); shadow of the upper liver line below the right hemidiaphragm.

Table 2
Diaphragmative, hepatic, and intestinal predisposing factors for Chilaiditi's sign and syndrome [4]

Predisposing factors
Diaphragmatic
Elevated right hemidiaphragm due to:
muscular degeneration
injured *nervous phrenicus*
Hepatic (reduced liver size)
cirrhoses
right lobe agenesis
ptoses
absent or weak suspensory ligaments
Intestinal
abnormal motility of the intestines
long intestines with long mesentherium
absence of peritoneal ligaments
malrotation of congenital malposition of the intestines

Most frequently, there is a malposition of the hepatic flexure of the colon, ascending or transversal colon, more rarely of the cecum, independently or in combination with the small intestine [4]. Depending on the position of the intestines in relation to the liver, the anterior and posterior type are differentiated [4], which may be either temporary or permanent [6, 7, 9].

Chilaiditi's sign is easily diagnosed, analyzing the standard chest X-ray finding. CT is the imaging technique of choice here, as it concurrently excludes a rupture of the diaphragm, intestinal perforation, congenital malformations, as well as other conditions and diseases [7–9]. The differential diagnosis includes renal or biliary colics, subphrenic abscess, pneumoperitoneum, or congenital diaphragmatic hernias [1, 5–7]. The most important radiologic indicators excluding these serious complications, particularly pneumoperitoneum, are the presence of the intestinal *haustrum* and the persisting pseudoperitoneum position at changing bodily postures.

The timely diagnosis is important to prevent the complications which may arise while performing various diagnostic procedures, including percutaneous liver biopsy, pleural puncture, and colonoscopy [4, 5].

Chilaiditi's syndrome is a rare condition manifested by diverse gastrointestinal symptoms, and a radiologically verified Chilaiditi's sign. It occurs very rarely, in elderly males four times as frequently as in elderly females, and the cases in children have also been reported [4]. It is manifested by the symptoms differing in intensity and frequency – abdominal pains, flatulence and "pouring" in the bowel, nausea, vomiting, altered discharge habits, more rarely retrosternal pains, heart arrhythmia, dyspnea, and respiratory distress [4, 5]. Different diseases and conditions may be additionally prolonged if accompanied with this syndrome; these most often include chronic obstructive pulmonary disease, sclerodermia, congenital hypothyroidism, paralytic ileus, *melanosis coli*, mental retardation, more rarely lung and colon cancer, bariatric surgery, gastric probe placement, colonoscopy [4].

The treatment of the syndrome is initiated by conservative measures including rest, rehydration, high content of plant fibers in the diet, nasogastric decompression, laxatives and/or antiemetics [7]. If the conservative treatment fails to result in adequate clinical and/or radiologic improvement or obstruction, ischemia or perforation are suspected, surgical treatment should be carried out [4, 7]. Cases requiring urgent surgery were rarely reported in the literature, which included volvulus of the cecum or colon, subphrenic appendicitis, intestinal perforation, intraabdominal herniation [4, 9]. No unique attitude to the most adequate surgical approach has been formulated yet. Invasive surgeries, including colon resection, hepatopexy, colonopexy, right hemicolectomy, sigmoidectomy, and subtotal colectomy have been successfully carried out, as well as less invasive laparoscopic colonopexies [4, 7, 9].

Conclusion

Chilaiditi's syndrome is a benign condition which rarely requires surgery. The clinical relevance of Chilaiditi's syndrome lies in the possibility to recognize and prevent various complications causing acute abdominal symptoms, such as obstruction, perforation or ischemia of the intestines.

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CASE REPORT

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Pleural empyema caused by *Salmonella enteritidis* in a patient with non-Hodgkin lymphoma

Empijem pleure prouzrokovan salmonelom enteritidis kod bolesnika sa nehodžkinovim limfomom

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Abstract

Introduction. Extraintestinal manifestations of nontyphoidal salmonellosis are usually seen in patients with cellular immunodeficiency. Pleural empyema caused by nontyphoidal *Salmonella* is very rare clinical presentation of salmonellosis and there are just a few cases described in a literature. We presented a very rare case of pleural empyema caused by *Salmonella enteritidis* in a patient with non-Hodgkin limphoma. **Case report.** A 60-year-old male with low grade B-cell lymphoma, mucosa associated lymphoid tissue (MALT) type in IV clinical degree, manifested with infiltration of stomach, bronchus, pleura and peritoneum was admitted to the hospital. Initially the patient was presented with non-specific symptoms and signs, suggesting poor general condition. During the hospitalization his pleural fluid became purulent and changes in blood counts were registered with the increase of leukocytes, especially neutrophils. A large number of leukocytes was found by microscopic evaluation of pleural fluid and *Salmonella enteritidis* was isolated by its culture. There were no pathogenic bacteria in stool culture and hemoculture remained sterile. Toxins A and B of *Clostridium difficile* were not detected in stool. The patient was treated by ciprofloxacin and cefrtiaxone for 14 days with drainage of the purulent content, what was followed by the resolution and organization of the pleural fluid. After the stabilization of his general condition, chemotherapy with cyclophosphamide, vincristine, prednisone (COP) was introduced, with complete response. **Conclusion.** Although rare, pleural empyema caused by nontyphoidal *Salmonella* should be considered in patients with severe immunosuppression, because appropriate antimicrobial therapy with surgical measures are very important for the outcome in these patients.

Key words:
empyema, pleural; diagnosis; salmonella enteritidis; lymphoma, non-hodgkin.

Apstrakt

Uvod. Ekstraintestinalne manifestacije netifusnih salmoneloza obično se sreću kod bolesnika sa imunodeficijencijom ćelijskog tipa. Pleuralni empijem prouzrokovan netifusnim salmonelama predstavlja veoma retku kliničku prezentaciju infekcije salmonelama i postoji svega nekoliko ovakvih slučajeva objavljenih u literaturi. Prikazali smo veoma redak slučaj empijema pleure izazvanog sojem *Salmonella enteritidis* kod bolesnika sa nehodžkinovim limfomom. **Prikaz bolesnika.** Šezdesetogodišnji muškarac sa niskogradusnim B-ćelijskim limfomom, tipa limfom limfnog tkiva mukoze (MALT), u IV kliničkom stadijumu, sa inflitracijom želuca, bronha, pleure i peritoneuma, primljen je na bolničko lečenje. U početku se bolest manifestovala nespecifičnim simptomima i znacima i lošim opštim stanjem. Tokom hospitalizacije pleuralna tečnost je postala purulentna, uz promene u krvnoj slici sa leukocitozom i neutrofilijom. Mikroskopskim pregledom pleuralne tečnosti uočen je veliki broj leukocita, a kulturom je izolovana *Salmonella enteritidis*. Koprokulturom nisu izolovane patogene bakterije. Hemokultura je ostala sterilna. Toksini A i B *Clostridium difficile* nisu nađeni u stolici. Bolesnik je lečen ciprofloksacinom i ceftriaksonom uz drenažu purulentnog sadržaja, što je bilo praćeno rezolucijom i organizacijom pleuralne tečnosti. Po stabilizaciji opšteg stanja bolesnika uvedena je hemioterapija po protokolu ciklofosfamid, vinkristin, prednizon (COP), sa kompletnim odgovorom. **Zaključak.** Iako redak, pleuralni empijem prouzrokovan netifusnim salmonelama trebalo bi razmotriti kod bolesnika sa teškom imunodeficijencijom, budući da su adekvatna i pravovremena antimikrobna terapija, uz hirurške mere, veoma važni za ishod lečenja ovih bolesnika.

Ključne reči:
empijem, pleuralni; dijagnoza; salmonella enteritidis; limfom, nehodžkinov.

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Introduction

Nontyphoidal *Salmonella* is widely spread in nature and usually presents as gastroenteritis in immunocompetent persons [1]. However, in immunocompromised patients, extraintestinal manifestations are possible, especially in patients with cellular immunodeficiency. For the last two decades the prevalence of nontyphoid salmonellosis has been increasing [2, 3]. The most important risk factors for extraintestinal salmonellosis are: extremes of age, malignancy, HIV infection, diabetes mellitus, sickle cell disease and therapeutic immunosupression [1, 2]. About 5% of symptomatic salmonellosis develop bacteriemia while less than 1% are focal infections like osteomyelitis, soft tissue infection, urinary tract infections or endocarditis [1]. Pleural empyema caused by non-typhoid *Salmonella* is an extremely rare condition and there are just a few cases described in the literature [4–6].

We presented a very rare case of pleural empyema caused by *Salmonella enteritidis* in a patient with non-Hodgkin lymphoma.

Case report

A 60-years-old male was admitted to the Clinic for Gastroenterology and Hepatology of the Military Medical Academy (MMA) in Belgrade on December 15 2013, because of swelling of the abdomen, sensation of early filling, noticed six months earlier, and intensified in the last three months, and were followed by extensive night sweating, lost of weight, fatigue and dyspnea. The patient was dismissed in good condition, he used no contaminated food, and there was no diarrheal illness in his surroundings, nor in himself.

At admission the patient was pale, dyspnoic with impaired respiratory sound on the left, silent cardiac sounds and systolic murmurs of the aortic confluence. The patient was normotensive with abdominal distension due to a large volume of ascites.

Laboratory data at admission were (as follows): red blood cells (RBC) 5.78 × 10^{12}/L (reference range 4.50–6.50 × 10^{12}/L), white blood cells (WBC) 10.6 × 10^{9}/L (reference range 4.00–11.00 × 10^{9}/L), neutrophils (Ne) 72.4% (reference range 40–74%), platelets (PLT) 578 × 10^{9}/L (reference range 160–370 × 10^{9}/L), glucosae 4.8 mmol/L (reference range 4.1–5.9 mmol/L), urea 4.4 mmol/L (reference range 2.5–7.5 mmol/L), creatinine 58 mmol/L (reference range 62–115 mmol/L), proteins 60 g/L (60–83 g/L), albumins 32 g/L (reference range 32–50 g/L), bilirubin 10 mmol/L (reference range 0–18 mmol/L), Na^{+} 140 mmol/L (reference range 136–145 mmol/L), K^{+} 5.2 mmol/L (reference range 3.5–5.1 mmol/L), Ca^{2+} 2.56 mmol/L (reference range 2.10–5.60 mmol/L), aspartate aminotransferase (AST) 33 U/L (reference range 0–37 U/L), alanine aminotransferase (ALT) 39 U/L (reference range 20–65 U/L), lactate dehydrogenase (LDH) 392 U/L (reference range 85–227 U/L), gama glutamyl transpeptidase (GGT) 147 U/L (reference range 0–73 U/L), alkaline phosphatase (ALP) 591 U/L (reference range 90–360 U/L), INR 0.97 (reference range 0.9–1.2), serum ascites albumin gradient (SAAG) 12 g/L; in ascites: albumins 20 g/L, LDH 392 U/L, GGT 147 U/L, ALP 591 U/L.

Chest radiography showed a pleural effusion in organisation on the left. A liver cyst 3 cm in diameter, was diagnosed on abdominal echotomography. On chest and abdominal multislice computed tomography (MSCT) massive pleural effusions on the left and smaller effusion on the right with compressive atelectasis were registred. The liver was enlarged, 17 cm in diameter, with massive ascites and the gastric thickened wall up to 19 mm (Figure 1).

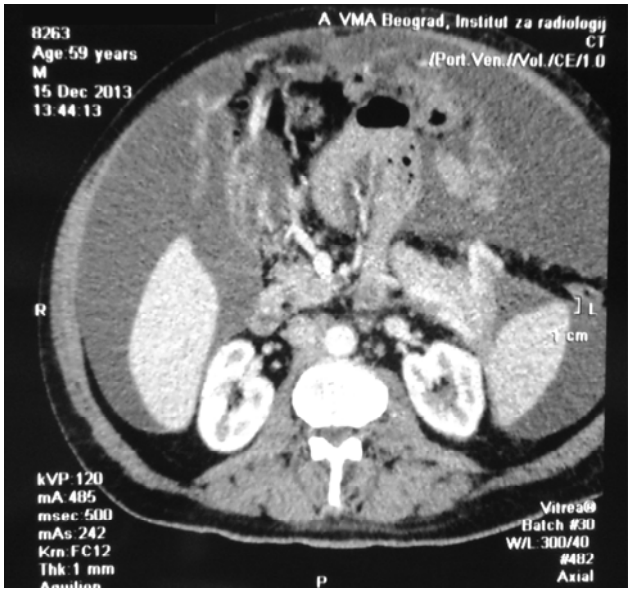

Fig. 1 – Multislice computed tomography of the abdomen revealed ascites in a patient with non-Hodgkin lymphoma.

On esophagogastroduodenoscopy many irregular, partially affiliated ulcerations in stomach were seen. Low grade B-cell lymphoma of the marginal zone was proved by its pathohistological examination. Bronchial infiltration for the left lower lobe was seen on bronchoscopy, whose histopathological examination proved the same type of lymphoma. Malignant lymphoma cells were also found by cytological examination of pleural and peritoneal fluid. Drainage of the thoracic cavity was made. Methylprednisolone in a dose of 1 mg/kg was started and the patient was transferred to the Clinic for Haematology of MMA on December 29, 2013. Just after the admission the patient had large-volume diarrhoea and fever. The patient received a short course of metronidazole until the arrival of microbiological analyses. In laboratory findings, the increase in the number of WBC was noticed up to 21.18 × 10^{9}/L, with the predominance of granulocytes (Ne 19.8 × 10^{9}/L). In the same time the pleural fluid became purulent (Figure 2), and microscopic evaluation showed a large number of polymorphonuclear leucocytes. Its bacteriological culture was positive for *Salmonella enteritidis*. Hemoculture remained sterile. Pathogenic bacteria were not isolated by stool culture. Stool was, also, negative for toxins A and B of *Clostridium difficile*.

The patient was treated by pareteral antimicrobial therapy, ciprofloxacin and ceftriaxone, simultaneously, according to the antibiogram, during 14 days, with drainage, that was followed by resolution and organisation of the pleural effusion. In further course the patient was pale, adynamic,

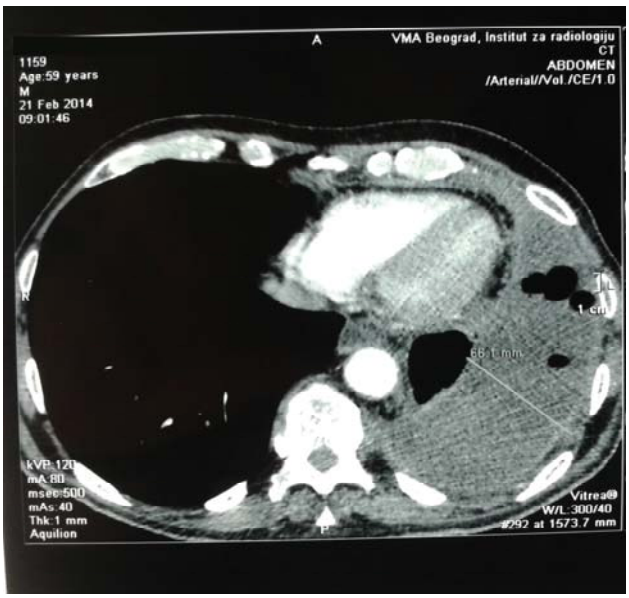

Fig. 2 – Empyema of the pleura in a patient with non-Hodgkin lymphoma detected using chest multislice computed tomography.

with deterioration of general condition, with radiographic picture indicating perforation of the hollow organ. On chest and abdomen MSCT, signs of pneumoperitoneum were found (Figure 3a). The surgeon decided not to operate the patient because of his poor general condition, but to go on with wide spectrum antimicrobal therapy (meropenem, metronidazole and fluconazole), supportive measures and drainage. After stabilisation of general condition, chemotherapy with cyclophosphamide, vincristine, prednisone (COP) was introduced. The patient recieved eight cycles of chemotherapy, with complete response. During chemotherapy there were no infectious complications, no neutropenia in our patient.

therapeutical procedures, the number of immunocompromised persons has increased. That is the reason for the incidence of extraintestinal nontyphoidal *Salmonella* infection to incease, also. Most of the patients with pleuropulmonary salmonellosis have additional lung or pleural disease [4, 5, 7]. Although, the presented patient was severely immunocompromised by his disseminated malignant disease and immunosuppressive treatment, he had also local immunosuppression, because of his infiltrated bronchus and pleura. Combination of systemic and local immunosuppression could be the explanation for this rare form of disease [8].

According to the literature, the most frequent serotypes of *Salmonella* isolated from pleural empyema are: *Salmonella typhimurium*, *Salmonella cholerasuis* and *Salmonella paratyphi*, while in just a few cases was isolated *Salmonella enteritidis* [9]. In most patiens the causative bacteria is isolated from stool, blood and pleural empyema. The way by which salmonella reaches the pleural fluid from intestinal tract can be haematogenous or *per continuitatem*. In the presented patient *Salmonella* was not isolated from blood, but it did not exlude hematogenous dissemination *via* transitory bacteriemia. In that case reticuloendothelial system coud be the source of *Salmonella* [5]. Although the causative agent was not detected in stool, we did not exlude acute salmonellosis, because the appearance of fever and diarrhea were time-related with the appearance of pleural empyema. Since we suspected microperforation of upper intestinal tract, direct spreading through diaphragm was also possible. Because the patient had thoracic drainage, before the contents became purulent, external aquisition was not to be excluded [10].

The most frequent clinical symptoms and signs of pleural empyema are fever, cough, dyspnea and pleuritic pain [11]. Clinical characteristics of our patients were non-specific, because they were masked by his poor general condition and the main illness.

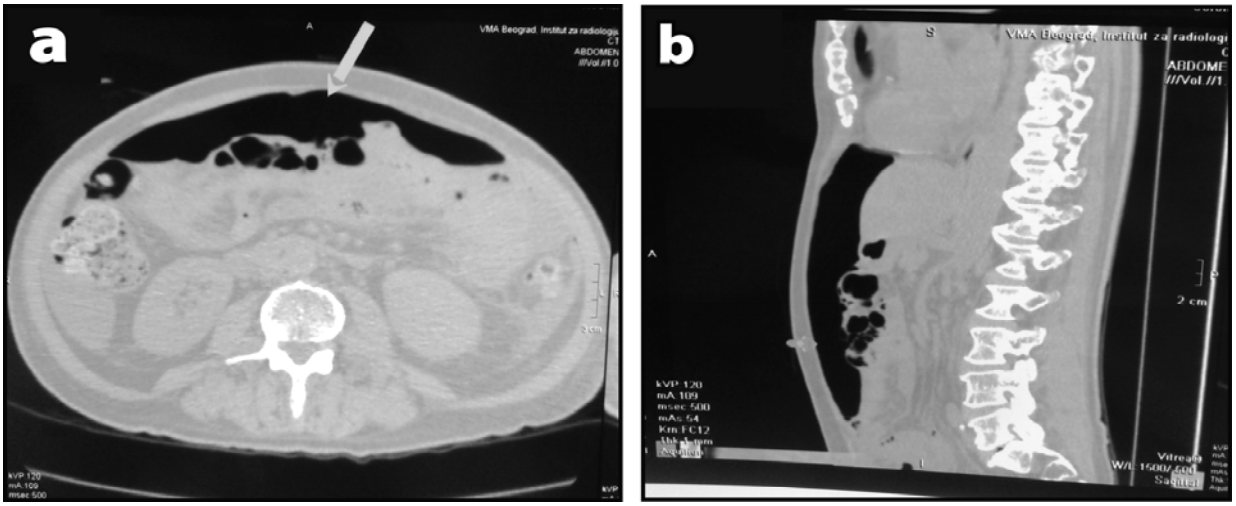

Fig. 3 – Pneumoperitoneum in a patient with non-Hodgkin lymphoma on a) transveral and b) longitudinal section of chest and abdominal multislice computed tomography.

Discussion

This case is an extreme rare form of extraintestinal nontyphoidal salmonellosis in a person with impaired cellular immunity. With the development of new diagnostic and

Most of the authors are consistent that treatment should include antimicrobial therapy and evacuation of pleural empyema by thoracocentesis, open drainage or pleural decortication [11]. Most *Salmonella* respond well to ciprofloxacin and third-generation cephalosporins [4]. The presented patient had a two-

week course of antimicrobial therapy with ciprofloxacin and cefriaxone, followed by open drainage of the thoracic cavity, followed by regression of empyema, despite the advanced disease. Dual antimicrobial therapy was introduced before the results of antibiogram were seen and it was continued after the antibiogram arrival, due to specificity of the affected area and patients poor general condition.

The most important predictive factors for the outcome are: age over 60, the presence of underlying disease and apropriate antimicrobial therapy [9]. The presented patient had more negative predictive factors for the outcome, but he survived with the help of appropriate and early antimicrobial therapy and the other supportive measures.

Conclusion

Althoug rare, nontyphoidal *Salmonella* should be considered as possible etiological factor of pleural empyema in patients with celullar immunodeficiencies, especially if a patient has underlying pulmonary disease. Namely, apropriate antmicrobial therapy with surgical measures can improve the outcome in these patients.

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CASE REPORT

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Iatrogenic dissection of the left main coronary artery during elective diagnostic procedures – A report on three cases

Jatrogena disekcija glavnog stabla leve koronarne arterije tokom elektivne dijagnostičke procedure

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Abstract

Introduction. Left main coronary artery dissection is a rare and potentially life-threatening complication of coronary angiography and angioplasty which requests urgent revascularization. **Case report.** During the period between 2010 and November 2014 at single healthcare center we did totally 8,884 coronary procedures, out of which 2,333 were percutaneous coronary interventions (PCI). In this period we had a total of 3 (0,03%) left main coronary artery dissections, and all of them were successfully treated by PCI. We presented three cases with iatrogenic dissection of the left main coronary artery, occurred during elective diagnostic procedures, successfully treated with PCI with different techniques. **Conclusion.** PCI could be fast and life-saving approach in iatrogenic dissections of the left main coronary artery.

Key words:
iatrogenic disease; coronary angiography; percutaneous coronary intervention; treatment outcome.

Apstrakt

Uvod. Disekcija glavnog stabla leve koronarne arterije je retka i potencijalno po život opasna komplikacija tokom koronarne angiografije i angioplastike i zahteva hitnu revaskularizaciju. **Prikaz bolesnika.** Tokom perioda između 2010. i novembra 2014. u našoj ustanovi sprovedene su ukupno 8 884 koronarne procedure, od kojih su 2 333 bile perkutane koronarne intervencije (PKI). U tom periodu imali smo ukupno 3 (0,03%) disekcije glavnog stabla leve koronarne arterije i sve su uspešno rešene putem PKI. U radu su prikazana tri bolesnika sa jatrogenom disekcijom glavnog stabla leve koronarne arterije, nastale tokom elektivnih dijagnostičkih procedura, koje su uspešno rešene pomoću PKI, korišćenjem različitih tehnika. **Zaključak.** PKI može biti brz pristup kojim se spasava život pri lečenju jatrogenih disekcija glavnog stabla leve koronarne arterije.

Ključne reči:
jatrogena bolest; angiografija koronarnih arterija; perkutana koronarna intervencija; lečenje, ishod.

Introduction

Iatrogenic dissection of the left main coronary artery (LMCA) is a rare and potentially life-threatening complication of coronary angiography and angioplasty, which requires urgent revascularization, using percutaneous coronary intervention (PCI) or surgery revascularization, also known as coronary artery bypass graft (CABG) [1].

LMCA dissection often leads to abrupt occlusion causing a great deal of myocardial ischemia, which results in acute heart failure with hemodynamic collapse. Prior to 1993, when PCI of iatrogenic dissection of LMCA was first done, urgent CABG surgery was the only treatment option [2]. It has been shown that the above mentioned complications are significantly more likely to occur during PCI procedures comparing to diagnostic catheterization (0.10% vs. 0.06%) [3]. When it comes to cardiac surgical care of these complications (for successful CABG) according to available data, 30-day mortality rate in this group of patients is slightly more than 26% [4].

Case report

Between 2010 and November 2014 at Military Medical Academy (Belgrade, Serbia) we did totally 8,884 interventi-

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ons, out of which 2,333 were PCIs. In this period we had a total of 3 (0.03%) LMCA dissections, and all of them were successfully treated by PCI (Figures 1–3). Common characteristics of all the patients were: they were women aged 61–72 years, with hypertension, angina pectoris complaints, and unprotected LMCA, and dissection occurred during elective diagnostic procedures as shown in Table 1.

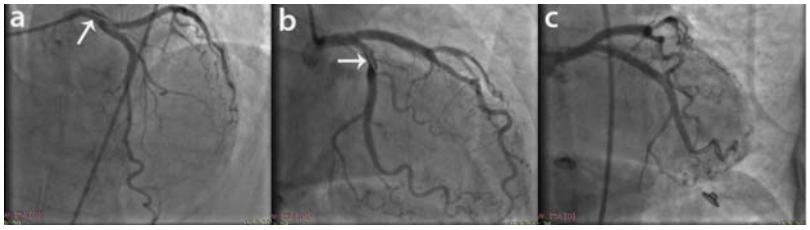

Fig. 1 – a) (arrow) Spiral dissection line of the left main coronary artery (LMCA), and b) (arrow) Circumflex artery (CXA); c) After stents implantation no dissection lines and coronary flow disturbance could be seen.

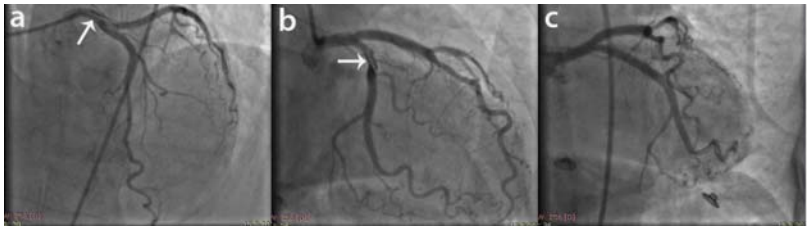

Fig. 2 – a) (arrow) Occlusive dissection of the left main coronary artery (LMCA); b) (arrow) Spreading down to significant stenosis in proximal left anterior descending artery (LAD) compromising coronary flow in mid and distal LAD; c) After stent implantation in LMCA, complete left system coronary flow has been achieved.

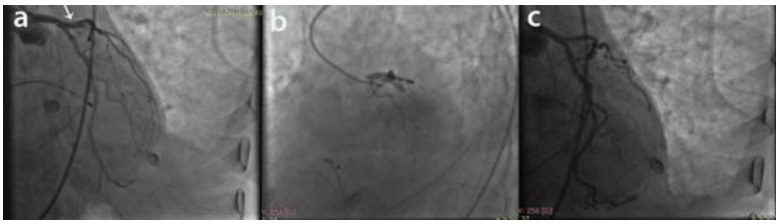

Fig. 3 – a) Occlusive spiral dissection of the left main coronary artery (LMCA) with b) Complete flow obstruction in the entire left coronary artery system; c) After stent implantation in LMCA, complete left system coronary flow has been achieved.

Discussion

Left main coronary artery dissection is a rare but potentially fatal complication that requires emergency care and coordination of cardiologist, cardiac surgeon and cardiopulmonary resuscitation team. If these complications occur, clinical picture, depending on the remaining anterograde blood flow, varies from asymptomatic in patients with preserved TIMI 3 flow, to clinical image of cardiogenic shock in patients with completely compromised flow behind the point of dissection. However, even in cases with initially preserved TIMI 3 flow and hemodynamic stability, rapid deterioration can be quickly followed by progression of aortic dissection or thrombus formation, which is always an urgent situation and requires immediate revascularization.

In patients with compromised hemodynamic status, an intra-aortic balloon pump could be a useful alternative for improving blood flow, and increasing oxygen delivery of the patient [5].

Dissection is the result of mechanical injury of the arterial wall due to manipulation of the catheter, either diagnostic or guided one, when the potential risk for dissection is much greater, as we already stated. Catheter type also plays an important role. For example, Amplatz, or small Judkins catheters can go deep into LMCA, therefore should be avoided for deeper positioning within the LMCA. Also, attention should be paid when using wires with higher penetration index (because of the potential sub-intimal route) and concomitant contrast injection, with constant and careful monitoring of hemodynamic status. In addition, good judgment is needed regarding unusual anatomy of the left coronary artery (abnormal artery location or origin), as well as, atherosclerosis or potential calcification and plaque in the LMCA, or more expressed angulation of LMCA and LAD / circumflex artery (CXA) joint. Careful positioning of the catheter in the artery coaxial level, contrast strength, and experience of the operator are also very important assumptions in the prevention of these complications.

Special group of patients like those with structural heart damage (bicuspid aortic valve) or the patients with hereditary connective tissue abnormalities (Marfan syndrome, cystic medial necrosis) are of special concern when performing elective diagnostic procedures [6].

Notwithstanding the foregoing, in certain cases it is impossible to prevent LMCA dissection.

All the three cases of LMCA dissection caused by diagnostic catheter, shown here, were successfully treated using PCI method, with the right selection of bifurcation techniques (Cullote, one stent or double stent technique), depending on

Table 1

Series of tree cases with left main coronary artery dissection during elective coronarography procedure

Parameters	Case No 1	Case No 2	Case No 3
Characteristics of the patient	Female, 72-year-old, arterial hypertension, former smoker, had CVI four years ago	Female, 61-year-old with arterial hypertension, hyperlipoproteinemia; smoker.	Female, 69-year-old with arterial hypertension, Basedow's disease; had myocarditis four years ago.
Reason for coronarography	Chest pain on moderate effort, positive stress ECG test.	Unstable angina pectoris	Unstable angina pectoris and AV block grade III. After implantation of temporary pacemaker, on day 3 she developed atrial fibrillation and acute heart failure with pulmonary edema. On echo, hypokinetic distal anteroseptum and distal portion of anterior wall were noticed.
Type of complication	Non-occlusive spiral dissection of LMCA spreading to midsection of CXA (Figures 1a and 1b)	Catheter tip-caused occlusive dissection of LMCA spreading through proximal LAD and stopped on luminal stenosis of 90%. Obstruction of LAD was apparent (Figures 2a and 2b)	On second projection a catheter tip-caused occlusive dissection of LMCA with propagation to both LAD and CXA was noticed with complete loss of flow. (Figures 3a and 3b).
How complication is resolved	Guide catheter Launcher® EBU 3.5 6F (Medtronic). First wire Asahi Sion (Asahi Intecc) in CXA and second one in LAD. First BMS Commander 3.5 × 32 mm (Alvimedica) implanted in CXA. Second BMS Constant 4.0 × 17 mm (Alvimedica) implanted in LMCA and proximal LAD	Guide catheter Launcher® EBU 3.5 6F (Medtronic). Wire Run-through® (Terumo) was plased in LAD. One BMS Constant 3.0 × 21 mm (Alvimedica) was implanted from mid-portion of LMCA down to proximal part of LAD. Another BMS Constant 3.0 × 17 mm (Alvimedica) was implanted in continuation to cover stenosis in LAD	Guide catheter JL 4.0 7F (Cordis). Wire InterFlex (Kimal) was placed in CXA. One BMS Omega 3.5 × 24 mm (Boston Scientific) was implanted from ostium of LMCA down to CXA.
Final result	TIMI 3 flow. Patient has fully recovered. Figure 1c.	TIMI 3 flow in all arteries. Small intramural hematoma in LMCA. Patient has fully recovered. Figure 2c	TIMI 3 flow in all arteries and hemodynamic stabilization (Figure 3c). After permanent DDDR pacemaker implantation and on day 10, patient was discharged recovered.

CVI – cerebrovascular insult; AV – atrioventricular; LMCA – left main coronary artery; LAD – left anterior descending; CXA – circumflex artery. TIMI – thrombolysis in miocardial infarction flow grade; BMS – bare metal stent.

the estimated time, the patient's hemodynamic status, coronary anatomy, dissection type (extensive or localized, occlusive or preserved with TIMI 3 flow), as well as, in accordance with the experience of operators in specific techniques.

Ending the bifurcation procedure with kissing technique is the recommended way and the final step in standard procedures. However, in the presented cases, we emphasize that those were patients with marked hemodynamic instability, but with the obtained adequate angiographic effect after placement of a stent in the left main branch. Due to the immediate excellent angiographic effect, after the state of extreme instability, operator's evaluation was that additional methods as final kissing or morphological assessment as intravascular ultrasound (IVUS) or optical coherence tomography (OCT) could be done in the second act, and after stabilization of the clinical status, with the aim to speed up the completion of the intervention, and to give as less as possible amount of contrast in the situations when the patient was extremely endangered.

Conclusion

If recognized in time, LMCA dissection can be successfully treated with stent implantation, resulting in a favorable short-term and long-term outcome. PCI could be fast and life-saving approach in iatrogenic disseactions of the left main coronary artery.

Conflict of interest

All the authors declare no conflict of interest.

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CASE REPORT

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Treatment of teeth in the esthetic zone in a patient with *amelogenesis imperfecta* using composite veneers and the clear matrix technique: A case report

Zbrinjavanje zuba u estetskoj zoni kod pacijenta sa *amelogenesis imperfecta* primenom kompozitnih faseta i matriks tehnike

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Abstract

Introduction. Restorative dental treatment of patients with a generalized form of *amelogenesis imperfecta* (AI) remains a challenge even today. The treatment approach is multidisciplinary and includes action of several dental disciplines such as restorative, orthodontic, and prosthetic dental specialties. **Case report.** A 18-year-old female patent was referred to the Department of Restorative Dentistry and Periodontology at the Military Medical Academy of Belgrade, Serbia. She was diagnosed with AI and formerly had been treated for a long period of time at the Department of Pediatric Dentistry and Orthodontics. Her primary concern upon arrival was discomfort and concern for the esthetic appearance of the anterior teeth. The treatment was done with the modified clear matrix technique used in composite veneer restoration of teeth in the esthetic zone. **Conclusion.** Because fixed prosthetic restoration with crowns, is the final treatment of AI patients it involves severe tooth structure loss. The clear matrix method which was done in this case allowed for greater comfort, functionality, simplicity, speed, greater economic efficiency and tooth structure preservation.

Key words:
amelogenesis imperfecta; esthetics, dental; dental veneers; dental materials; treatment outcome.

Apstrakt

Uvod. Restorativno lečenje zuba kod osoba sa opštom *amelogenesis imperfecta* (AI) i danas predstavlja problem. Terapijski pristup je multidisciplinaran i uključuje nekoliko specijalista iz oblasti stomatologije, kao što su restorativna stomatologija, ortodoncija i protetika. **Prikaz bolesnika.** Ženska osoba, stara 18 godina, primljena je na Odeljenje konzervativne stomatologije i parodontologije Vojnomedicinske akademije u Beogradu. Ona je obolela od AI, a pre toga je bila dugogodišnji pacijent na Odeljenju dečje stomatologije i ortodoncije. Njen primarni razlog posete bio je nelagodnost i zabrinutost zbog izgleda svojih zuba, naročito u zoni osmeha. Lečenje je sprovedeno korišćenjem kompozitnih vinira i modifikovanom *clear matrix* tehnikom. **Zaključak.** S obzirom na to da protetsko zbrinjavanje kao finalno rešenje kod osoba sa AI podrazumeva brušenje velikog broja zuba, modifikovana *clear matrix* metoda koja je primenjena u ovom slučaju je znatno komfornija, funkcionalnija, jednostavnija, brža, ekonomski prihvatljivija, a omogućava očuvanje zubnih struktura.

Ključne reči:
amelogenesis imperfecta; estetika, stomatološka; fasete, zubne; stomatološki materijali; lečenje, ishod.

Introduction

Amelogenesis imperfecta (AI) belongs to a group of hereditary syndromes that affect enamel formation during tooth development and create a functional and esthetic problem for both the patient and the dental professional. It generally affects all teeth, but it has been sometimes seen in a localized form where it affects only a portion of dentition. AI occurs 1 : 700 to 1 : 16,000 in a population depending on the analytic standards used [1, 2]. The transmission of this syndrome is

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genetic and can be autosomal dominant, autosomal recessive, X-linked or irregular [3]. AI affects primary and secondary dentition. Patients with AI usually have the following symptoms: teeth hypersensitivity, abnormal enamel structure, reduced crown esthetics, reduced crown size, occlusal dimension pathology, delayed eruption, tooth impaction, gingival inflammation and taurodontism [4–6].

In the literature it has been found that malformed enamel is closely related to lower or no production of enamelin, tuftelin, amelogenin and ameloblastin [7]. The appearance of teeth especially in the esthetic zone has a special psychological effect on a young adolescent patient. Parekh et al. [8] reported that several psychological problems arise in children and adolescent patients diagnosed with AI, which they differentiate into two major psychological archetypes. They include personal dissatisfaction and fear from comments made by their peers that refer to their appearance. The patients in that study reported similar dissatisfaction with the appearance of their smile, which was equivalent to the amount of discontent they had with the functionality aspect of their dentition. Therefore, it can be inferred that AI has a major influence on the patient's everyday life both esthetically and functionally.

The classification of AI divides the syndrome into four basic phenotypic types: hypomaturation, hypoplastic, hypocalcified and hypomaturation-hypoplastic types [9]. However, it is known today that there are 14 subtypes of AI [10]. In this case study the patient's teeth had an opaque mottled appearance with light brown to yellow discoloration of frontal teeth and darker brown to black discolorations of posterior teeth. Concerning posterior teeth in both the maxillary and mandibular arches, some of the enamel chipped in larger pieces of the occlusal teeth surfaces and had been previously restored. This type of AI is a product of a faulty maturation phase in amelogenesis. Generally, the hypomaturation type has some of the following dental complications: increased caries incidence, impactions, open bite deformity and taurodontism [11].

With most AI patients it is important to correct the occlusion as much as possible with a proper orthodontic treatment, and reduce the number of carious teeth to a minimum with proper restorative and preventive dental care. The final treatment in any case will be with fixed crowns and circular bridges in both arches. Today, with the advances in composite material technology, it is possible to esthetically restore teeth in patients with AI with composite veneers, which reduces cost, psychologically helps the patient carry on every day life and delays costly and invasive fixed prosthodontic treatment.

This case report focused on oral rehabilitation with composite materials in the 18-year-old patient with one of the hypomaturation subtypes of AI. The restoration included the novel type of treatment of the esthetic zone of the mouth – the modified clear matrix technique for composite veneers in the esthetic zone of both dental arches.

Case report

A female patent was referred to the Department of Restorative Dentistry and Periodontology at the Military Medical Academy of Belgrade. She was diagnosed with AI and was a long-term patient at the Departments of Pediatric Dentistry and Orthodontics. Her primary concern upon arrival at our office was discomfort and the appearance of teeth, particularly in the esthetic zone of smile.

Her medical history was exceptional as she negated any systemic medical problems. However, she did report a family history of similar conditions. During an oral examination it was noticed that the teeth had an opaque white color with light brown to yellow pigmentation that generated the mottled appearance of all tooth surfaces. The teeth had dark brown spots with black pigmentation on teeth 14, 24, and 33. The facial surfaces of the anterior teeth had white opaque enamel color with yellow vertical lines, which were sporadically situated. The diastema was not significantly present, except between teeth 11 and 12. Also, the tooth 12 was slightly rotated and the shape of the teeth in the esthetic zone generally did not stand out as irregular. Palatal surfaces of the frontal teeth did not have any anomalies except the opaque color and slight pigmentation (Figures 1 a and b).

The patient did not report increased sensitivity to cold (air blow test) or warm (warm gutta-percha test). The vertical dimension of occlusion was previously corrected through an orthodontic treatment and brought within class I occlusal po-

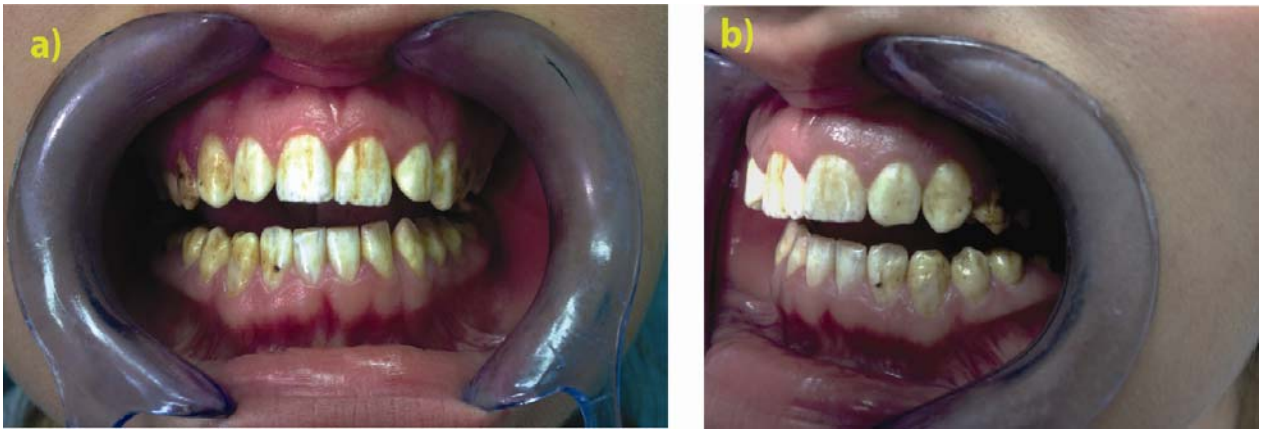

Fig. 1 – a) Anterior and b) side view of preoperative dental condition.

sition. The level of oral hygiene was satisfactory based on the levels of dental plaque index and gingival bleeding index. The gingiva was without any visible signs of inflammation and the rest of the oral mucosa was without any visual pathology. The patient negated any functional masticatory problems. All of the teeth present were previously restored with fillings and there were no carious teeth left. The classification of the main pathology, the AI, was done according to Witkop's classification. According to this classification it was found that the AI in question was type IIA, hypomaturation autosomal recessive form of AI [10].

The treatment course was planned according to the needs and financial capabilities of the patient. It concerned primarily a low cost composite restoration of the facial surfaces of all teeth in the esthetic zone with as less visits as possible and as little chair time as possible. She desired the restoration in the whole esthetic zone to be finished in one session. After consultation it was decided that the treatment was to be done with the modified clear matrix technique used in composite veneer restoration of the esthetic zone. The dental team concluded that it is too early for fixed crowns, at least in the esthetic zone, and that it was necessary to prolong the beginning of the fixed prosthetic treatment of this patient. The treatment could include fabrication of ceramic veneers, but the cost was not satisfactory for the patient. In agreement with the patient it was decided that the best solution was to restore the facial aspects of the teeth in the esthetic zone with composite veneers.

Impressions were made with alginate (Neocolloid alginate, Zhermarck®, Badia Polesine, Italy), and alabaster cast models were fabricated (Alabaster, PoliDent®, Volcja Draga, Slovenia). The facial aspects of all the predetermined teeth on the cast model in the smile area were slightly prepared with fine conical dental bur and a wax up of the future veneers was modeled with light-curing dental wax (LC Block-Out Resin, Ultradent®, South Jordan, USA). We decided to utilize this type of wax because of its easy manipulation, consistency and durability, which was needed during the thermo-vacuum transparent plate fabrication. The transparent splint plate (Duran®, Scheu, Iserlohn, Germany) was fabricated on the wax-up cast model and each tooth on the splint was separated with a disk burr (Galaxy® Cutting Wheel, Ortho Technology, Tampa, Florida, USA) so that each separated piece matched one tooth in the mouth.

The tooth facial surfaces were minimally prepared so that the proper roughness of the facial tooth surface was achieved first with a flame rough black ring 18 µm diamond burr (Diamond bur, New Tecnology Instruments NTI®, Thuringia, Germany) and then with a finer modified beveled cylinder yellow ring diamond 20 µm bur. After the proper roughness was achieved, retention slots were made parallel to the axial line of the tooth, which were 0.5 to 0.6 mm in depth for the frontal, and posterior teeth respectively. Therefore, for molars and the premolars, the thickness of the diamond bur used was 1.2 mm and for the frontal teeth it was 1.0 mm. The slot depth was determined by achieving depth of half of the thickness of the dental bur.

Upon the completion of the facial surface preparation, rubber dam – OptraDam (OptraDam® Plus Medium, Ivoclar Vivadent Corporate, Schaan, Liechtenstein) was positioned with a proper clamp (Rubber Dam Clamp, Dentsply®, Islandia, NY USA), one tooth at a time, with the inner plastic ring removed form the rubber dam. It was decided on a two-step bonding system. With the clamp and rubber dam in position each tooth was air-dried and was etched with 37% phosphoric acid (Scotchbond® Etchant, 3M/ESPE, St. Paul, MN, USA) for 15 sec, as indicated by the manufacturer. After this, each tooth was treated with an adhesive bonding agent (Filtek® LS System Adhesive, 3M/ESPE, St. Paul, MN, USA) using a brush, air dried for 5 sec and light cured with a LED light (Elipar® S10 LED Curing Light, 3M/ESPE, ST. Paul, MN, USA) for 10 sec. The appropriate piece of the matrix foil was removed from the cast model and fitted on the proper tooth (Figure 2).

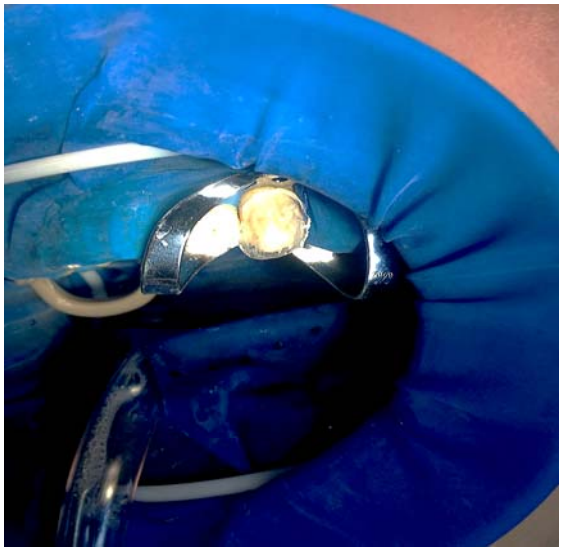

Fig. 2 – Matrix placed on the tooth 23 after acid etching and bond polymerization.

All the restorations were done with micro-hybrid composite material (Filtek® Universal Restorative Z250, 3M/ESPE, St. Paul, MN, USA). The composite material was inserted into the piece of the matrix foil in the pocket created from the labial wax-up only and was pressed on to the tooth so that the excess material would be pushed out. The excess material was removed with an interproximal explorer and the tooth was light cured for 20 sec as indicated by the manufacturer. The matrix was removed, the final touch up was done by adding more material and the tooth was further light cured for another 20 sec. The composite veneer was further prepared with fine-fissured dental burs and polished with polishing burs (NTI® Dia Gloss set, New Technology Instruments NTI®, Thuringia, Germany). Upon finishing polishing of each tooth, the rubber-dam clamp was removed and placed on the next tooth. When each dental arch was completed, the veneer symmetry, midline, height of the incisal edges of all teeth, and the position of the occlusal line to the bi-pupillary line were checked (Figure 3).

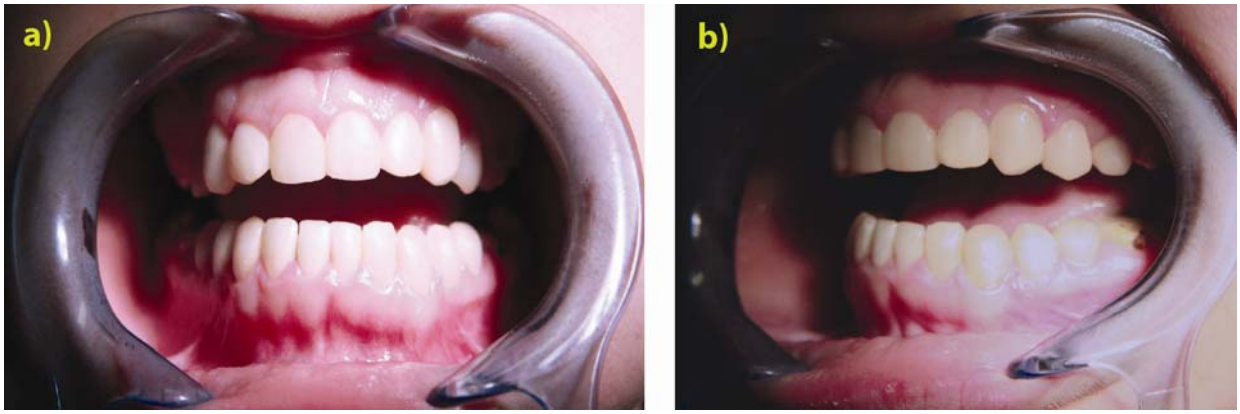

Fig. 3 – a) Anterior and b) side view of the postoperative result.

The patient was instructed how to maintain oral hygiene with brush, floss, and interdental brushes to control the level of dental plaque until the next visit. Fluoride application to all teeth was done after the completion of the procedure.

Post-op check-ups confirmed a solid and firm bond between the composite material and enamel surface on all teeth with AI. The three-month post-op checkup did reveal a small amount of discoloration on teeth 13, 15, 25 and 32, which were made by food and liquid staining as the patient is a non-smoker. The stains were removed with disc burs and new material was added with ease where needed.

Discussion

This case study focuses on the AI hypomaturation type, which exhibited white opaque enamel, with yellow-brown discolorations that easily chips from the underlying dentine.

AI represents a challenge for clinicians in dentistry because it creates great functional and esthetic problems that are hard and costly to restore, requiring multiple visits to the dental office [2]. In dentistry today, the final solution for AI patients are metal-ceramic or full ceramic crowns and bridges. However, it is important to understand all the aspects of the problem that the patient with AI may have and their desires before deciding upon the final treatment [5]. Restorative materials today have excellent esthetic and functional properties that allow the clinician and the patient to have more options for the treatment [6]. The attempt to restore the AI affected teeth with composite materials represents a novel approach in dentistry today and must be approached with caution. It is important to critically analyze the patient's, psychological status, economic status and the type of the AI present in the dentition so that the final result is both esthetically and functionally pleasing [11]. Sabatini and Guzmán-Armstrong [12] report that the new method called "modified clear matrix technique" allows for the simplification of the restorative process with composite materials in the patient with a generalized form of AI. They point out the possibility of using the matrix foil to create a good composite foundation for the final restoration. This, in turn, reduces the time spent on each tooth, chair time, the number of visits to the dental office and the cost of the whole procedure.

When planning this procedure it is important to examine the age, socioeconomic status, subtype of AI in the mouth, the level of oral hygiene, the presence of occlusal anomalies, and the time of completion of the procedure that best suits both to the patient and the clinician [13]. The female adolescent patient in this case report was primarily concerned with physical appearance of her smile and the cost needed to achieve the proper esthetic result. The modified clear matrix method for esthetic composite veneers was chosen for her because of the proper cost, promptness, and good esthetic results. The positive aspect of this technique is the good esthetic and functional restoration of the facial surfaces of teeth directly in the patient's mouth within one visit. Furthermore, it is possible to administer corrections if any problems should arise during the course of mastication after the procedure, using the same matrix that had been originally utilized [14]. The teeth in the esthetic zone had sufficient enamel for minimal preparation and the analysis of cast models indicated that the proper thickness of the composite restoration could be placed on the facial aspects of all teeth so that all the discolorations on them would be hidden. Minimal preparation included removing a minimal amount of enamel required to realize the proper roughness of the surface and to achieve good depth for the slots, which in turn provided good retention of the final restoration. Both of these conditions allow for a good bond between the enamel and the composite veneer, which was agreed upon during the check-ups ten days and three months after the procedure. During the checkups no composite restoration was detached from the underlying enamel. The discolorations on some restorations did arise but this was quickly removed with dental disc burs. The patient did not report any sign of discomfort, hypersensitivity during and after the procedure or any trouble with mastication. The shape of all restorations dictated a good esthetic result but also needed to be such so that the patient could maintain a proper oral hygiene. The patient received proper oral hygiene training related to composite veneers.

Using the clear matrix technique the patient's needs in terms of prompt procedure and cost were satisfied. Furthermore, good esthetics and functionality were properly achieved in a short time period, which benefits the clinician as well as the patient. The time period between check-ups

gives the patient time to adapt to the changes and to point out if any of the teeth need to be changed in appearance. For all postoperative treatments, the original matrix foils were used and provided an easy retreatment. It is important to indicate the need for a rubber dam (OptraDam®) that creates the proper dry conditions for a good bond between the composite resin and the enamel. Also, it is important to follow closely the actual manufacturer's instructions for each bonding step. The fabrication of wax-up models in the laboratory allowed for making of clear matrix foils for both mandibular and maxillary arches. That in turn provided fast and precise veneer restorations during the actual operative session in the dental office. This was important since a great number of teeth needed to be prepared and composite veneers fabricated in one session.

Advantages of our modified method are the following: clear matrix method generates composite veneers; prefabricated wax ups allow for easy matrix fabrication; minimal amount of tooth structure needs to be removed; minimal free handed modification needed; dividing the matrix foil into individual pieces, where each piece fitts one tooth at a time, enables that interdental contact points are not affected; fluoride application upon completion of the restoration is possible.

Conclusion

This case report describes oral rehabilitation with composite materials of an 18-year-old female patient who presented with one of the hypomaturation subtypes of the *amelogenesis imperfecta*. Restoration included the esthetic zone of the mouth and the procedure described was the modified clear matrix technique for composite veneers for both the maxillary and the mandibular arches. A new dental material technology, allows today, to simplify the process of restoring *amelogenesis imperfecta* patients permitting evasion of radical prosthetic rehabilitation. Our modified clear matrix method produces better comfort, functionality, treatment speed, esthetic results, and cost efficiency in comparison to fixed prosthetic rehabilitation.

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CASE REPORT

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Leptomeningeal carcinomatosis can be presenting manifestation of breast carcinoma

Leptomeningealna karcinomatoza kao manifestacija karcinoma dojke

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Abstract

Introduction. Leptomeningeal carcinomatosis (LC) is a serious complication occuring in solid cancer patients with rather poor prognosis. **Case report.** We presented a 47-year-old woman with the 6-month history of diffuse headache, nausea and visual obscuration. Initially, clinical status and brain magnetic resonance imaging (MRI) indicated syndrome of idiopathic intracranial hypertension. Due to clinical progression and high papillary stasis, cerebrospinal fluid (CSF) examination was performed only after ventriculoperitoneal shunt was implanted. This led to a significant although transient clinical improvement. Futher investigations led to the diagnosis of invasive lobular breast carcinoma and repeated CSF analysis revealed malignant breast carcinoma cells. In this case LC was an initial presentation of a malignant disease. **Conclusion.** In the presence of a high clinical suspicion of LC, in spite of initially negative findings, a clinician should persist in repeating relevant tests, such are MRI with larger amounts of gadolinium and high-volume cytological CSF analyses in order to make the diagnosis.

Key words:
breast neoplasms; neoplasm metastasis; meningeal neoplasms; diagnosis, differential.

Apstrakt

Uvod. Leptomeningealna karcinomatoza (LK) je ozbiljna komplikacija koja se javlja kod bolesnika sa karcinomima i ima lošu prognozu. **Prikaz bolesnika.** Prikazali smo 47-godišnju ženu sa 6-mesečnom anamnezom difuzne glavobolje, mučnine i zamagljenja vida. Na početku, klinički status i magnetna rezonanca mozga ukazali su na sindrom idiopatske intrakranijalne hipertenzije. Usled kliničkog pogoršanja i visoke staze papile, pregled cerebrospinalne tečnosti (CST) mogao je biti obavljen tek posle plasiranja ventrikuloperitonealnog šanta. Ovo je dovelo do značajnog, mada prolaznog, kliničkog poboljšanja. Dalje ispitivanje ukazalo je na postojanje invazivnog lobularnog karcinoma dojke, a ponavljanje analize CST otkrile su postojanje malignih ćelija karcinoma dojke. U ovom slučaju LK je bila početna prezentacija maligne bolesti. **Zaključak.** Ukoliko, uprkos početnim negativnim nalazima, postoji visoka klinička sumnja na LK, kliničar bi trebalo da ponavlja relevantne analize, kao što su magnetna rezonanca sa većom količinom gadolinijuma i analize većeg volumena CST, da bi se postavila dijagnoza.

Ključne reči:
dojka, neoplazme; neoplazme, metastaze; meninge, neoplazme; dijagnoza, diferencijalna.

Introduction

Leptomeningeal carcinomatosis (LC) is a serious complication of cancer associated with substantial morbidity and mortality. Leptomeningeal metastases ooccur in 5–15% of patients with cancer but the diagnosis has become increasingly common with the routine use of high-quality magnetic resonance imaging (MRI) and lengthening survival of patients with more effective treatments [1–3]. The most common primary cancers, excluding hematological malignancies, are breast, lung, gastrointestinal tumors and melanoma [1–3]. The disease typically involves multiple levels of the neuraxis, and obstruction of normal cerebrospinal fluid (CSF) flow pathways may result in increased intracranial pressure and hydrocephalus [2]. Identification of malignant cells by CSF cytology had been the diagnostic gold standard, with high specificity but limited sensitivity [1, 2]. In spite of

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advances in diagnosis and treatment, the prognosis in patients with LC remains poor.

Case report

A 47-year-old woman was admitted with a 6-month history of headache, bilateral tinitus, nausea with occasional vomitting and visual obscurations. The patient had been previously in good health with the negative past and family history. In particular, the family history of breast carcinoma was negative.

On admission, her general status was normal. Small painless lump in the upper lateral quadrant of the right breast was noted. No lymphadenopathy was found on clinical examination. On neurological examination the patient was well-oriented, with bilateral papillary edema (4 Dpt), decreased visual acuity bilateraly (20/25) and gait ataxia. Complete blood count and standard biochemistry were normal. A mildly increased D-dimer level (215 μg/L, reference < 125 μg/L) was noted. Brain computed tomography (CT) was normal.

Brain MRI indicated dilatation of both optic nerves with increased perineural CSF, flattening of the posterior sclera, empty sella syndrome, dilatated lateral ventriculs with periventricular edema; conclusion was made that these are typical signs of idiopathic intracranial hypertension (IIH). MR venography (MRV) revealed slower blood flow through sinus rectus without signs of trombosis.

Full coagulation assessment, immunological analyses with antinuclear, antiDNA and anticardiolipin antibodies, ELISA HIV antibodies and serology for syphilis were negative. Repeated D-dimer was normal. Abdominal ultrasound examination was unremarkable. Chest radiography indicated enlarged mediastinal lymph nodes, but thorax CT with contrast showed normal finding in the mediastinum; however, it revealed small lesion in the right breast and enlarged lymph nodes in the right axilla. Mammography confirmed tumorous formation in the upper lateral quadrant of the right breast. The biopsy was performed and histopathological analysis showed invasive lobular breast carcinoma. There was no metastatic lesions on radiography examination of the skull and spinal column.

Signs of clinical progression, comprising increased headache and vomiting, generalized tonic-clonic seizures and papillary stasis progressing to 6 Dpt, precluded CSF examination. An intense antiedematous therapy was administered (dexamethasone, mannitol). A repeat MRI showed the same finding. The ventriculoperitoneal (VP) shunt was implanted, leading to significant clinical improvement, decrease in papillary stasis (2.5 Dpt) and enabled CSF sampling. First CSF analyses showed normal biochemistry with one lymphocyte and polymorphonuclear each. CSF cytology indicated rare mononuclears with signs of atypia in some, but the finding was inconclusive. Oligoclonal bands were absent. CSF bacterial and fungal cultures were negative. A week later CSF analysis was repeated, showing mildly increased proteins (0.6 g/L), hypoglicorachia 2.3 mmol/L (glyceamia 6.8 mmol/L), 3 polymorphonuclears and 3 lymphocytes *per* 1 mL. Larger amount of CSF was sent to second cytological analysis, which revealed a large number of malignant cells corresponding to breast carcinoma (Figure 1). The patient was referred to the Oncology Unit for further treatment. Standard symptomatic and palliative care was applied and the patient died 4 months later.

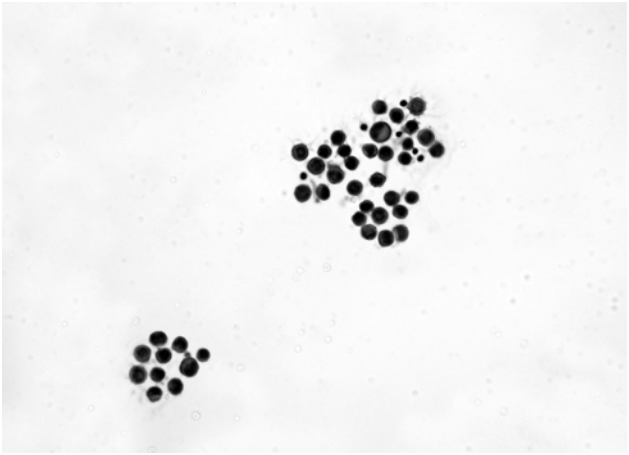

Fig. 1 – Clusters of breast lobular carcinoma cells in cytospin preparation of cerebrospinal fluid sample (haematoxylin and eosin, × 400).

Discussion

Breast cancer is the leading nonhematologic cause of LC [3]. We reported an illustrative case of a patient presenting with LC as initial manifestation of breast malignant disease. Initially, possible dural sinus thrombosis were suspected. However, no direct signs of sinus thrombosis were visualised, D-dimer normalized and no prothrombotic condition was identified. Only VP shunting led to a significant clinical improvement and enabled the diagnosis of metastasing breast neoplasma.

Althought CSF cytology, the gold standard of LC diagnosis, was immediately required, examination could not be performed untill VP shunt was placed. VP shunt is shown to significantly improve symptoms in patient with LC, which was the case in our patient as well [1, 3]. Only the second high-volume CSF analysis confirmed the diagnosis of LC. Positive CSF cytology is found on the initial examination in only 50% of LC patients, and in about 85% of those undergoing three high-volume (> 10 mL) lumbar punctures [1, 2]. The specificity of the test is excellent but there is a substantial frequency of false-negative results [2, 4].

There were no clear signs of meningeal pathology on MRI scan in this case. MRI of the brain and spine with a T1-weighted gadolinium-enhanced sequence, combined with fat-suppression T2-weighted scans, is recommended examination in LC, with the sensitivity reported to be up to 71% [2, 5]. However, sensitivity can depend on the quantity of gadolinium used [1, 5].

Our patient presented with several features atypical for LC. LC is the presenting symptom of malignant disease, seen in only 5–10% of the cases [5]. LC from solid tumors is usually detected in an advanced stage of systemic disease, in the setting of widespread metastases, which were absent in the presented patient [1, 2, 5]. Also, there was long symptoms duration of 6 months before the diagnosis and only 4 months after, while

median survival of treated patients with LC is known to be overall only 2–6 months [2, 5]. Nevertheless, 1-year survival rate can be as high as 25% [3]. MRI indicated IIH but not meningeal pathology, which is seen in at least 30% of cases [5].

Conclusion

Leptomeningeal carcinomatosis is one of the most serious complications occurring in solid cancer patients with rather poor prognosis. It can occasionally be the first manifestation of malignant disease. VP shunting can result in considerable clinical stabilization of the patient and can be vital in the diagnostic process. Lower sensitivity of conventional MRI and CSF examination can be diagnostic pitfalls, but in the setting of a strong clinical suspicion of LC, the clinicians should persist in repeating MRI with larger quantity of gadolinium and high-volume cytological CSF analyses.

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The list of authors should read as: M Ostojić, Tatjana S Potpara, Marija M Polovina, Mladen M Ostojić

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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

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a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

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Primeri referenci:

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

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Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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