YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2015; March Vol. 72 (No. 3): p. 211-302.

Vojnosanitetski Pregled 2015 March Vol. 72 (No. 3): p. 211-302.

YU ISSN 0042-8450 vol. 73, br. 3, 2015.

VOJNOSANITETSKI PREGLED

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

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

IZDAVAČ
Uprava za vojno zdravstvo MO Srbije

Adresa redakcije: Vojnomedicinska akademija, Institut za naučne informacije, Crnotravska 17, poštanski fah 33–55, 11040 Beograd, Srbija. Telefoni: glavni i odgovorni urednik 3609 311, glavni menadžer časopisa 3609 479, pretplata 3608 997. Faks 2669 689. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, Index Medicus (Medline), Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 1227423129552141 5. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € (u dinarskoj protivvrednosti na dan uplate) za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40 b.

YU ISSN 0042-8450 vol. 72 No. 3, 2015

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

PUBLISHER
Military Health Department, Ministry of Defence, Serbia

Editorial Office: Military Medical Academy, INI; Crnotravska 17, PO Box 33–55, 11040 Belgrade, Serbia. Phone: Editor-in-chief +381 11 3609 311; Main Journal Manager +381 11 3609 479; Fax: +381 11 2669 689; E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, Index Medicus (Medline), Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-314849-70 Ministry of Defence – Total means of payment – VMA (for the Vojnosanitetski pregled), refer to number 12274231295521415. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40 b.

CONTENTS / SADRŽAJ

EDITORIAL / UVODNIK

Silva Dobrić
The Author and the Reviewer of the Year 2014 Award by *Vojnosanitetski pregled*
Priznanje časopisa „Vojnosanitetski pregled" najplodnijem autoru i recenzentu u 2014. godini 215

ORIGINAL ARTICLES / ORIGINALNI RADOVI

Ranko Golijanin, Bojan Kujundžić, Zoran Milosavljević, Dragan R. Milovanović, Zlatibor Andjelković, Miroslav Obrenović, Radivoje Nikolić
Morphometric analysis of collagen and inflammatory cells in periodontal disease
Morfometrijska analiza kolagena i inflamatornih ćelija u periodontalnoj bolesti 219

Zoran Trifunović, Slobodan Obradović, Bela Balint, Radoje Ilić, Zoran Vukić, Marija Šišić, Jelena Kostić, Siniša Rusović, Milan Dobrić, Gordana Ostojić
Functional recovery of patients with ischemic cardiomyopathy treated with coronary artery bypass surgery and concomitant intramyocardial bone marrow mononuclear cell implantation – A long-term follow-up study
Funkcionalni oporavak bolesnika sa ishemijskom kardiomiopatijom lečenih implantacijom mononuklearnih ćelija koštane srži tokom aortokoronarne bajpas hirurgije 225

Borka Mandić, Zoran Lazić, Aleksa Marković, Bojan Mandić, Miška Mandić, Ana Djinić, Biljana Miličić
Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: A 6-week split-mouth clinical study
Uticaj postoperativne terapije laserom male snage na oseointegraciju samourezujućih implantata u bočnoj regiji gornje vilice: šestonedeljna *split-mouth* klinička studija 233

Radovan Milošević, Novak Milović, Predrag Aleksić, Miodrag Lazić, Snežana Cerović, Vladimir Bančević, Branko Košević, Predrag Marić, Aleksandar Spasić, Dejan Simić, Božidar Kovačević
Difference in recurrence frequencies of non-muscle-invasive-bladder tumors depending on optimal usage of intravesical immunotherapy of bacillus Calmette-Guérin
Razlika u učestalosti recidiviranja mišićno-neinvazivnih tumora mokraćne bešike zavisno od optimalne primene intravezikalne imunoterapije bacilom *Calmette-Guérin* 241

Vesna Martić
Concoradance of clinical and neurophysiologic diagnoses of carpal tunnel syndrome
Podudarnost kliničke i neurofizološke dijagnoze sindroma karpalnog kanala 247

Uroš Babić, Ivan Soldatović, Dejana Vuković, Milena Šantrić Milićević, Mihailo Stjepanović, Dejan Kojić, Aleksandar Argirović, Vinka Vukotić
Comparative analysis of the current payment system for hospital services in Serbia and projected payments under diagnostic-related groups system in urology
Komparativna analiza aktuelnog načina plaćanja bolničkih usluga u Srbiji i projektovanog plaćanja po sistemu dijagnostički srodnih grupa u urologiji 251

Aleksandar I Kiralj, Zlata Janjić, Jelena Nikolić, Borislav Markov, Marija Marinković
A 5-year retrospective analysis of necrotizing fasciitis – A single center experiences
Petogodišnja retrospektivna studija nekrotizujućeg fasciitisa – iskustvo jednog centra 258

GENERAL REVIEW / OPŠTI PREGLED

Dragana Daković, Ivan Mileusnić, Zoran Hajduković, Saša Čakić, Miloš Hadži-Mihajlović
Gingivitis and periodontitis in children and adolescents suffering from type 1 diabetes mellitus
Gingivitis i parodontopatija kod dece i adolescenata obolelih od dijabetesa melitusa tipa 1 265

CURRENT TOPIC / AKTUELNA TEMA

Vesna Putić, Jasmina Jović-Stošić
Intravenous fat emulsion in clinical practice: nutrient and antidote
Intravenska emulzija masti u kliničkoj praksi: nutrijent i antidot 274

CASE REPORTS / KAZUISTIKA

Maja Radić, Darka Hadnadjev
***Epidermolysis bullosa* of the esophagus – A case report**
Bulozna epidermoliza jednjaka 280

Tamara Alempijević, Ana Balović, Aleksandra Pavlović-Marković, Dino Tarabar, Miodrag Krstić, Predrag Miljić, Miloš Bjelović
Efficacy of long-acting somatostatin analogs in recurrent variceal bleeding in a patient with prehepatic portal vein thrombosis
Delotvornost leka dugotrajnog dejstva analognog somatostatina kod bolesnice sa ponovljenim varikoznim krvarenjem i trombozom prehepatične PORTNE vene 283

Dragan Vuković, Sanja Petrović, Predrag Paović
Secondary surgical management of suprachoroidal hemorrhage during *pars plana* vitrectomy
Sekundarno hirurško rešavanje suprahoroidalne hemoragije nastale u toku *pars plana* vitrektomije 287

Radoslav Romanović, Nenad Ratković, Žaklina Davičević, Radoje Ilić
Massive right atrial myxoma with dyspnea at rest in an elderly patient: A case report
Veliki miksom desne pretkomore sa dispnejom u miru kod bolesnice u starijem životnom dobu 291

Milena B. Ilić, Dalibor V. Jovanović, Miloš Z. Milosavljević, Vesna Stanković, Gordana Djordjević, Zoran Protrka, Jasmina Nedović, Slobodanka Lj. Mitrović
Hypercalcemic tipe of small cell carcinoma of the ovary
Hiperkalcemični tip sitnoćelisjkog karcinoma jajnika 295

CORRIGENDA / ISPRAVKA 299

INSTRUCTIONS TO THE AUTHORS / UPUTSTVO AUTORIMA 300

Theodoor Rombouts (1597–1637): The tooth puller (extractor), oil on canvas, 235.5 cm × 156.5 cm; Museum of Fine Arts, Ghent, Netherlands.

The Publisher and the Editorial Board of the *Vojnosanitetski pregled* (VSP) already 10 years successively, on March 2, as a part of marking the Day of the Military Medical Academy in Belgrade, in which the Editorial Office of the Journal is located, assign acknowledgment to the most productive author and reviewer in the previous year. Laureates of the VSP for 2014, are for the first time experts in the field of dental medicine, which proves the fact that more and more articles from this field have recently been published in our Journal (see Editorial, pages 215–8).

Theodoor Rombouts (1597–1637): Čovek koji vadi zub, ulje na platnu, 235,5 cm × 156,5 cm; Muzej lepih umetnosti, Gent, Holandija.

Izdavač i Uredništvo časopisa „Vojnosanitetski pregled“ (VSP), već 10 godinu zaredom, 2. marta, u sklopu obeležavanja Dana Vojnomedicinske akademije u Beogradu, u kojoj je smeštena Redakcija VSP, dodeljuju priznanje najplodnijem autoru i recenzentu časopisa za prethodnu godinu. Laureati časopisa za 2014. godinu, po prvi put su stručnjaci iz oblasti stomatologije, što samo potvrđuje činjenicu da se poslednjih godina na stranicama našeg časopisa objavljuje sve više radova iz ove oblasti (vidi Uvodnik, str. 215–8).

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

The Author and the Reviewer of the Year 2014 Award by *Vojnosanitetski pregled*

Priznanje časopisa „Vojnosanitetski pregled“ najplodnijem autoru i recenzentu u 2014. godini

Silva Dobrić

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

The *Vojnosanitetski pregled* (VSP) was initiated as the official professional and scientific journal of medical doctors, stomatologists and pharmacists of the Army of Serbia, so it has been publishing papers on medicine, dental medicine and pharmacy from the very beginning. Over the years, according to the field of research the highest number of published papers come from clinical medicine, then there come those on the problems of dental medicine, while the smallest number of papers come from pharmacy. It is no surprise then that from the start of awarding the prize the Author of the Year by *Vojnosanitetski pregled* (in 1996 for 1995) up to the last year, the most numerous prizes were awarded to the medical doctors (15), two times to pharmacists, and to molecular biologists (2). It coincides with the fact that since 2011, when the prize the Reviewer of the Year was established, this title has been received by medical doctors. This is the first time, however, that this year both prizes rush to the hands of the experts on dental medicine. The fact characteristic for what we have been noticed for the last few years, is the increasing influx of papers on dental medicine.

The Author of the Year 2014 is Asisst. Prof. Tatjana Čutović, DMD, PhD, from the Clinic of Dental Medicine, Department of Orthopedics of the Jaw, Military Medical Academy, Belgrade. She is Assistant Professor at the Medical Faculty of the Military Medical Academy, University of Defence, in Belgrade. The Reviewer of the Year 2014 is a retired Professor of the Faculty of Dental Medicine, University of Belgrade, Dr. Ljubomir Todorović, DMD, PhD.

The applicable diplomas will be awarded to them on March 2, on the occasion of marking the Day of the Military Medical Academy, Belgrade, where the Editorial Office of the VSP is situated.

As mentioned many times before, the criteria for the prize the Author of the Year are based on the number of published papers within the year in question, the category of the papers, as well as the order of the authors, and the great number of points is received for the category of original papers and being the first among the authors.

Budući da je Vojnosanitetski pregled (VSP) osnovan kao zvanični stručni i naučni časopis lekara, stomatologa i farmaceuta Vojske Srbije, u njemu su od samog početka objavljivani radovi iz oblasti medicine, stomatologije i farmacije. Posmatrano po oblastima, već godinama najveći broj objavljenih članaka pripada oblasti kliničke medicine, zatim slede članci koji se odnose na stomatološku problematiku, dok najmanje članaka dolazi od pripadnika farmaceutske struke. Zbog toga ne treba da čudi što je, od početka dodele priznanja za autora godine VSP (1996. godine za 1995) do zaključno sa prošlom godinom, najveći broj njih pripao lekarima (15 puta), u dva navrata farmaceutima, i isto toliko molekularnim biolozima. U skladu sa ovim je i podatak da su, od 2011. godine kada je ustanovljeno i priznanje za Recenzenta godine VSP, tu titulu poneli pripadnici lekarske profesije. Ove godine, međutim, po prvi put priznanje i za Autora i za Recenzenta godine VSP ide u ruke stručnjacima iz oblasti stomatologije. Ovaj podatak , na neki način, odslikava ono što uočavamo već par godina unazad, a to je sve veći priliv radova iz oblasti stomatologije.

Autor godine za 2014. jeste dr sci. stom. Tatjana Čutović iz Klinike za stomatologiju (Odeljenje za ortopediju vilice) Vojnomedicinske akademije (VMA) u Beogradu i docent Medicinskog fakulteta VMA Univerziteta odbrane u Beogradu, a Recenzent godine VSP za 2014, dr sci. stom. Ljubomir Todorović, redovni profesor Stomatološkog fakulteta Univerziteta u Beogradu u penziji. Njima će 2. marta, u sklopu obeležavanja Dana Vojnomedicinske akademije u Beogradu, u kojoj je smeštena Redakcija VSP, biti uručene odgovarajuće diplome.

Kao što je već više puta spominjano, kriterijumi za izbor Autora godine baziraju se na broju radova objavljenih u godini za koju se priznanje dodeljuje, kategoriji kojoj rad pripada i redosledu među autorima, pri čemu najveći broj bodova donosi kategorija Originalni članci i prvo mesto među autorima. Kada je posredi izbor za Recenzenta godina, onda se u obzir uzima broj urađenih recenzija, poštovanje datog roka za

Correspondence to: Silva Dobrić, Institute for Scientific Information, Military Medical Academy, Crnotravska 17, 000 Belgrade, Serbia. Phone: +381 11 3609 311. E-mail: vsp@vma.mod.gov.rs

Regarding the Reviewer of the Year, the number of peer reviewed papers is what matters, meeting time limits, and above all critical recognizing of a paper.

Assistant Professor Tatjana Čutović, DMD, PhD, published three original papers in the Vojnosanitetski pregled last year, in the two of them being the first author, and the coauthor in the third one (Table 1). It has to be pointed out that Assistant Professor Tatjana Čutović has been one of the most fruitful authors of our Journal for a longer time, thus this prize is quite a logical result of that.

For years Professor Ljubomir Todorović, DMD, PhD, is, also, one of the most involved peer reviewers in our Journal. From 2002 on he has been the member of the Editorial Board of the VSP, as well, contributing significantly by his wide experience to increasing the quality and reputation and international affirmation of the Journal. That makes me particularly pleased with the prize awarded just to him.

njihovo dostavljanje, kao i poštovanje opšte prihvaćenih principa za kritičko sagledavanje naučnog članka.

Doc. dr Tatjani Čutović prošle godine na stranicama VSP objavljena su tri rada iz kategorije Originalni članci. U dva od njih bila je prvi autor, a u trećem koautor (Tabela 1). Treba istaći da je doc. dr Tatjana Čutović već duže vreme jedan od najplodnijih autora našeg časopisa, pa ne čudi što je za 2014. godinu njena publicistička produktivnost nagrađena ovim priznanjem. Takođe, prof. dr Ljubomir Todorović, već godinama jedan je od naših najangažovanijih recenzenata. Od 2002. godine on je redovni član Uredništva VSP i svojim bogatim iskustvom, i kao urednik i kao recenzent, značajno je doprineo podizanju kvaliteta i ugleda samog časopisa, kao i njegovoj međunarodnoj afirmaciji. Zbog toga mi je posebno zadovoljstvo što je priznanje Recenzent godine ovaj put pripalo, upravo njemu.

Table 1

Articles of Asisst. Prof. Tatjana Čutović published in the *Vojnosanitetski pregled* in 2014 / / Članci doc. dr Tatjane Čutović objavljenih u Vojnosanitetskom pregledu u 2014. godini

No./ Br.	Article category/ Kategorija članka	Authors and title of article/ Autori i naslov članka
1	Original article/ Originalni članak	*Tatjana Čutović, Nebojša Jović, Ljiljana Stojanović, Julija Radojičić, Irena Mladenović, Stevo Matijević, Ružica Kozomara.* A cephalometric analysis of the cranial base and frontal part of the face in patients with mandibular peognathism. Vojnosanit Pregl 2014; 72(6): 534–41.
2	Original article/ Originalni članak	*Tatjana Čutović, Nebojša Jović, Ružica Kozomara, Julija Radojičić, Mirjana Janošević, Irena Mladenović, Stevo Matijević.* Cephalometric analysis of the middle part of the face in patients with mandibular prognathism. Vojnosanit Pregl 2014; 71(11): 1026–33.
3	Original article/ Originalni članak	*Julija Radojičić, Tatjana Tanić, Nebojša Jović, Tatjana Čutović, Konstatinos Papadopoulos.* Presurgical orthodontic treatment of patients with complete bilateral cleft lip and palate. Vojnosanit Pregl 2014; 71(7): 693–9.

What follows are the concise biographies of the 2014 laureates by the VSP. It is my true pleasure to congratulate to them for these recognitions in the name of the Publisher, members of the Editorial Board and Editorial Office and of me personally, hoping that many similar occasions will come.

U nastavku će, kao i do sada, biti date kratke biografije laureata VSP za 2014. godinu. Ja koristim priliku da im u ime Izdavača, Uredništva i Readakcije VSP, kao i u svoje lično ime, čestitam na ovim priznanjima, sa željom za još mnogo sličnih uspeha u narednim godinama.

The short biography of the Author of the Year 2014 by the VSP – Asisst. Prof. Tatjana Čutović, DMD, PhD.

Dr. Tatjana Čutović (born September 28, 1964 in the town of Čačak) graduated from the Faculty of Dental Medicine, University of Belgrade in 1990, where se, also, passed specialization in the orthopedics of jaws in 1995.

Kratka biografija Autora godine Vojnosanitetskog pregleda za 2014. godinu.

Dr Tatjana Čutović rođena 28.09.1964. godine u Čačku. Diplomirala je na Stomatološkom fakultetu Univerziteta u Beogradu 1990. godine, gde je završila i specijalizaciju iz ortopedije vilica 1995. godine

Assist. Prof. Tatjana Čutović, DMD, PhD – The Author of the Year 2014 by the *Vojnosanitetski pregled* / Doc. dr sc. stom. Tatjana Čutović – Autor godine Vojnosanitetskog pregleda za 2014.

She received her M.A. degree in 2007 and PhD degree in 2012 at the Faculty of Medicine, University of Prishtina/Kosovska Mitrovica (Title od dissertation: The significance of cranio-dento-facial characteristics of various types of mandibular prognathism).

She has been employed in the Military Medical Academy, Belgrade ever since 1996, at the Clinic for Dental Medicine (Department for the Orthopedics of Jaws). She is an Assistant Professor in Oral Medicine at the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade.

Her scientific and professional interests are: orthodontia in adults and in those with advanced periodontal disease, studies of skeletal abnormalities class III, especially mandibular prognathism, its etiology, diagnostics and the aspects of multidisciplinary therapy.

Together with colleagues from the Clinic for Maxillofacial Surgery, Military Medical Academy in Belgrade, Dr. Čutović got many years of experience with patients presented with serious acquired dentofacial deformities, where she is a steady member of the Consultation Team for the orthopedics of jaws.

Regarding advanced training, Dr. Čutović passed 18 international courses. She is the author and coauthor of numerous scholarly papers on the orthopedics of jaws (out of which 12 were published in the journals covered by Science Citation Index Expanded database), as well as two monographs.

The short Biography of the Reviewer of the Year 2014 by the VSP – Prof. Ljubomir Todorović, DMD, PhD.

Prof. Ljubomir Todorović, DMD, PhD (born 1947, Belgrade) graduated from the Faculty of Dental Medicine, University of Belgrade, in 1971 where he also passed specialization exam in 1979, received M.A. degree in 1975 (Master thesis: Clinical and laboratory assessment of the interaction of amitriptyline with some local anesthetic solutions), and PhD degree in 1985 (Dissertation: Changes in reactivity of tissue following supervoltage radiotherapy for malignant tumors of the mouth cavity and the neck). Specific fields of his work are oral surgery and dental anesthesiology.

Na Medicinskom fakultetu Univerziteta u Prištini, sa sedištem u Kosovskoj Mitrovici odbranila je magistarski rad 2007. godine, a doktorat 2012. godine (Naziv doktorskog rada: Značaj kranio-dento-facijalnih karakteristika različitih oblika mandibularnog prognatizma).

Od 1996. godine zaposlena je u VMA, na Klinici za stomatologiju (Odeljenje za ortopediju vilica). Docent je iz uže naučne oblasti Oralna medicina na Medicinskom fakultetu VMA Univerziteta odbrane u Beogradu.

Uža oblast njenog stručnog i naučnog rada su: ortodoncija odraslih pacijenata i pacijenata sa uznapredovalom parodontalnom bolešću i proučavanje etiologije, dijagnostike i aspekata multidisciplinarne terapije skeletnih nepravilnosti III klase, posebno mandibularnog prognatizma.

U saradnji sa kolegama iz Klinike za maksilofacijalnu hirurgiju VMA, već duži niz godina učestvuje u lečenju pacijenta sa teškim urođenim i stečenim dento-facijalnim deformitetima, a kao iskusan ekspert u toj oblasti stalni je član konzilijuma za deformitete lica i vilica.

U okviru profesionalnog usavršavanja završila je 18 međunarodnih kurseva. Autor je i koautor brojnih naučnostručnih radova iz oblasti ortopedije vilica, od čega 12 u časopisima sa SCI listi, kao i dve monografije.

Kratka biografija Recenzenta godina Vojnosanitetskog pregleda za 2014. godinu

Prof. dr Ljubomir Todorović rođen je u Beogradu 1947. godine. Diplomirao na Stomatološkom fakultetu Univerziteta u Beogradu 1971. Na istom fakultetu 1979. godine položio je specijalistički ispit iz oralne hirurgije, odbranio magistarski rad (Kliničko i laboratorijsko ispitivanje interakcije amitriptilina i pojedinih lokalnih anestetičkih rastvora) 1975. godina, i doktorski rad (Promene reaktivnosti tkiva posle supervoltažne radioterapije malignih tumora usne šupljine i vrata) 1985. Uža oblast njegovog stručnog i naučnog rada jeste oralna hirurgija i stomatološka anesteziologija.

Prof. Ljubomir Todorović, DMD, PhD – the Reviewer of the year 2014 by the *Vojnosanitetski pregled* / Prof. dr sc. stom. Ljubomir Todorović – Recenzent godine Vojnosanitetskog pregleda za 2014.

Prof. Todorović has been the Professor at the Faculty of Dental Medicine, Belgrade since 1995 (retired on October 1, 2012). He was the Head of the Clinic for Oral Surgery in Belgrade in a period 1996-2000, the Vice Dean of the Faculty of Dental Medicine, Belgrade from 2006 to 2012 (two mandates). In 1985 he passed advanced training at the Eastman Dental Institute, University College Hospital, London, and in 1986 at John Radcliffe Hospital, Oxford, Great Britain.

Prof. Todorović is a Professor of Oral Surgery and Dental Anesthesiology at undergraduate studies, as well as of the Methodology of Research and Publishing in Biomedical Sciences at PhD and Academic Specialization studies. Also, used to be a Visiting Professor of Oral Surgery at the Faculty of Dental Medicine, Benghazi, Libya, the town of Foča (Bosnia and Herzegovina) and Podgorica (Montenegro).

Within his long career, Prof. Todorović used to be a Mentor, a Chairman to the Commission for the Specialization Exams of Oral Surgery, a President and a Member of numerous commissions for M.A. and PhD exams. He is, also, the author of six textbooks, one atlas, and two chapters in two scientific monographs. Prof. Todorović published 83 papers (43 in Serbian journals, and 40 in foreign ones), took part in reports and inviting lectures at home (92), and abroad (39), and was cited totally 88 times.

Prof. Todorović has been the member of the Serbian Medical Society since 1972, the Secretary of the Section of Dental Medicine (1981–1984), the President of the Section of Oral Surgery (1995–1999), and the Associate Member of the Academy of Medical Sciences of the Serbian Medical Society (since 2000). He received numerous recognitions in the Serbian Medical Society.

Also, Prof. Todorović is the member of the International Association of Oral and Maxillofacial Surgeons, British Association of Oral and Maxillofacial Surgeons, Groupement International pour la Recherche Scientifique en Stomatologie et Odontologie, and Balkan Stomatological Society; also used to be Deputy Editor-in-Chief of Dental Medicine Journal of Serbia (1986-1993) and the Editor-in-Chief of this Journal (1994–2001) and the Oral Surgery Bilten of Serbia (1998–2002); he is also Editor-in- hief of the Balkan Journal of Dental Medicine starting from its foundation in 1997. Since 2002 Prof. Todorović has been an ordinary member of the Editorial Board of the Vojnosanitetski Pregled.

Za redovnog profesora Stomatološkog fakulteta Univerziteta u Beogradu biran je 1995. (penzionisan je 01.10.2012). Bio je nastavnik iz predmeta Oralna hirurgija i Stomatološka anestezija na dodiplomskim studijama, a na doktorskim i akademskim specijalističkim studijama iz predmeta Metodologija naučno-istraživačkog rada i Publikovanje u biomedicinskim naukama. Kao gostujući profesor držao je nastavu iz oralne hirurgije na Stomatološkim fakultetima u Bengaziju, Libija, Foči (Bosna i Hercegovina) i Podgorici (Crna Gora).

Upravnik Klinike za oralnu hirurgiju u Beogradu bio je od 1996–2000, a prodekan Stomatološkog fakulteta u dva mandata, od 2006. do 2012. godine. Usavršavao se na Eastman Dental Institute *University College Hospital* u Londonu (1985) i *John Radcliffe Hospital* u Oksfordu (1986) u Velikoj Britaniji.

Tokom dugogodišnje karijere više puta je bio mentor studentskih i specijalističkih radova, kao i magistarskih i doktorskih teza. Bio je predsednik Komisije za specijalističke ispite za oblast oralna hirurgija, predsednik ili član većeg broja komisija za odbranu magistarskih i doktorskih teza. Autor je 6 udžbenika, 1 atlasa i 2 poglavlja u dve naučne monografije. Objavio je 83 rada (43 u domaćim časopisima, 40 u inostranim), a učestvovao je saopštenjima i pozivnim predavanjima na brojnim domaćim (92) i inostranim (39) skupovima. Njegovi radovi citirani su 88 puta.

Aktivan je član Srpskog lekarskog društva (SLD) od 1972. godine, a od 2000. godine i član Akademije medicinskih nauka SLD. Bio je sekretar Stomatološke sekcije SLD (1981–1984) i predsednik Sekcije za oralnu hirurgiju SLD (1995–1999). Za svoj rad u Društvu dobio je veći broj priznanja.

Takođe, član je *International Association of Oral and Maxillofacial Surgeon, British Association of Oral and Maxillofacial Surgeons, Groupement International pour la Recherche Scientifique en Stomatologie et Odontologie' i Balkan Stomatological Society*. Bio je zamenik glavnog i odgovornog urednika Stomatološkog glasnika Srbije (1986-1993), a njegov glavni i odgovorni urednik od 1994. do 2001. godine. Takođe, uređivao je i Bilten oralnih hirurga Srbije od 1998. do 2002. godine. Glavni je i odgovorni urednik *Balkan Journal of Stomatology* (sada *Balkan Journal of Dental Medicine*) od njegovog osnivanja – 1997. godine. Ovu funkciju vrši punih 18 godina i dalje. Od 2002. godine stalni je član Uređivačkog odbora Vojnosanitetskog pregleda.

ORIGINAL ARTICLES

UDC: 616.311.2-098.8
DOI: 10.2298/VSP130627076G

Morphometric analysis of collagen and inflammatory cells in periodontal disease

Morfometrijska analiza kolagena i inflamatornih ćelija u periodontalnoj bolesti

Ranko Golijanin*†, Bojan Kujundžić†, Zoran Milosavljević‡, Dragan R. Milovanović§††, Zlatibor Andjelković‖, Miroslav Obrenović¶, Radivoje Nikolić††**

***Department of Dentistry, ‡Department of Histology and Embriology, §Department of Pharmacology and Toxicology, **Department of Surgery, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; †Faculty of Dental Medicine, ¶Faculty of Medicine, University of East Sarajevo, Foča, Republic of Srpska, Bosnia and Herzegovina; ††Clinical Center "Kragujevac", Kragujevac, Serbia**

Abstract

Background/Aim. Periodontal disease affects gingival tissue and supporting apparatus of the teeth leading to its decay. The aim of this study was to highlight and precisely determine histological changes in the gum tissue. **Methods.** Gingival biopsy samples from 53 healthy and parodontopathy-affected patients were used. Clinical staging of the disease was performed. Tissue specimens were fixed and routinely processed. Sections, 5 μm thin, were stained with hematoxylin and eosin, histochemical Van-Gieson for the collagen content, Spicer method for mast-cells and immunochemical method with anti-CD68 and anti-CD38 for the labelling of the macrophages and plasma-cells. Morphometric analysis was performed by a M42 test system. **Results.** While the disease advanced, collagen and fibroblast volume density decreased almost twice in the severe cases compared to the control ones, but a significant variation was observed within the investigated groups. The mast-cell number increased nearly two times, while the macrophage content was up to three times higher in severe parodontopathy than in healthy gingival tissue. However, the relative proportion of these cells stayed around 6% in all cases. Plasma-cells had the most prominent increase in the number (over 8 times) compared to the control, but again, a variation within investigated groups was very high. **Conclusion.** Gingival tissue destruction caused by inflammatory process leads to significant changes in collagen density and population of resident connective tissue cells. Although inflammatory cells dominated with the disease advancing, a high variation within the same investigated groups suggests fluctuation of the pathological process.

Key words:
peridontal diseases; gingiva; histological techniques; collagen; macrophages; plasma cells.

Apstrakt

Uvod/Cilj. Parodontopatija utiče na tkivo desni i potpornog aparata zuba i dovodi do njegovog propadanja. Cilj ovog istraživanja bio je da se istaknu i precizno odrede histološke promene u tkivu desni. **Metode.** Korišćeni su uzorci biopsija gingiva kod 53 zdrave osobe i osoba sa parodontopatijom. Izvršena je klinička gradacija oboljenja. Uzorci tkiva su fiksirani i rutinski obrađeni. Preparati debljine 5 μm bojeni su hematoksilin-eozin metodom, histohemijski po Van-Gizonu za sadržaj kolagena, Špicerovom metodom za mastocite i imunohemijski sa anti-CD68 i anti-CD38 za obeležavanje makrofaga i plazmocita. Morfometrijska analiza je izvršena upotrebom M42 test sistema. **Rezultati.** Dok je bolest napredovala, volumenska gustina kolagena i broj fibroblasta u teškim slučajevima smanjili su se skoro dva puta u odnosu na kontrolu, ali su uočene značajne razlike u ispitivanim grupama. Broj mastocita povećao se skoro dva puta, dok je sadržaj makrofaga bio i do tri puta veći u teškoj parodontopatiji u odnosu na zdravu gingivu. Međutim, relativni udeo tih ćelija ostao je oko 6% u svim slučajevima. Porast broja plazma ćelija bio je najizrazitiji (preko 8 puta) u odnosu na kontrolu, ali ponovo, varijacije u okviru ispitivanih grupa bile su veoma visoke. **Zaključak.** Destrukcija gingivalnog tkiva izazvana zapaljenskim procesom dovodi do značajnih promena u gustini kolagena i broju ćelija vezivnog tkiva. Iako inflamatorne ćelije dominiraju sa napredovanjem oboljenja, velike varijacije u okviru istih ispitivanih grupa sugerišu promenljivost patološkog procesa.

Ključne reči:
periodontalne bolesti; gingiva; histološke tehnike; kolagen; makrofagi; plazma ćelije.

Correspondence to: Ranko Golijanin, Department of Dentistry, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Zmaj Jovina 32, 34 000 Kragujevac, Serbia. Phone: +381 34 366 530. E-mail: direktorzstomkg@open.telekom.rs

Introduction

Periodontal disease represents a significant health problem today and many morphological studies performed so far have yield a lot of evidence about general histological changes within gingival tissue. During the decades of the research a chain of principal pathophysiological events in the disease have been revealed which had, in essence, progressive nature. Both the tissue arrangement and the cellular composition display particular pattern during evolution of periodontal disease. In early stages, gingival inflammation predominates due to activation of involved cells and secretion of pro-inflammatory cytokines in contact with bacterial products of gingival plaque. In this phase, monocytes, macrophages and some other cells (including fibroblasts) play crucial role [1]. However, in chronic gingival lesions the cellular pattern is changed with predomination of lymphocytes, primarily of plasma-cell type [2, 3]. All these processes result in the final event, a loss of collagen, which ultimately causes the loss of gingival tissue and thoughtlessness [4, 5]. Although we know much about principal mechanism underlying the initiation and the development of gingival destruction, some issues concerning pathogenesis of periodontal disease are less investigated. The exact distribution of collagen fibers within different anatomical sites of periodontal tissue remains unresolved for fine details. Furthermore, few reports are available in the literature on *in situ* quantification of infiltrating inflammatory cells [6–8]. The role of some inflammatory cells such as macrophages, mast-cells as well as plasma-cells is investigated, but the results are somewhat controversial [9, 10]. Therefore the aim of our study was to investigate the changes in collagen distribution and quantity, the connection between volume density of inflammatory cells and the clinical stage of the periodontal disease as well as the distribution and quantification of the less investigated types of inflammatory cells – mast cells.

Methods

In the study gingival biopsy samples from 53 patients aged 14–60 years were used. Ethical approval for the research protocol was issued by the Institutional Ethics Committee and the informed consent from the study participants were obtained. The first act in this study was clinical examination performed in order to determine the condition of the periodontal apparatus. Community periodontal index of treatment needs [11], Muhlemann-Sulcus bleeding index [12] as well as assessment of the periodontal pocket depth were used to classify the patients. According to the periodontal disease classification [13] the patients were divided into four groups: the control group (12 healthy donors), the group 1 (10 patients with gingivitis), the group 2 (14 patients with moderate periodontal disease) and the group 3 (17 patients with severe periodontal disease). All gingival tissue samples were fixed in 10% buffered formalin for 48 hours, routinely processed and embedded in paraffin. Sections 5 μm thin were stained with classic hematoxylin/eosin for verification of the pathological changes, Van-Gieson for highlight of the collagen fibers and histochemical Spicer method for identification of the mast cells. Routine immunohistochemical staining was performed with anti-CD68 (Sigma-Aldrich, USA) and anti-CD38 (Dako, Denmark) antibodies for visualization of macrophages and plasma cells respectively. Quantification of collagen fibers and morphometric analysis of the labeled cells was performed using the M42 test system calibrated for proper magnification. All the values were calculated *per* unit area (1 mm^2).

The results were presented in Tables and Figures as mean ± standard deviation. Statistical analysis was performed with SPSS software. Estimation of statistical significance between the mean values was performed by independent Student's *t*-test and Mann-Whitney U-test. The level of significance of statistical differences was set at $p \leq 0.05$ with the double-sided approach.

Results

According to the design of the study, our results comprised of histological examination, characterization of collagen and morphometric analysis of fibroblasts, mast-cells, macrophages and plasma cells.

Histological examination

Microscopic examination of the human gingiva in the control group showed a well-known structure of mucous membrane. Stratified epithelium was subdivided into 3 sections: oral, sulcular and junctional. Underlying *lamina propria* showed thin and short collagen fibers in the papillary layer and thick, dense and long fibers in the reticular layer. In the group 1 only junctional epithelium showed signs of lymphocyte and polymorphonuclear leukocyte infiltration. Scarce inflammatory fields located just below this epithelium were present in, sometimes edematous, *lamina propria* with rare fragmentation and lysis of the collagen fibers. Histological characteristics of moderate and severe periodontal disease were quite different from the gingivitis cases. The increased number and extent of inflammatory lesions, lysis of collagen fibers as well as the loss of fibroblast were verified. In rare cases it was almost impossible to discern the moderate and severe form of the disease because the extent of the pathological changes was inconsistent. Nevertheless, severe forms of the disease showed confluent fields of inflammation sometimes located in the deeper gingival tissue and sometimes much nearer to the papillary layer or extended to either oral or junctional gingiva with the significant degradation of the collagen fibers. In two cases of severe illness focal destruction of the basal *lamina* with infiltration of immune cells into the gingival epithelium occurred. Fields of irregular and fairly regular re-deposition of collagen fibers by the fibroblasts were identified in two cases of severe periodontal disease which suggest reparation process. All these findings showed cyclic nature of periodontal disease, with the phases of deterioration and the phases of reparation of the gingival tissue.

Collagen fibers analysis

In the control group collagen fibers did not show any visible changes. In the gingivitis group the findings were similar, but perivascular spaces were deprived of collagen content. Slight tissue edema and the scarce regions of thinned collagen

fibers were the only difference comparing with the control group. In the groups 3 and 4 a large variation of collagen content (high standard deviations) was observed and this was not so much related to the clinical stage, but mostly to the phase of the disease. It was obvious that the quantity and quality of collagen fibers were inversely related to the extent of the inflammatory lesions. In the severe cases, inside the confluent inflammatory fields, collagen fibers were absent and located only on its rim in the form of thin, dense bundles preventing the penetration of inflammatory cells into the healthy part of the gingiva (Figure 1A). Quantitative analysis (Table 1) showed a significant decrease of the collagen volume density especially in the moderate and severe cases compared to the control and the gingivitis group. Large variations of collagen content were observed in the two parodontopathy groups.

Distribution, number and density of fibrocytes/fibroblasts

In our study the staining method did not allow us to discern these two cell types, so they were observed as a unique cell population. In healthy gingiva fibrocytes/fibroblasts they were distributed primarily within the papillary layer while poorly present in the reticular layer. In the gingivitis group these cells can be observed mainly in the regions with preserved collagen fibers, while they were absent from the inflammatory fields. Stereological analysis of the fibrocytes/fibroblasts showed a gradual loss of their number and a significant decrease in the relative proportion of these cells in a pathologically altered gingiva (Table 1). The difference in the absolute number of fibrocytes/fibroblasts in the examined groups was not significant because of a high variation, but their differences in relative proportion (the number of fibrocytes/fibroblasts per overall number of cells) were highly significant in the parodontopathy groups compared to the control as well as gingivitis group.

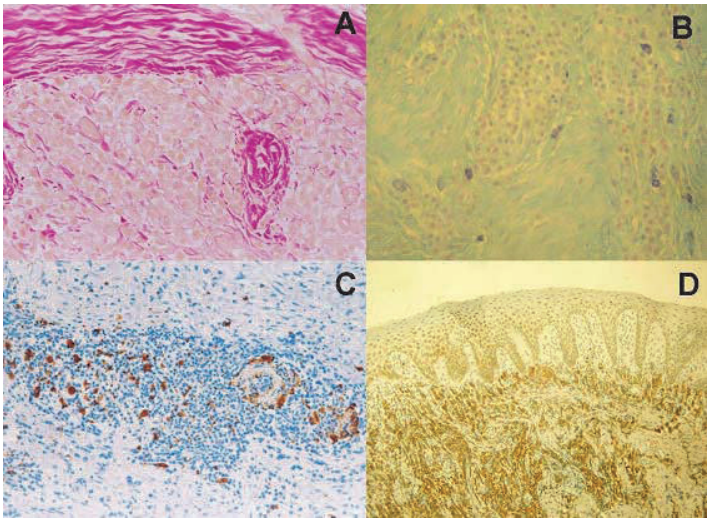

Fig. 1 – A) Bundles of collagen fibers surrounding the inflammatory focus. Inside the focus fibers are present in traces, (Van-Gieson, ×400); B) Mast cells on the periphery of the inflammatory foci (Spicer, ×400); C) Macrophages inside the inflammatory foci and around the field of collagen fibers degradation (Anti-CD68, ×200); D) Plasma-cells delineating the healthy papillary and the affected reticular layer of *lamina propria* (Anti-CD38, ×400).

Table 1

Morphometric data of collagen and inflammatory cells volume density

Variable	Healthy gingiva (n = 12)	Gingivitis (n = 10)	Moderate parodontopathy (n = 14)	Severe parodonthopathy (n = 17)
Collagen volume density	57.33 ± 5.27	46.48 ± 9.34[1]	32.72 ± 14.59[1, 2]	26.93 ± 13.71[1, 2]
Fibrocytes/Fibroblasts (n/mm^2)	539.11 ± 171.34	396.77 ± 192.23[1]	367.08 ± 264.57[1]	303.32 ± 236.06[1]
percent	55.22 ± 6.25	23.51 ± 9.18[1]	15.49 ± 12.74[1, 2]	13.25 ± 11.42[1, 2]
Mast cells (n/mm^2)	62.56 ± 21.87	94.56 ± 27.32[1, 2]	141.41 ± 31.14[1, 2]	134.13 ± 29.93[1, 2]
percent	6.35 ± 2.25	5.56 ± 1.76	5.97 ± 2.07	5.85 ± 2.37
Macrophages (n/mm^2)	58.74 ± 18.56	116.55 ± 54.78[1]	147.73 ± 81.14[1]	157.41 ± 77.56[1]
percent	6.02 ± 0.83	6.90 ± 2.28	6.23 ± 2.48	6.86 ± 2.85
Plasma cells (n/mm^2)	77.63 ± 61.11	356.42 ± 224.84[1]	788.53 ± 861.90[1, 2]	731.34 ± 651.74[1, 2]
percent	12.67 ± 4.52	21.09 ± 14.04[1]	33.26 ± 15.49[1]	31.93 ± 11.75[1]
All cells (n/mm^2)	976.32 ± 284.45	1688.46.46 ± 602.41[1]	2359.43 ± 1404.57[1]	2289.73 ± 1345.74[1]
percent	27.52 ± 1.16	20.71 ± 7.32[1]	18.73 ± 9.47[1]	16.96 ± 8.42[1]

The values are given as mean ± standard deviation of the number or percent of the cells *per* mm^2 of gingival tissue; 1 – $p < 0.05$ *vs* healthy control; 2 – $p < 0.05$ *vs* gingivitis *vs* healthy gingiva

Distribution, number and density of mast-cells

Mast-cells of healthy gingiva were mainly located in the perivascular spaces, almost always as a single cell. In gingivitis, the number of mast cells increased particularly within reticular layer of the *lamina propria* and around the inflammatory focuses. In the groups 3 and 4, the total num-

ber of mast cells was further increased and their main location was on the edge of the inflammatory fields (Figure 1B). Stereological and statistical analysis (Table 1) showed that the number of mast cells was significantly higher in the gingivitis group compared to healthy gingiva and also in the parodontopathy groups compared to the gingivitis group. On the other hand, their relative proportion was not changed and these cells comprised ~6% of total cells in all the examined groups (Table 1). Additionally, across all the four investigated groups mast cells remained with the similar granular content.

Distribution, number and density of macrophages

Macrophages are scattered throughout the healthy gingival *lamina propria* as rare, isolated cells with dominant location below the sulcular and junctional epithelium. In gingivitis tissue specimens they were positioned mainly between the papillary and reticular layer of *lamina propria* and they were not numerous inside the fields of inflammation. In parodontopathy cases, macrophages were always present inside the inflammatory foci, but their number was greater around the collagen fibers facilitating their degradation (Figure 1C). Only in severe cases they can be verified in the stratified epithelium, also. Stereological examination showed that, similarly to mast cells, the absolute number of macrophages was increasing with advancing of the pathological process, but relative contribution to the total cell population remained the same, around 6% (Table 1).

Distribution, number and density of plasma-cells

In healthy gingiva, plasma cells were rare, primarily distributed in small groups, around the vasculature, within reticular layer of the *lamina propria*. In gingivitis, plasma cells density was related to inflammatory foci if they were the dominant cell population. In moderate and severe cases of periodontal disease, plasma cells almost completely expel other cells within inflammed focuses, but were absent from the spaces between the foci. The papillary region and tissue near and around collagen fibers contained very rare, scattered plasma cells. When the severe inflammatory process was located deeper in the gingival lamina propria, plasma cells seemed to demarcate healthy papillary and affected reticular layer (Figure 1D). Morphometric analysis showed that the number of these cells was increased in both, absolute value and relative contribution (Table 1) in parodontopathy cases and the difference was statistically significant compared to the gingivitis and the control group. The relative increase in the plasma cell number was, in one part, in correlation with the decrease in the fibrocytes/fibroblasts number. In the other part, their relative contribution in the overall cell population was the consequence of the overgrowth of the other two types of inflammatory cells (mast-cells, macrophages). Hence, plasma-cells represented the dominant cell population of inflammatory foci in the periodontal disease.

Discussion

Parodontopathy represents a significant health problem for patients causing pain, discomfort and gums decay. Changes in gingival tissue include inflammation, collagen degradation and loss of protective function. In our study, with the progression of the disease, gingival collagen volume density decreases, with the loss of collagen fibers within inflammatory foci and its increase around the infiltrates. Our finding are similar to previous reports [4, 8, 14] and taking into account data from all studies, the mean collagen volume density in the healthy gingiva was in the range from 54% to 63%, in gingivitis cases from 38% to 46%, and in the specimens from severe parodontopathy from 25% to 27%. However, in our samples much greater variability in collagen content in the same study groups was found, approximately about 2–3 times (expressed as standard deviation from the mean) than in previous reports, being the highest in severe cases of the disease. Human and animal studies confirmed that, during the evolution of periodontal disease, alteration in collagen types occurred including collagen type I, III, IV, V and VI [14, 15]. Dynamism of these alterations in qualitative and quantitative terms could cause heterogeneity of exact collagen content in different phases of the disease. Further studies are required to better characterize the factors causing the novel observation in our study, e.g. progressive variability of collagen changes in different stages of parodontopathy.

Population of fibroblasts in our study is decreasing in both, absolute and relative numbers (relating to disease stages) and it is associated with the collagen volume density loss and the observed high variability of collagen content. Surprisingly, a few published papers dealt specifically with comparative morphometric analysis (quantification *in situ*) of these cells in the healthy gingiva and different stages of periodontal disease in humans. An old study referred equal proportion of fibroblast to other cell population in healthy gingival connective tissue [16] and our finding confirmed that report. Since then, researchers focused more on changes in fibroblast synthetic function [17, 18] and their characterization in a particular patient population such as diabetic patient with periodontal disease [19]. Seymour and Greenspan [3] described the frequent association of plasma-cells with fibroblasts suggesting that they were important in the pathogenesis of periodontal disease. Indeed, later studies confirmed the pleiotropic role of fibroblasts during the development of parodontopathy which encompassed several pieces of inflammatory cascades, key enzymatic processes in collagen synthesis and degradation and the regulation of their own life-cycle events [20].

Some studies addressed the role of mast cells in human periodontal disease, but a few of them dealt with morphometric analysis. Researchers found that the number of these cells and their degranulation is progressively increasing with advancing stages of the pathological process, placing them in the vicinity to mononuclear cells [21, 22]. However, others did not verify that findings and suggested that the mast cell population was progressively decreasing with weak migration to inflammatory foci [23]. In our specimens the absolute count of mast cell indeed increased, but in relative proportion they were greatly over-numbered by the rising population of plasma-cells. In addition, mast cells in our study were located around the inflammatory lesion showing weak

histological signs of degranulation. Taking into account the presented evidence we could argue that mast cells are not the primary factor in gingival destruction pathways but, rather, contributing or secondary causes in evolution of the periodontal disease.

Morphometric analysis of macrophages in our study, in general, confirms previous findings. It is well-documented that the population of these cells increases in different pathological stages of periodontal disease and that they produced a variety of biologically active substances playing the crucial role in gingival tissue destruction and function of the other immune cells [24, 25]. In comparison to healthy gingiva, the number of macrophages increases ~2.5 times in severe parodontopathy cases and they were grouped within inflammatory foci as well as in the vicinity of collagen-destructing fibers which is similar to the results of the previous study [8]. The absolute number of these cells in our samples is, however less, compared to the mentioned report but it seems that some methodological differences could contribute to this variability. Firstly, we used manual cell counting approach instead of semi-automated imaging analysis and, secondly, our patient population is somewhat different, being, for example younger, with magnitude about a decade. Many risk factors influence the dynamics of periodontal disease including sociodemographic ones like age, gender, income and education [26]. All these factor could contribute to the heterogenity of study subjects in different researchers and consequently in results variability. The findings that the macrophage density in gingivitis was greater than in advanced periodontal destructions further documented the observed fluctuations [27].

The dominant cell population in our study are plasma cells which confirm a well-known fact about histological properties of the periodontal lesions [2, 28]. However, a few studies quantify plasma cells relative to other inflammatory cell subsets in gingival tissue during the different phases of the periodontal disease [8]. In general, evidence revealed a progressive increase in plasma cell density but in ~2 times less magnitude than in our samples. The above-discussed issue about research methods heterogeneity probably contributes to these differences. Taking into account the tissue distribution of plasma cells, we also confirmed that they are primary located within inflammatory lesions. One interesting finding is their placement in the demarcating zone between healthy papillary and affected reticular gingival tissue in the few most severe cases of disease.

Conclusion

Our study described histological and morphometric patterns of periodontal disease during its progression. The existance of severe inflammatory lesions deep in the gingival tissue could create conditions for underlying bone destruction. Integrating our findings with the existing knowledge in this topic highlights much greater fluctuations in disease onset and progression than previously thought.

REFERENCES

1. *Page RC*. Gingivitis. J Clin Periodontol 1986; 13(5): 345−55.
2. *Ohlrich EJ*, *Cullinan MP, Seymour GJ*. The immunopathogenesis of periodontal disease. Aust Dent J 2009; 54(Suppl 1): 2−10.
3. *Seymour GJ, Greenspan JS*. The phenotypic characterization of lymphocyte subpopulations in established human periodontal disease. J Periodontal Res 1979; 14(1): 39−46.
4. *Séguier S, Godeau G, Brousse N*. Collagen Fibers and Inflammatory Cells in Healthy and Diseased Human Gingival Tissues: A Comparative and Quantitative Study by Immunohistochemistry and Automated Image Analysis. J Periodontol 2000; 71(7): 1079−85.
5. *Ejeil A, Gaultier F, Igondjo-Tchen S, Senni K, Pellat B, Godeau G,* et al. Are Cytokines Linked to Collagen Breakdown During Periodontal Disease Progression. J Periodontol 2003; 74(2): 196−201.
6. *Lappin DF, MacLeod CP, Kerr A, Mitchell T, Kinane DF*. Anti-inflammatory cytokine IL-10 and T cell cytokine profile in periodontitis granulation tissue. Clin Exp Immunol 2001; 123(2): 294−300.
7. *Séguier S, Gogly B, Bodineau A, Godeau G, Brousse N*. Is Collagen Breakdown During Periodontitis Linked to Inflammatory Cells and Expression of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Human Gingival Tissue. J Periodontol 2001; 72(10): 1398−406.
8. *Younes R, Ghorra C, Khalife S, Igondjo-Tchen-Changotade S, Yousfi M, Willig C,* et al.. Pertinent cell population to characterize periodontal disease. Tissue Cell 2009; 41(2): 141−50.
9. *Bendeck MP*. Macrophage Matrix Metalloproteinase-9 Regulates Angiogenesis in Ischemic Muscle. Circ Res 2004; 94(2): 138−9.
10. *Kessenbrock K, Brown M, Werb Z*. Measuring matrix metalloproteinase activity in macrophages and polymorphonuclear leukocytes. Curr Protoc Immunol 2011; 14: 14−24.
11. *Ainamo J, Löe H*. Anatomical Characteristics of Gingiva: A Clinical and Microscopic Study of the Free and Attached Gingiva. J Periodontol 1966; 37(1): 5−13.
12. *Mühlemann HR, Son S*. Gingival sulcus bleeding: a leading symptom in initial gingivitis. Helv Odontol Acta 1971; 15(2): 107−13.
13. *Armitage GC*. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 2004; 34(1): 9−21.
14. *Hillmann G, Krause S, Ozdemir A, Dogan S, Geurtsen W*. Immunohistological and morphometric analysis of inflammatory cells in rapidly progressive periodontitis and adult periodontitis. Clin Oral Investig 2001; 5(4): 227−35.
15. *Lorencini M, Silva JA, Almeida CA, Bruni-Cardoso A, Carvalho HF, Stach-Machado DR*. A new paradigm in the periodontal disease progression: Gingival connective tissue remodeling with simultaneous collagen degradation and fibers thickening. Tissue Cell 2009; 41(1): 43−50.
16. *Daniel A, Dupont M*. Stereological analysis of human gingival connective tissues. Clinically healthy gingiva. J Biol Buccale 1980; 8(2): 141−53.
17. *Vardar-Sengul S, Arora S, Baylas H, Mercola D*. Expression Profile of Human Gingival Fibroblasts Induced by Interleukin-1β Reveals Central Role of Nuclear Factor-Kappa B in Stabilizing Human Gingival Fibroblasts During Inflammation. J Periodontol 2009; 80(5): 833−49.
18. *Oyarzún A, Arancibia R, Hidalgo R, Peñafiel C, Cáceres M, González MJ,* et al. Involvement of MT1-MMP and TIMP-2 in human periodontal disease. Oral Dis 2010; 16(4): 388−95.
19. *Seppälä B, Sorsa T, Ainamo J*. Morphometric analysis of cellular and vascular changes in gingival connective tissue in long-term insulin-dependent diabetes. J Periodontol 1997; 68(12): 1237−45.

20. *Havemose-Poulsen A, Holmstrup P.* Factors Affecting IL-1-Mediated Collagen Metabolism By Fibroblasts and the Pathogenesis of Periodontal Disease: A Review of the Literature. Crit Rev Oral Biol Med 1997; 8(2): 217−36.
21. *Batista AC, Rodini CO, Lara VS*. Quantification of mast cells in different stages of human periodontal disease. Oral Dis 2005; 11(4): 249−54.
22. *Huang S, Lu F, Chen Y, Huang B, Liu M*. Mast Cell Degranulation in Human Periodontitis. J Periodontol 2013; 84(2): 248−55.
23. *Gemmell E, Carter CL, Seymour GJ*. Mast Cells in Human Periodontal Disease. J Dent Res 2004; 83(5): 384−7.
24. *Beklen A, Ainola M, Hukkanen M, Gurgan C, Sorsa T, Konttinen YT*. MMPs, IL-1, and TNF are Regulated by IL-17 in Periodontitis. J Dent Res 2007; 86(4): 347−51.
25. *Wallace AM, Sandford AJ, English JC, Burkett KM, Li H, Finley RJ,* et al. Matrix metalloproteinase expression by human alveolar macrophages in relation to emphysema. COPD 2008; 5(1): 13−23.
26. *Kocher T, Schwahn C, Gesch D, Bernhardt O, John U, Meisel P,* et al. Risk determinants of periodontal disease - an analysis of the Study of Health in Pomerania (SHIP 0). J Clin Periodontol 2005; 32(1): 59−67.
27. *Lins RD, Figueiredo CR, Queiroz LM, da Silveira EJ, Freitas RA*. Immunohistochemical evaluation of the inflammatory response in periodontal disease. Braz Dent J 2008; 19(1): 9−14.
28. *Kim YC, Ko Y, Hong SD, Kim KY, Lee YH, Chae C*, et al. Presence of Porphyromonas gingivalis and plasma cell dominance in gingival tissues with periodontitis. Oral Dis 2010; 16(4): 375−81.

Received on June 27, 2013.
Accepted on December 27, 2013.
OnLine-First November, 2014.

ORIGINAL ARTICLE

UDC: 616.12-089
DOI: 10.2298/VSP140109071T

Functional recovery of patients with ischemic cardiomyopathy treated with coronary artery bypass surgery and concomitant intramyocardial bone marrow mononuclear cell implantation – A long-term follow-up study

Funkcionalni oporavak bolesnika sa ishemijskom kardiomiopatijom lečenih implantacijom mononuklearnih ćelija koštane srži tokom aortokoronarne bajpas hirurgije

Zoran Trifunović*†, Slobodan Obradović†‡, Bela Balint†§, Radoje Ilić*†, Zoran Vukić¶, Marija Šišić||, Jelena Kostić, Siniša Rusović††, Milan Dobrić**, Gordana Ostojić†§**

***Cardiac Surgery Clinic, ‡Clinic for Emergency and Internal Medicine, §Institute for Transfusiology and Hemobiology, ¶Clinic for Anestesiology and Intensive Care, ||Nuclear Medicine Institute, ††Radiology Institute, Military Medical Academy, Belgrade, Serbia; **Department of Cardiology, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia**

Abstract

Background/Aim. Intramyocardial bone marrow mononuclear cells (BMMNC) implantation concomitant to coronary artery bypass grafting (CABG) surgery as an option for regenerative therapy in chronic ischemic heart failure was tested in a very few number of studies, with not consistent conclusions regarding improvement in left ventricular function, and with a follow-up period between 6 months and 1 year. This study was focused on testing of the hypothesis that intramyocardial BMMNC implantation, concomitant to CABG surgery in ischemic cardiomyopathy patients, leads to better postoperative long-term results regarding the primary endpoint of conditional status-functional capacity and the secondary endpoint of mortality than CABG surgery alone in a median follow-up period of 5 years. **Methods**. A total of 30 patients with ischemic cardiomyopathy and the median left ventricular ejection fraction (LVEF) of 35.9 ± 4.7% were prospectively and randomly enrolled in a single center interventional, open labeled clinical trial as two groups: group I of 15 patients designated as the study group to receive CABG surgery and intramyocardial implantation of BMMNC and group II of 15 patients as the control group to receive only the CABG procedure. All the patients in both groups received the average of 3.4 ± 0.7 implanted coronary grafts, and all of them received the left internal mammary artery (LIMA) to the left anterior descending (LAD) and autovenous to other coronaries. **Results.** The group with BMMNC and CABG had the average of 17.5 ± 3.8 injections of BMMNC suspension with the average number of injected bone marrow mononuclear cells of 70.7 ± 32.4 × 10^6 in the total average volume of 5.7 ± 1.5 mL. In this volume the average count of CD34+ and CD133+ cells was 3.96 ± 2.77 × 10^6 and 2.65 ± 1.71 × 10^6, respectively. All the patients were followed up in 2.5 to 7.5 years (median, 5 years). At the end of the follow-up period, siginificantly more patients from the group that received BMMNC were in the functional class I compared to the CABG only group (14/15 *vs* 5/15; $p = 0.002$). After 6 months the results on 6-minute walk test (6-MWT) were significantly different between the groups (435 m in the BMMNC and CABG group and 315 m in the CABG only group; $p = 0.001$), and continued to be preserved and improved on the final follow-up (520 m in the BMMNC and CABG group *vs* 343 m in the CABG only group; $p < 0.001$). Cardiovascular mortality was also significantly reduced in the BMMNC and CABG group ($p = 0.049$). **Conclusion**. Implanatation of BMMNC concomitant to CABG is a safe and feasible procedure that demonstates not only the improved functional capacity but also a reduced cardiac mortality in a 5-year follow-up in patients with ischemic cardiomyopathy scheduled for CABG surgery.

Key words:
coronary artery bypass; bone marrow transplantation; myocardium; intraoperative period; physical endurance; mortality.

Correspondence to: Zoran Trifunović, Vlajkovićeva 28/12, 11 000 Belgrade, Serbia. Phone: +381 11 324 64 15, +381 64 111 4104. E-mail: drtrifun@yahoo.com

Apstrakt

Uvod/Cilj. Intramiokardna implantacija mononuklearnih ćelija koštane srži (MNĆKS) tokom hirurške revaskularizacije miokarda aortokoronarnim premošćivanjem (HRMAKP), kao pokušaj regenerativne terapije u lečenju hronične ishemijske srčane slabosti, testirana je u manjem broju kliničkih studija sa nekonzistentnim zaključcima po pitanju popravljanja funkcije leve komore i sa periodom praćenja najčešće od šest meseci do godinu dana. Primarni cilj studije bio je da pokaže bolji i dugotrajniji funkcionalni kapacitet za vežbanje bolesnika sa ishemijskom kardiomiopatijom (IK) operisanih kombinacijom implantacije MNĆKS i HRMAKP u odnosu na bolesnike operisane samo HRMAKP. Sekundarni cilj bio je da pokaže njihovo duže preživljavanje i kvalitetniji život u nižoj NYHA klasi u dugoročnom praćenju, prosečno pet godina posle operacije. **Metode**. Trideset bolesnika sa IK i srednjom ejekcionom frakcijom leve komore od 35,9 ± 4,7% bili su prospektivno i nasumično uključeni u otvorenu interventnu kliničku studiju jednog centra i podeljeni u dve grupe: prva grupa od 15 bolesnika označena kao studijska grupa i predviđena da dobije uz HRMAKP i implantaciju MNĆKS i druga grupa od 15 bolesnika, označena kao kontrolna grupa, kojoj je rađena samo HRMAKP. **Rezultati**. Svi bolesnici obe grupe dobili su prosečno 3,4 ± 0,7 aortokoronarnih graftova, i to svi po graft leve unutrašnje torakalne arterije na prednju silaznu međukomorsku granu leve koronarne arterije (LIMA-LAD) i po potrebi autovenske graftove na druge koronarne arterije. Grupa kojoj je rađena i implantacija MNĆKS imala je u proseku 17,5 ± 3,8 injekcija rastvora MNĆKS sa prosečnim brojem ubrizganih mononuklearnih ćelija od 70,7 ± 32,4 × 10^6 u totalnom prosečno ubrizganom volumenu od 5,7 ± 1,5 mL. Izračunato je da je prosečno ugrađeno 3,96 ± 2,77 × 10^6 CD34+ ćelija i 2,65 ± 1,71 × 10^6 CD133+ ćelija. Svi bolesnici su postoperativno praćeni od 2,5 do 7,5 godina (prosečno 5 godina). U vreme poslednje postoperativne kontrole statistički značajno veći broj bolesnika iz grupe HRMAKP uz intramiokardnu implantaciju MNĆKS nalazio se u NYHA I funkcionalnoj klasi u poređenju sa bolesnicima iz grupe kojoj je rađena samo hirurška revaskularizacija (14/15 *vs* 5/15; $p = 0.002$). Pređeno rastojanje izmereno šestominutnim testom hoda pokazalo je statistički značajnu razliku već na postoperativnoj kontroli posle šest meseci (435 m u grupi MNĆKS i HRMAKP u odnosu na 315 m u grupi HRMAKP; $p = 0{,}001$) i održavala se sve vreme praćenja i neznatno povećavala do poslednje kontrole (520 m u odnosu na 343 m; $p < 0{,}001$). Kardiovaskularni mortalitet bio je takođe statistički značajno manji u grupi lečenoj implantacijom MNĆKS uz HRMAKP nego u grupi tretiranoj HRMAKP ($p = 0{,}049$). **Zaključak**. Implantacija mononuklearnih ćelija koštane srži uz hirurške revaskularizacije miokarda aortokoronarnim premošćivanjem bezbedna je i izvodljiva procedura kojom se postiže bolji funkcionalni oporavak bolesnika i smanjuje kardiovaskularni mortalitet.

Ključne reči:
aortokoronarno premošćavanje; transplantacija kostne srži; miokard; intraoperativni period; sposobnost, fizička; mortalitet.

Introduction

Despite long time improvements in treating ischemic cardiomyopathy, disability and mortality rates remain high and 50% of the diagnosed, will die within 5 years [1]. Alongside of improved medical therapy and surgical and interventional revascularization, regenerative therapy emerged as one of promising additional options over the last decade [2–8]. One of the first clinically utilized options for regenerative attempts in myocardium was bone marrow aspirate mononuclear cells (BMMNC) intracoronary injections in the early postinfarctional recovery period for improvement in left ventricular function [9, 10]. Few smaller scale randomized studies have proved that intramyocardial application of BMMNC in ischemic cardiomyopathy is safe and feasible, however, the results in a short-term follow-up period of 6–12 months were different regarding improvement in ventricular function and patients condition [11–15].

The aim of this study was to test hypothesis that intramyocardial BMMNC implantation concomitant to coronary artery bypass grafting (CABG) surgery in ischemic cardiomyopathy patients leads to better postoperative results than CABG surgery alone, regarding the primary endpoint of patients functional capacity and secondary endpoint of cardiovascular mortality in the median follow-up period of 5 years.

Methods

Between June 2006 and April 2011, 30 consecutive patients with ischemic cardiomyopathy (at least one previous myocardial infarction) were scheduled for CABG surgery and planned for prospective interventional, single center, open labeled, randomized clinical trial. The patients were randomized into two groups: group I of 15 patients designated as the study group to receive CABG surgery and intramyocardial implantation of BMMNC and group II of 15 patients as the control group to receive only CABG. In the both groups CABG was performed predominantly in "on pump" fashion, with the heart arrested by cold crystalloid cardioplegia or in "off pump" method on beating heart, and with left interval mammary artery (LIMA) graft on the left arterior descending artery (LAD) and venous aortocoronary bypasses to other coronary arteries if needed. All the participating patients provided informed consent and signed a form approved by the local Ethics Committee. Inclusion criteria for patient enrolment in the study were as follows: scheduled for CABG surgery due to LAD occlusion or multi-vessel coronary disease, age between 35 and 72 years, previous myocardial infarction older than 30 days, established diagnosis of ischemic cardiomyopathy with left ventricular ejection fraction (LVEF) < 40% and in the New York Heart Association (NYHA) III–IV functional class; full medical treatment for heart failure [β-blockers, angiotensin converting enzyme (ACE) inhibitors, diuretics].

Exclusion criteria were any of the following: non-ischemic dilatative cardiomyopathy, aneurism of the left ventricle, chronic obstructive pulmonary disease, valves insufficiency or stenosis indicated for surgical correction, serious and chronic cardiac rhythm disturbances, hepatic or renal dysfunction, malignancy, cerebrovascular insult in the previous 3 months, hematological diseases, unable to understand explanation of the procedure.

The heart team consisting of the interventional cardiologist/radiologist, heart surgeon and clinical cardiologist evaluated coronary angiography and all clinical and imaging data and made decisions on coronary revascularization and stem cell implantation.

Echocardiography was performed on Vivid 7 GE Ultrasound Systems. LVEF was calculated using the biplane method of discs (modified Simpson's rule) in the apical 4- and 2-chamber views, as recommended by the American Society of Echocardiography [16].

Single-Photon Emission Computed Tomography (SPECT) study was performed 60 min from intravenous injection of 740MBq Technetium labeled contrast media Tc99m MIBI (methoxyisobutylisonitrile) et rest. Perfusion defect extent was determined by a semiquantitative method using Auto Quant software, Cedars-Sinai (QPS/QGS component of Auto Quant) on 20 segmented heart model and was expressed in percentage.

The level of brain natriuretic peptide (BNP) was evaluated preoperatively and in every postoperative control.

The severity of symptoms was assessed using the New York Heart Association functional classification [17]. NYHA functional class was estimated for all the patients preoperatively and on postoperative controls.

Six a minute walk test (6-MWT) was performed as a simple and useful test to evaluate functional capacity in patients with heart failure [18–20]. 6-MWT was not performed preoperatively due to poor condition, dyspnea and/or angina at rest. The results were assessed postoperatively after 6 months, 1 year and on final follow-up for all the patients.

Technical elements of the procedure

After induction of general endotracheal anesthesia, before proceeding with CABG, bone marrow was obtained by multiple aspirations from the posterior iliac crest in the amount of 150 mL, mixed with 25 mL of heparinized saline, and transferred into a sterile bag to the Institute for Transfusiology and Hemobiology, Military Medical Academy in Belgrade. After filtering, 10 mL of heparinized saline and 25 mL of citrate phosphate dextrose (CPD) anticoagulant solution were added and centrifuged on 370 g force spin for 10 min in a Hettich–Roto Silenta RP (Hettich, Germany). After supernatant removal, 15 mL of hydroxyetil starch (HES) solution, 5mL of CPD and 10 mL of IWM-phosphate buffered saline were added in the sediment and incubated for 60 min at the temperature of 22 ± 2°C and then underwent to ''heavy-spin" centrifugation (g-force = 3890) for 10 min. After new removal of supernatant, in the sediment ("buffy coat" + erythrocytes) 5 mL of HES solution was added and left for 40 min to gravity sedimentation at the temperature of 22 ± 2°C. Supernatant was removed and whitish clouded sediment in the middle was aspirated, approximately 15 mL of "buffy coat" rich in BMMNC. This was the final 15 mL BMMNC product that was brought back in the operative room and injected intramyocardially. All the procedures from harvesting to cell injection were performed in a closed-circuit system using asterile conection equipment (Sterile Tubing Welder TSCD-Terumo, Japan) with a sterile plastic bag system designated for cell transplantation in peroperative conditions.

After finishing revascularization with LIMA to LAD and sufficient number of autovenous aortocoronary bypass grafts to achive total targeted revascularization (either "on pump" or "off pump", and if "on pump" when heart resumed its function from cardiopulmonary bypass), intramyocardial implantation was performed with a 1 mL insulin syringe through a 27G needle. BMMNC injection was targeted in to the hypocontractile periinfarcted viable myocardium that was visually identified and performed transepicardialy in 30–45 degree manner, intramyocardially by multiple, average 17.5 ± 3.8 injections, with a single injecting volume of 0.2–0.5 mL to the final injected volume of the average 5.7 ± 1.5 mL as shown in Figure 1.

From the rest of BMMNC suspension, the cell viability, precisely cell "membrane integrity" of mononuclear cells (MNC) was determined by trypan blue exclusion test, and was above 95% in each patient. The number of CD34+ and CD133+ cells was counted in a sample diluted with phosphate buffered saline and fixed with paraformaldehyde.

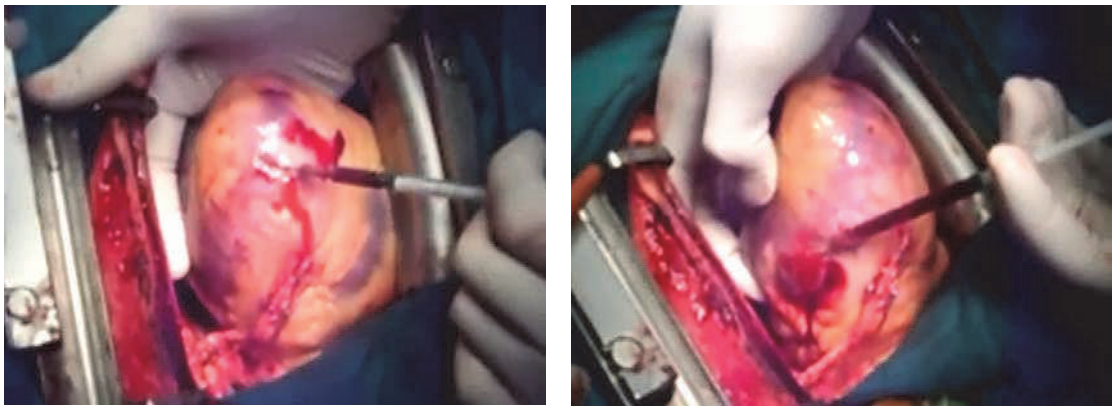

Fig. 1 – Sequences of bone marrow aspirate mononuclear cells implantation procedure in the anteroapical and anterolateral wall of the left ventricle during coronary artery bypass grafting.

Fixed cells were incubated with monoclonal antibodies specific for CD34+ and CD133+ surface antigens conjugated with FITC (fluorescein isothiocyanate) or PE (phycoerythrin) and then were investigated by the flow cytometry method with the EPICS XL–MCL device (Coulter, Germany).

Follow-up of the patients

All the procedures elapsed uneventfully. The patients had good postoperative course with hospital discharge on the 10th postoperative day. Clinical follow-up was performed 2 months, 6 months, one year, and every year after, during the follow-up period. Echocardiography study was performed preoperatively and during each follow-up visit. Gated SPECT was performed pre-operatively and 6 months postoperatively and if needed later at 2-year intervals. MSCT was performed to evaluate grafts after 1 year and if needed later at 2-year intervals. 6-MWT was performed after 6 months to test functional capacity of the patients and then after 1 year and every year after.

The median follow-up was 5 years (range 2.5–7.5 years).

The primary aim of the study was to determine patients postoperative functional capacity and also to find out the cardiac related mortality in the median follow-up period of 5 years.

Statistical analysis

The continuous variables were calculated as mean value ± standard deviation (± SD) and median values, whereas the absolute and relative frequencies were measured for categorical variables. Continuous variables distributions were determined by the Kolmogorov-Smirnov test. The differences between the groups in continuous data were examined by the Student *t*-test or the Mann-Whitney test as appropriate. In case of categorical variables, the group differences were examined by the Fisher exact test, mixed-design ANOVA for repeated measures or Friedman's test as appropriate. The correlations were assessed by the Pearsons correlation analysis. All the statistical tests were two-tailed. A p value < 0.05 was considered significant. Statistical analysis was performed with commercially available software (SPSS Statistics 17.0.).

Results

The study included 30 patients (28 males and 2 females) mean age 56.9 ± 9.0 years. The two groups were well matched as shown in Table 1, with no statistically significant differences between the patients in the group 1 (BMMNC and CABG) and the group 2 (CABG only; $p > 0.05$), except in a higher number of patients with hypercholesterolemia in the group 1 ($p = 0.035$). All the patients had at least one previous anteroseptal myocardial infarct, whereas 5 patients in the BMMNC and CABG, and 1 patient in the CABG only group had two previous myocardial infarctions at different locations of the myocard.

Table 1

Preoperative clinical and demographic characteristics of the patients

Patients characteristics	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
Gender – male, n (%)	14 (93.3)	14 (93.3)	1.000
Age (years), $\bar{x}$ ± SD	53.8 ± 10.1	60 ± 6.8	0.059
Number of previous MI, n (%)			
1	10 (66.7)	14 (93.3)	0.169
2	5 (33.3)	1 (6.7)	
Time from the first MI, $\bar{x}$ (range)	3.20 (6–12 months)	3.07 (6–12 months)	0.692
Localisation of the first MI, n (%)			
anteroseptal	9 (60.0)	11 (73.3)	0.857
anteroseptal and lateral	1 (6.7)	0 (0.0)	
inferoposterolateral	3 (20.0)	3 (20.0)	
inferior	2 (13.3)	1 (6.7)	
Hypertension, n (%)	7 (46.7)	10 (66.7)	0.462
Smoking status, n (%)			
active smoker	1 (6.7)	1 (6.7)	
previous smoker	11 (73.3)	11 (73.3)	1.000
non smoker	3 (20.0)	3 (20.0)	
Hypercholesterolemy, n (%)	14 (93.3)	8 (53.3)	0.035*
Diabetes mellitus, n (%)			
insulin dependent	3 (20.0)	2 (13.3)	0.762
oral hypoglycemic therapy	3 (20.0)	2 (13.3)	
NYHA class, n (%)			
III	11 (73.3)	13 (86.7)	0.651
IV	4 (26.7)	2 (13.3)	
BMI [kg/m^2], $\bar{x}$ ± SD	27.90 ± 3.35	27.40 ± 3.75	0.703
LVEF (%), $\bar{x}$ ± SD	35.3 ± 3.9	36.5 ± 5.3	0.490
SPECT defect extent (%), $\bar{x}$ ± SD	26 ± 9	28 ± 9	0.597
BNP (pg/mL), $\bar{x}$ ± SD	471.86 ± 375.79	632.85 ± 444.55	0.293

***Statistically significant difference; MI – myocardial infarction; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells; NYHA – the New York Heart Association; BMI – body mass index; LVEF – left ventricular ejection fraction; SPECT – Single Photon Emission Computed Tomography; BNP – brain natriuretic peptide.**

All the patients in both groups received the average of 3.4 ± 1.0 implanted coronary grafts, all of them LIMA to LAD and autovenous to the other coronaries. There were no significant differences in the number of grafts between the groups (3.3 ± 1.1 in the BMMNC and CABG group *vs* 3.5 ± 0.9 in the CABG only group; $p = 0.476$).

The group BMMNC and CABG had the average of 17.5 ± 3.8 injections of BMMNC suspension with the average number of injected bone marrow mononuclear cells 70.7 ± 32.4 × 10^6 in the total average volume of 5.7 ± 1.5 mL. In this volume the average count of CD34+ and CD133+ cells were 3.96 ± 2.77 × 10^6 and 2.65 ± 1.71 × 10^6, respectively, as shown in Table 2.

Table 2
Operative/procedural characteristics in the group BMMNC and CABG

Variable	x̄ ± SD
Injection, n	17.5 ± 3.8
Volume, mL	5.7 ± 1.5
MNC, n	70.7 ± 32.4
CD34+ (× 10^6), n	3.96 ± 2.77
CD133+ (× 10^6), n	2.65 ± 1.71

BMMNC – bone marrow aspirate mononuclear cells; CABG – coronary artery bypass grafting; MNC – mononuclear cells.

The early postoperative course was unevetful in both groups with no significant differences between them in regard to adverse side effects during hospital stay. There were no significant differences in cardiac specific enzymes activities after the operation or the number of atrial fibrilation episodes or appearance of pericardial effusion between the groups.

In a follow-up period, 6 and 12 months postoperatively, there were no statistically significant difference between the patients in the group 1 (BMMNC and CABG) and the group 2 (CABG only) in regard to the NYHA functional class ($p = 0.224$; $p = 0.169$). At the time of the last recorded control, that was between 2.5 and 7.5 (median 5) years after the cardiac surgery, statistically significantly more patients from the BMMNC and CABG group were in the NYHA functional class I *vs* the CABG only group (14 *vs* 5; $p = 0.002$) as presented in Table 3.

On the other hand, the distance that was measured by 6-MWT showed a significant difference between the groups even after 6 months postoperatively (435 ± 90 m in the BMMNC and CABG group and 315 ± 80 m in the CABG only group; $p = 0.001$), and continued to be preserved and improved after 12 months (499 ± 85 m in the BMMNC and CABG group *vs* 338 ± 103 m in the CABG only group; $p < 0.001$) and on final follow-up (520 ± 79 m in the BMMNC and CABG group *vs* 343 ± 114 m in the CABG only group; $p < 0.001$). The cardiac related mortality was also significantly reduced in the BMMNC and CABG group (0/15 *vs* 4/15, respectively; $p = 0.049$) as shown in Table 4.

There were no significant difference in mean LVEF preoperatively between the groups (35.3 ± 3.9% *vs* 36.5 ±

Table 3
Changes in the New York Heart Association (NYHA) functional class in a follow-up period of median 5 years

Variable NYHA class	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
after 6 months			
I	15 (100.0)	12 (80.0)	
II	0 (0.0)	2 (13.3)	0.224
III	0 (0.0)	1 (6.7)	
after 1 year			
I	14 (93.3)	10 (66.7)	0.169
II	1 (6.7)	5 (33.3)	
final control			
I	14 (93.3)	5 (33.3)	
II	1 (6.7)	8 (53.3)	0.002*
III	0 (0.0)	2 (13.3)	

Results are given as number (%) of patients.

***Statistically significant difference; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells.**

Table 4
Functional capacity measured by walking distance test (6-MWT) the and the mortality in a 5-year follow-up period

Variable	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
6MWT (m), x̄ ± SD			
after 6 months	435 ± 90	315 ± 80	0.001*
after 1 year	499 ± 85	338 ± 103	< 0.001*
final control	520 ± 79	343 ± 114	< 0.000*
Vital status at final follow-up, n (%)			
live	13 (86.7)	11 (73.3)	
death from cardiac event	0 (0.0)	4 (26.7)	0.049*
death from non cardiac event**	2 (13.3)	0 (0.0)	

***Statistically significant difference; **cerebrovascular hemorrhage 6 years after operation in 41-year old patient, and pulmonary malignancy 6 years after the operation in 78 year old patient; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells.**

5.3%, respectively; $p = 0.490$) but on final postoperative follow-up the mean LVEF in the BMMNC and CABG group was significantly higher than in the CABG only group (45.3 ± 4.9% *vs* 33.9% ± 8.8%, respectively; $p < 0.001$) as shown in Figure 2.

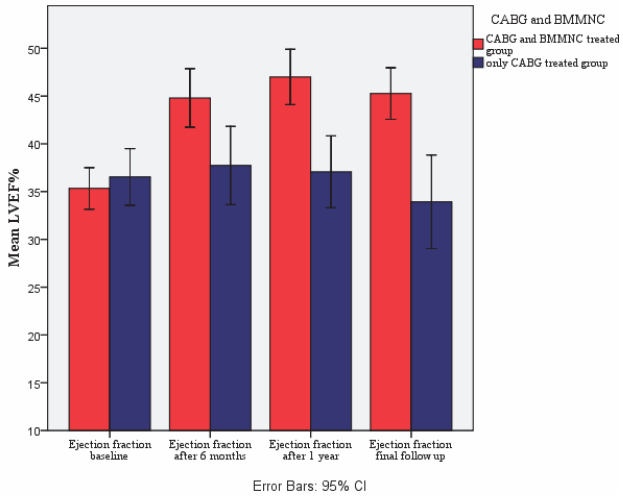

Fig. 2 – Differences in the mean left ventricular ejection fraction (LVEF) between the groups; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cell; CI – confidence interval.

Discussion

Since 2001 clinical trials have been initiated to investigate the safety and efficacy of cardiac cell therapy in patients. Most studies were conducted in patients with acute MI and intracoronary application of BMMNC (about 30 randomized trials) and demonstrated conflicting results [21–26]. On the other hand, 12 studies reporting inconsistent results were performed in the patients with chronic ischemic cardiomyopathy, among them several with intramyocardial implantation concomitant to CABG [27, 28]. In a meta-analysis performed and published by Donndorf at al. [29] six studies were included with the sample size of 20–40 and follow-up duration of only 3–6 months. All of the patients had improvement in LVEF and tending to reduced LVEDV sugesting that a decrease of cardiac remodeling was achieved. The authors also pointed out to methodologically heterogenity of the analized studies in few important moments, such as time from MI, preparing BMMNC, points of implantation, evaluation of ventricular function and the range of preoperative LVEF values. To overcome these limitations the authors launched a controlled, prospective, randomized, double blinded multicenter trial (PERFECT) in July 2009 [30]. The present status of this study is that currently recruiting participants (http://clinicaltrials.gov/show/NCT00950274).

In the present study, the approach was to use BMMNC due to the presumption that there is no favorable cell in bone marrow that can be isolated and that can behave better in myocardial recovery than the equilibrium between cells subpopulations and their products present in a mononuclear fraction of bone marrow and their influence on cardioprogenitor cells resided in myocardium. For delivery the method of intramyocardial transepicardial route was chosen. Since the patients were operated due to postinfarctional ischemic cardiomyopathy (median LVEF of 35.9 ± 4.7%) sheduled for CABG surgery, it was very natural to try to identify peri-infarction viable myocardium in the region of fibrous scared as a targeted area, and inject the amount of BMMNC that seemed to be acceptable not to disturb the milieu of myocardium, but to achieve the goal of cell engraftment. During the procedure there were no adverse side effects noted, and in the early postoperative period recovering was uneventfull, as it was in the control group. Markers of myocardial damage were in the same rank in both groups, so as postoperative pericardial effusion which were quite a rare event and did not require additional intervention other than the use of diuretics. According to the results from the patients this procedure seems to be safe, not aggravating the risk for CABG itself. Postoperatively, the procedure contributes to better quality of life and further reduces cardiovascular mortality. Postoperative improvement in the NYHA class is evident for all the patients, which is consistent with other studies and follow-up periods of 6–12 months [14, 31, 32]. Also, functional capacity of patients in the BMMNC group was significantly better according to the results achived by 6-MWT in all time frames during a long-term follow-up. This is in accordance with short-term results of others investigations for concomitant procedures of CABG and BMMNC implantation. Comparing long-term results to that of intracoronary implantation studies, these results might be superior, because in a meta-analysis of the results beyond 12 months good efects of intracoronary procedure vanish, and clinical benefit because marginal and transient, with no durable effect [33]. The possible reason could be cell retention and homing ability in different implantation technics. Despite some leakage from the point of injection, the amount of BMMNC that retained and engrafted the myocardium by intramyocardial method remained to be highest, 30–75% after 24 h, and more than 25% after 48 h of implantation. Compared to other methods, that wash-out to other organs is extremely high, even to 95% in 48h [34, 35]. Carefull attention in this study was paid to the appearance of new arrhythmias [12, 13]. There were no associated arrhythmias recorded in the BMMNC group of patients in this study.

There is still a number of open questions for actual and future clinical studies: does bone marrow stem cell implantation improve left ventricular function in all patients with heart failure; which group of patients can achieve the most benefit from the procedure; what is the optimal timing for conducting procedure after MI; what is the optimal number and population of cells to achieve most benefit from the procedure; is the intramyocardial transepicardial route best for cell administration; should it be performed simultaneously with CABG for all ischemic cardiomyopathy patients?

Conclusion

Implantation of stem cells during coronary artery bypass grafting surgery is a safe and feasible procedure that does not aggravate any additional risk of cardiac surgery. Intramyocardial stem cell implantation during coronary ar-

tery bypass grafting in comparison to coronary artery bypass grafting alone, improves functional capacity of patients with ischemic cardiomyopathy and reduces long-term cardiac mortality. However, larger randomized trials should confirm effectiveness of intramyocardial bone marrow mononuclear cells therapy during coronary artery bypass grafting operation.

REFERENCES

1. *Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB*, et al. Heart disease and stroke statistics - 2012 update: a report from the American Heart Association. Circulation 2012; 125(1): e2−e220.
2. *Urbanek K, Torella D, Sheikh F, De AA, Nurzynska D, Silvestri F*, et al. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proc Natl Acad Sci U S A 2005; 102(24): 8692−7.
3. *Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso* F, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 2004; 95(9): 911−21.
4. *Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B,* et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410(6829): 701−5.
5. *Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S,* et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114(6): 763−76.
6. *Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT*, et al.. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003; 107(18): 2294−302.
7. *Strauer B, Steinhoff G.* 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice. J Am Coll Cardiol 2011; 58(11): 1095−104.
8. *Bartunek J, Dimmeler S, Drexler H, Fernández-Avilés F, Galinanes M, Janssens S,* et al. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006; 27(11): 1338−40.
9. *Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV,* et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106(15): 1913−8.
10. *Obradović S, Rusović S, Balint B, Ristić-Andelkov A, Romanović R, Baskot B*, et al. Autologous bone marrow-derived progenitor cell transplantation for myocardial regeneration after acute infarction. Vojnosanit Pregl 2004; 61(5): 519−29.
11. *Stamm C, Westphal B, Kleine H, Petzsch M, Kittner C, Klinge H,* et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003; 361(9351): 45−6.
12. *Mocini D, Staibano M, Mele L, Giannantoni P, Menichella G, Colivicchi F*, et al. Autologous bone marrow mononuclear cell transplantation in patients undergoing coronary artery bypass grafting. Am Heart J 2006; 151(1): 192−7.
13. *Stamm C, Kleine H, Choi Y, Dunkelmann S, Lauffs J, Lorenzen B,* et al. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. J Thorac Cardiovasc Surg 2007; 133(3): 717−25.
14. *Zhao Q, Sun Y, Xia L, Chen A, Wang Z.* Randomized study of mononuclear bone marrow cell transplantation in patients with coronary surgery. Ann Thorac Surg 2008; 86(6): 1833−40.
15. *Patel AN, Geffner L, Vina RF, Saslavsky J, Urschel HC, Kormos R*, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: A prospective randomized study. J Thorac Cardiovasc Surg 2005; 130(6): 1631−8.
16. *Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA,* et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18(12): 1440−63.
17. *New York Heart Association.* Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th ed. Boston: Little, Brown and Company; 1994.
18. *Rostagno C, Gensini GF.* Six minute walk test: a simple and useful test to evaluate functional capacity in patients with heart failure. Intern Emerg Med 2008; 3(3): 205−12.
19. *Guazzi M, Dickstein K, Vicenzi M, Arena R.* Six-minute walk test and cardiopulmonary exercise testing in patients with chronic heart failure: a comparative analysis on clinical and prognostic insights. Circ Heart Fail 2009; 2(6): 549−55.
20. *Forman DE, Fleg JL, Kitzman DW, Brawner CA, Swank AM, McKelvie RS,* et al. 6-min walk test provides prognostic utility comparable to cardiopulmonary exercise testing in ambulatory outpatients with systolic heart failure. J Am Coll Cardiol 2012; 60(25): 2653−61.
21. *Obradović S, Balint B, Romanovic R, Trifunović Z, Rusović S, Baskot B*, et al. Influence of intracoronary injections of bone-marrow-derived mononuclear cells on large myocardial infarction outcome: quantum of initial necrosis is the key. Vojnosanit Pregl 2009; 66(12): 998−1004.
22. *Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA*, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007; 167(10): 989−97.
23. *Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T*, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006; 355(12): 1199−209.
24. *Lunde K, Solheim S, Forfang K, Arnesen H, Brinch L, Bjørnerheim R,* et al. Anterior myocardial infarction with acute percutaneous coronary intervention and intracoronary injection of autologous mononuclear bone marrow cells: safety, clinical outcome, and serial changes in left ventricular function during 12-months' follow-u. J Am Coll Cardiol 2008; 51(6): 674−6.
25. *Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W*, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006; 367(9505): 113−21.
26. *Obradovic S, Balint B, Trifunovic Z.* Stem Cell Therapy in Myocardial Infarction Clinical Point of View and the Results of the REANIMA Study (REgenerAtion of Myocardium with boNe Marrow Mononuclear Cells in MyocArdial Infarction). Rijeka: InTech; 2011.
27. *Zhao Q, Ye X.* Additive value of adult bone-marrow-derived cell transplantation to conventional revascularization in chronic ischemic heart disease: a systemic review and meta-analysis. Expert Opin Biol Ther 2011; 11(12): 1569−79.
28. *Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B.* Adult Bone Marrow Cell Therapy Improves Survival and Induces Long-Term Improvement in Cardiac Parameters A Systematic Review and Meta-Analysis. Circulation 2012; 126(5): 551−68.
29. *Donndorf P, Kundt G, Kaminski A, Yerebakan C, Liebold A, Steinhoff G,* et al. Intramyocardial bone marrow stem cell transplan-

tation during coronary artery bypass surgery: a meta-analysis. J Thorac Cardiovasc Surg 2011; 142(4): 911−20.

30. *Donndorf P, Kaminski A, Tiedemann G, Kundt G, Steinhoff G.* Validating intramyocardial bone marrow stem cell therapy in combination with coronary artery bypass grafting, the PERFECT Phase III randomized multicenter trial: study protocol for a randomized controlled trial. Trials 2012; 13: 99.
31. *Holinski S, Schmeck B, Claus B, Radtke H, Elgeti T, Holzhausen M,* et al. Encouraging experience with intracardiac transplantation of unselected autologous bone marrow cells concomitant with coronary artery bypass surgery after myocardial infarction. Ann Thorac Cardiovasc Surg 2011; 17(4): 383−9.
32. *Rivas-Plata A, Castillo J, Pariona M, Chunga A.* Bypass grafts and cell transplant in heart failure with low ejection fraction. Asian Cardiovasc Thorac Ann 2010; 18(5): 425−9.
33. *Wei HM, Wong P, Hsu LF, Shim W.* Human bone marrow-derived adult stem cells for post-myocardial infarction cardiac repair: current status and future directions. Singapore Med J 2009; 50(10): 936−42.
34. *Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET,* et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005; 112(Suppl): 1150−6.
35. *Li SH, Lai YY, Sun Z, Han M, Moriyama E, Wilson B*, et al. Tracking cardiac engraftment and distribution of implanted bone marrow cells: Comparing intra-aortic, intravenous and intramyocardial delivery. J Thorac Cardiovasc Surg 2009; 137: 1225−33e1.

Received on January 9, 2014.
Revised on January 31, 2014.
Accepted on February 5, 2014.
OnLine-First November, 2014.

ORIGINAL ARTICLE

UDC: 615.849.19::616.314-089.843
DOI: 10.2298/VSP131202075M

Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: A 6-week split-mouth clinical study

Uticaj postoperativne terapije laserom male snage na oseointegraciju samourezujućih implantata u bočnoj regiji gornje vilice: šestonedeljna *split-mouth* klinička studija

Borka Mandić*, Zoran Lazić†‡, Aleksa Marković*, Bojan Mandić§, Miška Mandić¶, Ana Djinić*, Biljana Miličić‖

***Clinic of Oral Surgery, §Clinic of Maxillofacial Surgery, ‖Department for Medical Statistics and Informatics, ¶Department of Orthodontics, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia; †Clinic of Dental Medicine, Military Medical Academy, Belgrade, Serbia; ‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Low-level laser therapy (LLLT) has been proven to stimulate bone repair, affecting cellular proliferation, differentiation and adhesion, and has shown a potential to reduce the healing time following implant placement. The aim of this clinical study was to investigate the influence of postoperative LLLT osseointegration and early success of self-tapping implants placed into low-density bone. **Methods.** Following the split-mouth design, self-tapping implants (n = 44) were inserted in the posterior maxilla of 12 patients. One jaw side randomly received LLLT (test group), while the other side was placebo (control group). For LLLT, a 637 nm gallium-aluminum-arsenide (GaAlAs) laser (Medicolaser 637, Technoline, Belgrade, Serbia) with an output power of 40 mW and continuous wave was used. Low-level laser treatment was performed immediately after the surgery and then repeated every day in the following 7 days. The total irradiation dose *per* treatment was 6.26 J/cm² *per* implant. The study outcomes were: implant stability, alkaline-phosphatase (ALP) activity and early implant success rate. The follow-up took 6 weeks. **Results.** Irradiated implants achieved a higher stability compared with controls during the entire follow-up and the difference reached significance in the 5th postoperative week (paired *t*-test, $p = 0.030$). The difference in ALP activity between the groups was insignificant in any observation point (paired *t*-test, $p > 0.05$). The early implant success rate was 100%, regardless of LLLT usage. **Conclusion.** LLLT applied daily during the first postoperative week expressed no significant influence on the osseointegration of self-tapping implants placed into low density bone of the posterior maxilla. Placement of self-tapping macro-designed implants into low density bone could be a predictable therapeutic procedure with a high early success rate regardless of LLLT usage.

Key words:
dental implants; oral surgical procedures; laser therapy, low-level; bone regeneration; alkaline phosphatase; treatment outcome.

Apstrakt

Uvod/Cilj. Terapija laserom male snage (TLMS) stimuliše reparatorne sposobnosti kosti utičući na ćelijsku proliferaciju, diferencijaciju i adheziju, i ima potencijal da skrati vreme zarastanja kosti nakon ugradnje implantata. Cilj ove kliničke studije bio je da se ispita uticaj postoperativne primene TLMS na oseointegraciju i rani uspeh ugradnje samourezujućih implantata u kost male gustine. **Metode.** Prateći *split-mouth* dizajn, samourezujući implantati (n = 44) ugrađeni su u posteriorne regije gornje vilice 12 pacijenata. Slučajnim izborom, jednoj od strana vilice je dodeljena TLMS (test grupa), dok je druga strana bila placebo (kontrolna grupa). Za TLMS korišćen je galijum-aluminijum-arsenid (GaAlAs) laser (Medicolaser 637, Technoline, Beograd, Srbija) talasne dužine 637 nm, snage 40 mW, neprekidnog režima rada. Tretman laserom male snage sprovodio se neposredno po ugradnji, a zatim svakodnevno, tokom narednih sedam dana. Ukupna zračna doza po tretmanu bila je 6,26 J/cm² po implantatu. Praćeni su stabilnost implantata, aktivnost alkalne fosfataze (ALP) i procenat rane uspešnosti implantatne terapije. Period praćenja bio je šest nedelja. **Rezultati.** Zra-

Correspondence to: Borka Mandić, Clinic of Oral Surgery, Faculty of Dentistry, University of Belgrade, Dr Subotića 4, 11 000, Belgrade, Serbia. E-mail: borkamandic@yahoo.com

čeni implantati imali su veću stabilnost u odnosu na kontrolne tokom celog perioda praćenja, a statistički značajno veća stabilnost bila je u petoj postoperativnoj nedelji (*t*-test za vezane uzorke, $p = 0.030$). Razlika u aktivnosti ALP između grupa nije bila statistički značajna ni u jednoj tački posmatranja (*t*-test za vezane uzorke, $p > 0.05$). Procenat rane uspešnosti terapije implantatima bio je 100%, bez obzira na primenjenu TLMS. **Zaključak.** Svakodnevna primena TLMS u prvoj postoperativnoj nedelji nije pokazala značajan uticaj na oseointegraciju samourezujućih implantata u kost male gustine bočne regije gornje vilice. Primena implantata samourezujućeg makrodizajna u kosti male gustine mogla bi predstavljati predvidljivu terapijsku proceduru sa visokim procentom rane uspešnosti, bez obzira na primenjenu TLMS.

Ključne reči:
implantati, stomatološki; hirurgija, oralna, procedure; lečenje laserom male snage; kost, regeneracija; alkalna fosfataza; lečenje, ishod.

Introduction

Low-level laser therapy (LLLT) has been used for more than 30 years in the medical field and no adverse effects have been reported [1]. It is defined as red beam or near-infrared laser therapies of low energy density and output power, with wavelengths between 500 and 1,200 nm, that do not increase normal tissue and body temperature [1]. Its effects are therefore nonthermal and biostimulative.

As LLLT affects various tissue responses such as blood flow, inflammation, cellular proliferation and/or differentiation [2], stimulation with LLLT creates a number of environmental conditions that appeared to have accelerated healing of bone defects in animal models and clinical investigations [2–5].

Though the exact mechanism of these effects is not elucidated yet, they are considered to be results of laser irradiation on the cell membrane, mitochondria, DNA and RNA synthesis, collagen synthesis, neovascularization, cell proliferation, and the production of ATP [6].

In oral implantology, research has been focused on the potential of LLLT to reduce the healing time following implant placement and to improve the potential for bone regeneration [2].

Previous experimental studies reported that low-level laser treatment stimulated proliferation and differentiation of osteoblasts [7–11] as well as their bonding to titanium implant [7]. It significantly increased alkaline phosphatase (ALP) activity, which is considered to be a marker of differentiated osteoblasts, in culture [8, 9, 11] and animal models [10]. When applied in the early postoperative period, LLLT lead to an enhancement of the mechanical strenght of bone-implant interface [12–14] and stimulation of bone matrix production and bone nodule formation [9].

There are a number of studies suggesting that low-level laser treatment in the early postoperative period after implant placement may lead to a positive clinical effect [2].

As low-density bone (D3 and D4 class of bone, Leckholm & Zarb classification [15]) is usually present in the molar region of the upper jaw, this has proven to be the region of lower success rates of dental implant therapy due to lack of primary stability that can be obtained [16]. Postoperative LLLT might have potential beneficial influence on dental implant treatment in this area, making it more predictable.

The aim of our study was to investigate the influence of postoperative LLLT on osseointegration of self-tapping implants placed into low density bone, by investigating and comparing clinical status – implant stability with the appearance of the marker of alkaline phosphatase in the periimplant crevicular fluid. The second aim was to evaluate early success rate of implants placed into the premolar/molar maxillary region, regarding LLLT.

Methods

The study was conducted in accordance with the 1975 Declaration of Helsinki, as revised in 2002. The protocol was approved by the Ethics Commitee of the Faculty of Dentistry, University of Belgrade (No.36/22), and the patients gave their written informed consent. Written patient's consent was also obtained to publish clinical photographs.

A total of 12 patients (6 males and 6 females) seeking implant therapy for bilateral reconstruction in the posterior maxilla were recruited for this study. All the patients were healthy adults, age 18 or older. The patients were selected in accordance with the following inclusion criteria: sufficient bone volume to receive implants without requiring bone augmentation (reconstruction) procedures and no history of previous tooth extraction in the last six months in the selected area. Exclusion criteria were: 1) systemic: pregnancy or lactation, systemic disease that affects osseointegration, anticoagulant therapy, systemic glucocorticoid therapy, history of radiotherapy in the craniofacial region within last 12 months, smoking habit of more than 10 cigarettes per day and 2) local: acute infection in the mouth, uncontrolled or untreated periodontal disease.

For patients' selection and treatment planning, panoramic radiographs and 3D computed tomography scans were required, followed by clinical intraoral examination.

Following split mouth design, a total of 44 self-tapping BlueSky® (Bredent, Germany) implants with diameter of 4 mm and length of 10 mm were inserted bilaterally and simetrically in the posterior maxilla of the selected patients.

Local anesthesia was induced by infiltration with 2% lidocaine hydrochloride and 1: 80 000 adrenaline. After crestal incision and mucoperiosteal flap elevation, preparations of implant recipient sites were performed under cooling with physiological solution, according to the protocol following the manufacturer's instructions (Bredent, Germany). The speed of 15 rpm with a torque of 35 Ncm was set for insertion of all implants. The implants were allowed to heal transmucosally and sutures were removed after 7 days.

Postoperatively all the patients were prescribed amoxicillin (1.5 g) or clindamycin (1.8 g) daily, for three days as well as nonsteroidal anti-inflammatory drugs for pain relief. The patients were also given detailed instructions with regard to oral hygiene. No temporary prosthesis was placed during the entire 6-week observation period.

After the surgery, one of the sides of the upper jaw of the patients was randomly (computer-generated random numbers) chosen to receive low-level laser treatment (test group). The other side of the jaw was placebo, without any treatment performed and served as a control (control group).

A 637 nm gallium-aluminum-arsenide (GaAlAs) laser (Medicolaser 637, Technoline, Belgrade, Serbia) with an output power of 40 mW and continuous wave was used. The implant on the chosen side was irradiated intraorally, orthoradially to the implant's longitudinal axis (Figure 1). Low-level laser treatment was performed immediately after the surgery and then repeated every day in the following 7 days. The total irradiation dose per treatment was 6.26 J/cm² per implant.

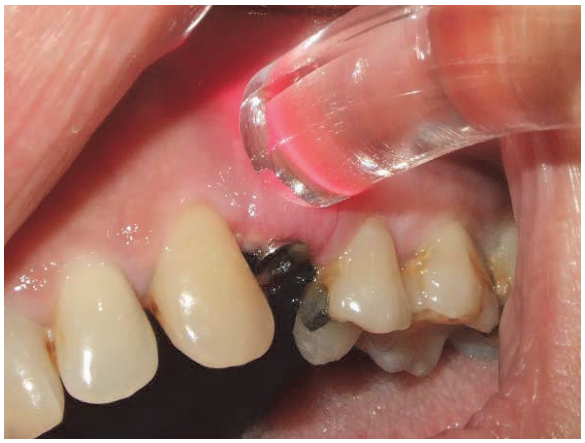

Fig. 1 – Postoperative low-level laser therapy. The operational field was irradiated by laser probe positioned intraorally, at a distance of 1 cm and orthoradially to the implant's longitudinal axis.

Evaluation of osseointegration of implants

All assessments of the study outcomes were preformed in a double blind manner, since neither patients (due to placebo) or assessors (not involved in LLLT) were aware of treatment allocation.

Resonance frequency analysis (RFA) was performed using the Osstell™ Mentor instrument (Integration Diagnostics, Göteborg, Sweden) by a trained calibrated operator who was unaware of which side would be irradiated. Measurements were recorded immediately after implant insertion and then postoperatively in a weekly manner during the following 6 weeks. A standardized abutment of fixed length (Smartpeg™ Integration Diagnostics, Göteborg, Sweden) was inserted and hand-tightened into each implant. The transducer probe (Osstell™ Mentor Probe) was held so that the probe tip was aimed at the small magnet on top of the Smartpeg™ at a distance of 2–3 mm (Figure 2). It was held still until the instrument beeped and displayed the implant stability quotient (ISQ) value. Each measurement was repeated until the same value was recorded twice, which was accepted as the authentic value. For the post-surgical stability measurements, abutments were removed from the implants.

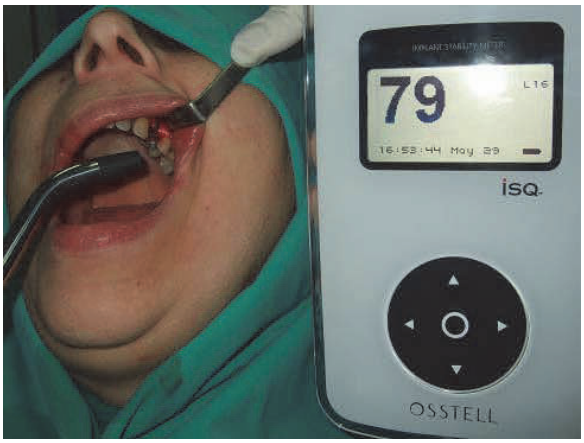

Fig. 2 – Implant stability measurement by means of resonance frequency analysis. The hand-held probe stimulates magnetically the transducer attached to the implant. The degree of implant stability is shown on the display as implant stability quotient value.

Evaluation of bone remodelation intensity and osteoblast differentiation

Peri-implant crevicular fluid (PICF) sampling was performed on the postoperative day 7, 14, 21 and 28.

To avoid mechanical irritation, blood contamination or stimulation of the PICF, PICF samples were collected before the clinical measurements. Briefly, following the isolation of the sampling area with sterile cotton rolls, supragingival plaque was removed and the sampling site was gently air dried to reduce any contamination with plaque and saliva. Extreme care was taken to minimize the level of mechanical irritation during PICF sampling as this is known to affect the actual fluid volume in a given site. Standardized sterile paper strip (Periopaper® N° 593525, Oraflow Inc, Amityville NY) was placed at the entrance of peri-implant sulcus and pushed until minimal resistance was felt (Figure 3). Sampling time was

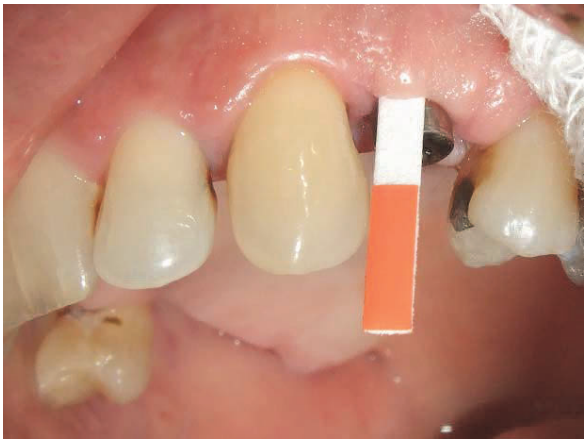

Fig. 3 – Peri-implant crevicular fluid collection. After the isolation of implant sites with cotton rolls, standardized paper strips were inserted into the sulci until a slight resistance was felt.

standardized as 60 s. Samples with visible blood contaminations were discarded. Paperstrips with PICF from single implants were immediately used for ALP activity determination.

A quantity of 20 µl of distilled water was added to each sample. The tubes were vigorously shaken for 1 min and then centrifuged at 2,000 g for 5 min with the strips kept at the collar of the tube in order to completely elute PICF components.

ALP activity was assayed spectrophotometrically with spectrophotometer at 405 nm (Secomam Basic, France). The principle of method is coloured reaction in which ALP hydrolyses p-nitrophenyl phosphate in the presence of magnesium ions to yellow product p-nitrophenol and inorganic phosphate. The reaction of 10 µl of the sample with 500 µl of the working reagent is at 37 °C, and the rate of increase in absorbance is read after 1 min, then in 1 min intervals and finally recorded after 4 minutes at 405 nm. ALP activity is expressed in U, where U (international unit) represents the amount of enzyme that catalyses release of 1 µmol of p-nitrophenol per min at 37 °C. The final results were reported as total ALP activity (U/sample).

Evaluation of early implant success

Early implant success was evaluated after the sixth postoperative week using the following criteria proposed by Buser et al. [17]: 1) the absence of recurring peri-implant infection with suppuration; 2) the absence of persistent subjective complaints such as pain, foreign body sensation, and/or dysesthesia, 3) the absence of a continuous radiolucency around the implant and 4) the absence of any detectable implant mobility.

Possible adverse events related to LLLT were also recorded during a 6-week follow-up.

Statistical analysis was performed using the SPSS® 17.0 software (SPSS Inc., Chicago, IL, USA). Implants were used as units of analysis. ISQ and ALP activity data were reported using measures of central tendency (mean, median) and variation (standard deviation, min, max, 95% confidence interval (CI). One-sample Kolmogorov–Smirnov test was used to assess the normality of data distribution. Repeated measures analysis of variance was performed to analyze changes of ISQ, as well as ALP activity data, during the observation period and was followed by *post hoc* least significant difference test to determine differences within groups between particular observation points. The statistical significance of differences in the observed parameters (ISQ and ALP activity) between the groups in each observation point was analyzed using paired samples *t*-test since data from strictly symmetrical positions of the implants were compared (split-mouth design). The statistical significance of all tests was defined as $p < 0.05$.

Results

Twelve eligible patients were enrolled in the study. They received a total of 44 implants. Since all 4 implants of one male patient aged 68 inserted bilaterally into the regions of the first and the second maxillary molars failed to achieve primary stability sufficient for the one- stage surgery approach, they were covered, not irradiated and excluded from the study. Eleven remaining patients of both genders (5 females and 6 males), mean age 61.28 years (55 to 75) enrolled in this study completed the study protocol. They received a total of 40 implants bilaterally inserted into premolar and/or molar maxillary regions, with 20 implants randomly and symmetrically attributed to each of the two groups, irradiated (test) or non-irradiated (control) group that were included in the analyses. A total follow-up period per patient was 6 weeks.

Resonance frequency analysis

Within the test group significant changes were recorded during a 6-week follow-up ($p = 0.016$) (Table 1, Figure 4).

Table 1

Descriptive statistics for implant stability measurements by means of resonance frequency analysis in test (irradiated) and control (non-irradiated) implants at baseline and during six postoperative weeks

Time	Side	x̄ ± SD	Med	Min	Max	95% CI
Baseline	test	76.00 ± 3.52	75.5	70	82	74.25–77.75
	control	72.89 ± 7.15	74.5	56	80	69.33–76.45
1st week	test	74.88 ± 3.40	75	70	82	73.06–76.69
	control	74.69 ± 4.80	74.5	67	84	72.13–77.24
2nd week	test	74.22 ± 3.93	74	68	81	72.27–76.18
	control	72.56 ± 5.67	72.5	61	80	69.74–75.37
3rd week	test	72.67 ± 3.65	73	61	77	70.85–74.48
	control	70.44 ± 6.16	70	55	80	67.38–73.51
4th week	test	72.50 ± 4.18	73	60	77	70.42–74.58
	control	69.22 ± 9.09	70	39	79	64.70–73.74
5th week	test	72.94 ± 3.92	73.5	63	79	71.00–74.89
	control	69.83 ± 7.03	71.5	48	78	66.34–73.33
6th week	test	72.67 ± 3.69	73.5	63	78	70.83–74.50
	control	70.61 ± 7.20	72	52	79	67.03–74.19

The results are presented as implant stability quotient values.

CI – confindence interval.

The maximum stability was achieved at baseline and afterwards significantly declined in the 2nd, 3rd and 4th week ($p = 0.029$; $p = 0.007$; $p = 0.008$; respectively) with the minimal recorded value in the 4th week. In the 5th week it started to rise insignificantly, but fell again in the 6th week, in both ob-

servation points still being significantly lower than the baseline stability ($p = 0.017$; $p = 0.005$; respectively). The differences in ISQ values between both consecutive weeks within the test group were not significant ($p > 0.05$).

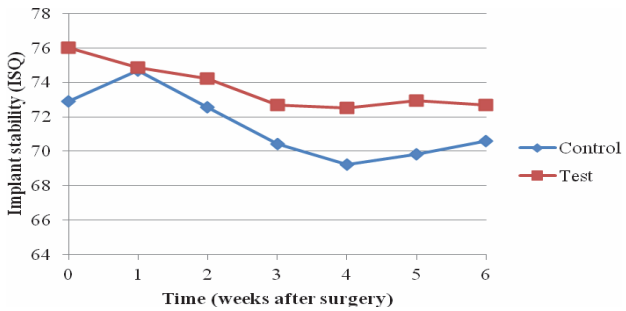

Fig. 4 – Effect of low-level laser therapy on implant stability measured by resonance frequency analysis.

In the control group significant changes in implant stability over time were revealed ($p = 0.023$) (Table 1, Figure 4). The maximum implant stability was achieved in the 1st week, and afterwards significantly decreased in the consecutive 2nd and 3rd week ($p = 0.047$; $p = 0.044$; respectively). An insignificant decrease continued in the 4th week ($p = 0.234$), when the minimum value was recorded and was significantly lower than baseline stability ($p = 0.039$). Afterwards it started to rise insignificantly during the 5th and 6th consecutive weeks ($p = 0.401$; $p = 0.110$; respectively) with ISQ values recorded in the 5th week being significantly lower compared to baseline stability ($p = 0.029$) whereas stability recorded in the 6th week was insignificantly different compared to baseline ($p = 0.074$).

Between group comparative analysis revealed higher ISQ values in the test group compared to the controls during the entire 6-week observation period with the difference being statistically significant in the 5th week ($p = 0.030$) (Table 2). The highest implant stability was recorded at baseline, in the test group. Both groups showed the "stability dip" (with the lowest ISQ values) in the 4th week, with the minimal recorded ISQ value in the control group (Figure 4).

Alkaline-phosphatase activity

Within the test group, statistically significant changes of ALP activity were observed during the 4-week observation period ($p < 0.0005$) (Table 3, Figure 5). The highest ALP activity was recorded in the 1st week and afterwards significantly decreased in the 2nd week ($p \leq 0.005$). An insignificant decrease continued from the 2nd week till the 3rd week ($p = 0.175$) followed by an insignificant increase recorded in the 4th week ($p = 1.000$). The ALP activity value in each observation point (2nd, 3rd and 4th week) was significantly lower than in the 1st postoperative week ($p \leq 0.0005$; $p \leq 0.0005$; $p = 0.010$; respectively).

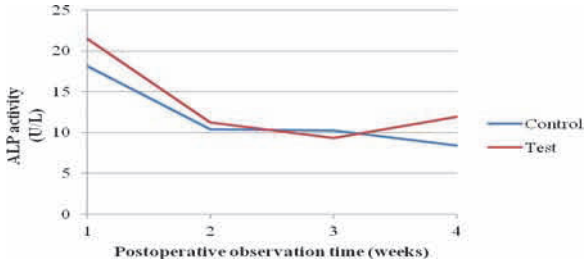

Fig. 5 – Effect of low-level laser therapy on alkaline phosphatase (ALP) activity in peri-implant crevicular fluid, measured spectrophotometrically during a 4-week observation period.

Table 2

Differences in implant stability between irradiated (test) and non-irradiated (control) implants

Time	Implant stability quotient ($\bar{x} \pm SD$)			*p*
	test	control	95% CI for MD	
Baseline	76.00 ± 3.52	72.89 ± 7.15	-0.78177 to 7.00399	0.110
1st week	74.88 ± 3.40	74.69 ± 4.80	-3.45378 to 3.82878	0.914
2nd week	74.22 ± 3.93	72.56 ± 5.67	-1.73616 to 5.06950	0.316
3rd week	72.67 ± 3.65	70.44 ± 6.16	-0.88360 to 5.32805	0.150
4th week	72.50 ± 4.18	69.22 ± 9.09	-0.72534 to 7.28089	0.102
5th week	72.94 ± 3.92	69.83 ± 7.03	0.34554 to 5.87668	0.030*
6th week	72.67 ± 3.69	70.61 ± 7.20	-0.60045 to 4.71157	0.121

MD – mean difference; **p* values (paired samples *t*-test) – statistically significant; CI – confidence interval.

Table 3

Descriptive statistics for alkaline phosphatase activity assayed spectrophotometrically in test (irradiated) and control (non-irradiated) implants during four week observation period.

Time	Side	Mean	Med	Min	Max	95% CI
1st week	test	21.53 ± 6.65	24.47	9.87	30.13	18.22–24.84
	control	18.16 ± 5.11	17.92	9.73	26.87	15.62–20.71
2nd week	test	11.26 ± 4.64	10.40	4.48	17.77	8.95–13.57
	control	10.39 ± 4.05	9.35	4.68	17.17	8.23–12.55
3rd week	test	9.36 ± 4.23	8.82	4.20	19.32	7.25–11.46
	control	10.22 ± 4.26	8.50	3.08	17.92	8.03–12.41
4th week	test	11.96 ± 8.34	8.89	5.46	39.92	7.81–16.10
	control	8.45 ± 3.46	7.47	3.05	18.97	6.73–10.17

The results are presented as U/sample, where U (international unit) represents the amount of enzyme that catalyses release of 1 µmol of p-nitrophenol *per* min at 37 °C; CI –confidence interval.

In the control group ALP activity values significantly changed during the 4-week follow-up ($p < 0.0005$) (Table 3, Figure 5). The maximum ALP activity was recorded in the 1st postoperative week and then continuously declined until the end of the 4th week. This decline was significant in the 2nd, 3rd and 4th week ($p = 0.006$; $p = 0.003$; $p < 0.0005$; respectively) in comparison with the 1st one. The decrease in ALP activity between the 1st and the 2nd week was statistically significant ($p = 0.006$) whereas no significant difference in ALP activity was observed between the 2nd and 3rd week ($p = 1.000$), neither between the 3rd and 4th postoperative week ($p = 0.743$).

The mean ALP values were higher in the test group during a 4-week follow-up, except in the 3rd postoperative week, but the difference between the groups was not statistically significant at any time of observation (Table 4). The pattern of ALP activity changes over time was different in the test and control groups (Figure 5). After the initial decline of ALP activity in the test group an increase in the 4th week was observed reaching values similar to those of the 2nd week ($p = 1.000$), whereas in the control group a continuous decrease was recorded.

Table 4

Differences in alkaline phosphatase (ALP) activity between irradiated (test) and non-irradiated (control) implants

Time	ALP ($\bar{x} \pm SD$)		95% CI of MD	*p*
	Test	Control		
1st week	21.53 ± 6.65	18.16 ± 5.11	-0.50252 to 7.24085	0.084
2nd week	11.26 ± 4.64	10.39 ± 4.05	-0.26683 to 3.42371	0.088
3rd week	9.36 ± 4.23	10.22 ± 4.26	-2.77642 to 1.61913	0.584
4th week	11.9 ± 8.34	8.45 ± 3.46	-0.85890 to 7.87234	0.108

ALP activity is presented in U/L; MD – mean difference; *p*- values (paired samples *t*-test) CI – confidence interval.

Early implant success

The early implant success rate after the first six weeks (prior to implant placement) was 100%, regardless of LLLT usage. No adverse event was recorded during the follow-up.

Discussion

Osseointegration is an essential prerequisite for the dental implants' long-term prognosis. Therefore, chemical, biological and biophysical adjunctive therapies to improve and accelarate healing at bone-implant interface have been widely investigated [18]. This randomized, double blind, split-mouth clinical study was focused on the effect of postoperative LLLT using a 637 nm GaAlAs laser with an output power of 40 mW and total irradiation dose *per* treatment of 6.26 J/cm² *per* implant, on osseointegration of self-tapping implants placed into posterior maxilla. Our intention was to explore this effect on bone healing after dental implant placement in the maxillary premolar and/or molar region, being the area of the least predictible success of implant therapy [16], where the use of LLLT might be of major clinical relevance. The results of our study suggest that LLLT did not significantly affect the osseointegration of self-tapping implants placed into low density bone of posterior maxilla.

A 637 nm GaAlAs laser has been chosen due to its beneficial effects on bone regeneration reported in animal [3] and clinical studies [4]. LLLT has been found to increase osteoblastic proliferation, collagen deposition, and bone neoformation in the irradiated comparing to non-irradiated bone [3, 9]. Studies using animal models and human osteoblast-like cells cultures, demonstrated that the use of low-level laser after titanium implant insertion promoted osseointegration due to rapid bone turnover [7, 12] and seemed to accelerate active bone replacement without causing tissue or implant damage [7]. Histomorphometric evaluation in animal models revealed more bone-implant contact in the irradiated groups as compared to the controls at 3 and 6 [19] and 16 weeks postoperatively [20]. These results suggest that LLLT may stimulate bone repair, affecting cellular proliferation, differentiation and adhesion [7–14, 19, 20].

In this study osseointegration was evaluated through its two indicators – secondary implant stability measured by means of RFA and ALP activity assayed spectrophotometrically. Secondary implant stability is a clinical reflection of cellular events in peri-implant healing department and therefore indicates the rate and extent of osseointegration [21]. We used RFA as a non-invasive method that has proved to be a reliable tool to assess implant stability, determine different healing phases of dental implants and predict success of implant treatment [21]. Longitudinal ISQ values in both groups followed the usual pattern of changes with "stability dip" in the 4th postoperative week that reflected bone remodeling process when primary spongiosa was being replaced with lamellar and/or parallel-fibered bone [16, 22]. The trend of higher ISQ values recorded in the test group compared to controls during the entire 6-week period of observation, reached a significant difference in the 5th postoperative week. This result might suggest biomodulatory effect of LLLT that increases cellular activity and bone apposition but still not clinically significant to provide an earlier and better anchorage of implants. Statistically significant regeneration of bone tissue around irradiated implants was recorded in an intermediate period, which was in agreement with literature data [13, 23]. It has been shown that although LLLT is capable to increase the number of osteogenic cells in the very initial stage of healing, its effect on implant stabilization in this stage is still insignificant [13, 23]. Conversely, previous reports of animal studies reported that postoperative LLLT improved

biomechanical characteristics of bone-implant interface [12–14]. The authors agreed that single [14] or multisession [12, 13] LLLT was beneficial to improve bone-implant interface strength, resulting in higher values of removal torque required to detach bone and implant in sites previously submitted to irradiation in comparison to non-irradiated sites [13, 14].

The only clinical study that investigated the stability of oral implants after LLLT was the study of Garcià-Morales et al. [24]. Under the conditions of their study, no evidence was found of any effect of LLLT on the stability of implants when measured by RFA. The authors remarked that potential beneficial effect of LLLT was perhaps masked by high initial stability attained in the posterior mandible region [24]. With regard to different irradiation protocol used in a Garcià-Morales study [24] (infrared laser with seven irradiations repeated every 48 h for the first 14 days), as well as different implantation sites, comparison with our results is difficult.

In our study, during the whole 6-week observation period in both irradiated and non-irradiated implants, implant stability rates were high (≥ 69 ISQ), which is interesting, since the implantation site was the posterior maxilla. These results could probably be explained by the self-tapping implant design as has been previously demonstrated by a recent randomized clinical trial [25]. Exceptionally, four implants of one male patient aged 68 inserted bilaterally into the regions of the first and the second maxillary molars failed to achieve primary stability sufficient for one stage surgery approach. Although the cause of poor implant stability remains unclear, the fact that all the implants were placed to the same patient indicates the probable systemic factor despite the inconspicuous medical history. Regardless of the possibility of LLLT to promote the osseointegration of implants with poor primary stability demonstrated in animal model [26] we decided to cover them and exclude from the study due to concerns that weekly RFA measurements during early healing might damage weak bone-implant interface resulting in implant failure.

We compared clinical status of the implant – its stability, with the appearance of the marker of ALP in the peri-implant crevicular fluid. ALP is considered to be a marker of differentiated osteoblasts and their activity, as early progenitor cells do not express ALP activity but differentiate through a defined number of cell divisions to express ultimately a mature osteoblast phenotype that is capable of bone formation [27]. Our results revealed significant changes in ALP activity longitudinally in time, i.e. during the 4-week observation period, within both groups. The significantly enhanced ALP activity in the early stage of bone tissue healing (first postoperative week) was found in both irradiated and non-irradiated implants. As new bone formation starts as early as 1 week after implant placement when the primary bone contacts are supplemented by newly formed secondary bone contacts [28], this result may indicate an intensive osteoblastic activity around implants, i.e. bone formation. On the other hand, a subsequent decrease of ALP activity from the second week and onwards, would therefore be the result of greater presence of differentiated cells (osteocytes) at the implant-bone interface. However, this is unlikely the case, as this is too early for the bone deposition process to decline. Apart from that released from osteoblasts during bone remodeling, ALP found in PICF can also derive from polymorphonuclear cells during inflamation [29] and periodontal fibroblasts during periodontal regeneration [30]. Increased ALP activity in the first postoperative week is therefore more likely the result of inflammation that occurs as a physiological response to operation trauma, and which presents the first phase of osseointegrating process.

Although our results showed no statisticaly significant difference in ALP activity between the test and control group in all observation points, the pattern of ALP activity changes over time was different. In contrast to the control group where continuous decrease of ALP activity was recorded, in the test group after the initial decline, an increase was observed in the 4th week. The increase in ALP activity in the laser group might be interpreted as an indication of enhanced osteoblast activity and therefore, improved bone neoformation and mineralization. This biochemical result was supported by our clinical finding from the 5th observation week when a significantly higher stability was recorded for irradiated implants compared to controls, suggesting beneficial effect of LLLT on osseointegration.

Previous *in vitro* [8, 9, 11] and animal [10] studies reported on enhancements in the ALP activity as well as matrix formation after LLLT, which the authors considered as an indication of increased osteoblastic activity after LLLT.

Generalisation of our results might be affected by bone density, implant macro design, as well as irradiation protocol we used. In the literature, there is no consensus regarding LLLT protocol. The ideal wave length, energy density and irradiation protocol are perhaps yet to be determined. Furthermore, we have used self-tapping implant macro design since it has been recommended for low density bone of posterior maxilla in order to achieve sufficient implant stability [25]. However, non self-tapping implants are not so effective in providing good primary stability into spongy bone and more pronounced effect of LLLT on the healing of such implants could be expected since the effect of LLLT in our study might be masked by self-tapping design.

Conclusion

Low-level-laser therapy applied daily during the first postoperative week using a 637 nm gallium-aluminum-arsenide (GaAlAs) laser with an output power of 40 mW and total irradiation dose *per* treatment of 6.26 J/cm² *per* implant expressed no significant influence on the osseointegration of self-tapping implants placed into low density bone of posterior maxilla. Placement of self-tapping macro-designed implants into low density bone could be predictable therapeutic procedure with a high early success rate regardless the low-level laser therapy use.

Acknowledgements

The study was financially supported in part by Bredent, Senden, Germany.

REFERENCES

1. *Harris DM*. Biomolecular mechanism of laser biostimulation. J Clin Laser Med Surg 1991; 9(4): 277−80.
2. *Stanford OT, Beirne R, Ellingsen JE*. Effects of Low-Level Laser Treatment on Bone Regeneration and Osseointegration of Dental Implants. Int J Oral Maxillofac Implants 2007; 22(5): 691−5.
3. *Markovic A, Kokovic V, Todorovic L*. The influence of low-power laser on healing of bone defects: An experimental study. J Oral Laser Applic 2005; 5: 169−72.
4. *Marković A, Todorović L*. The Influence of Low-power Laser on Healing of Bone Defects after Periapical Surgery: A Clinical Study. J Oral Laser Applic 2006; 6: 163−8.
5. *Pinheiro AL, Gerbi ME*. Photoengineering of bone repair processes. Photomed Laser Surg 2006; 24(2): 169−78.
6. *Karu T*. Photobiology of low-power laser effects. Health Phys 1989; 56(5): 691−704.
7. *Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K*. Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. Biomaterials 2005; 26(17): 3503−9.
8. *Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D*, et al. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wien Klin Wochenschr 2008; 120(3−4): 112−7.
9. *Ozawa Y, Shimizu N, Kariya G, Abiko Y*. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone 1998; 22(4): 347−54.
10. *da Silva AP, Petri AD, Crippa GE, Stuani AS, Stuani AS, Rosa AL*, et al. Effect of low-level laser therapy after rapid maxillary expansion on proliferation and differentiation of osteoblastic cells. Lasers Med Sci 2012; 27(4): 777−83.
11. *Abramovitch-Gottlib L, Gross T, Naveh D, Geresh S, Rosenwaks S, Bar I,* et al. Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix. Lasers Med Sci 2005; 20(3−4): 138−46.
12. *Khadra M, Rønold HJ, Lyngstadaas SP, Ellingsen JE, Haanaes HR*. Low-level laser therapy stimulates bone-implant interaction: an experimental study in rabbits. Clin Oral Implants Res 2004; 15(3): 325−32.
13. *Maluf AP, Maluf RP, da Brito CR, França FM, de Brito RB*. Mechanical evaluation of the influence of low-level laser therapy in secondary stability of implants in mice shinbones. Lasers Med Sci 2010; 25(5): 693−8.
14. *Boldrini C, de Almeida JM, Fernandes LA, Ribeiro FS, Garcia VG, Theodoro LH,* et al. Biomechanical effect of one session of low-level laser on the bone-titanium implant interface. Lasers Med Sci 2013; 28(1): 349−52.
15. *Lekholm U, Zarb GA*. Patient selection and preparation. In: *Branemark PI, Zarb GA, Albrektsson T*, editors. Tissue-Integrated Prostheses: Osseointegration in clinical dentistry. 1st ed. Chicago: Quintessence; 1985. p. 199−210.
16. *Bischof M, Nedir R, Szmukler-Moncler S, Bernard J, Samson* J. Implant stability measurement of delayed and immediately loaded implants during healing. Clin Oral Implants Res 2004; 15(5): 529−39.
17. *Buser D, Weber HP, Lang NP*. Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. Clin Oral Implants Res 1990; 1(1): 33−40.
18. *Mavrogenis AF, Dimitriou R, Parvizi J, Babis GC*. Biology of implant osseointegration. J Musculoskelet Neuronal Interact 2009; 9(2): 61−71.
19. *Pereira CL, Sallum EA, Nociti FH, Moreira RW*. The effect of low-intensity laser therapy on bone healing around titanium implants: a histometric study in rabbits. Int J Oral Maxillofac Implants 2009; 24(1): 47−51.
20. *Jakse N, Payer M, Tangl S, Berghold A, Kirmeier R, Lorenzoni M*. Influence of low-level laser treatment on bone regeneration and osseointegration of dental implants following sinus augmentation. An experimental study on sheep. Clin Oral Implants Res 2007; 18(4): 517−24.
21. *Meredith N*. Assessment of implant stability as a prognostic determinant. Int J Prosthodont 1998; 11(5): 491−501.
22. *Huwiler MA, Pjetursson BE, Bosshardt DD, Salvi GE, Lang NP*. Resonance frequency analysis in relation to jawbone characteristics and during early healing of implant installation. Clin Oral Implants Res 2007; 18(3): 275−80.
23. *Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Cristinamartins M*. Infrared laser light reduces loading time of dental implants: a Raman spectroscopic study. Photomed Laser Surg 2005; 23(1): 27−31.
24. *Garcia-Morales JM, Tortamano-Neto P, Todescan FF, de Andrade JC, Marotti J, Zezell DM*. Stability of dental implants after irradiation with an 830-nm low-level laser: a double-blind randomized clinical study. Lasers Med Sci 2012; 27(4): 703−11.
25. *Marković A, Calvo-Guirado JL, Lazić Z, Gómez-Moreno G, Ćalasan D, Guardia J*, et al. Evaluation of primary stability of self-tapping and non-self-tapping dental implants. A 12-week clinical study. Clin Implant Dent Relat Res 2013; 15(3): 341−9.
26. *Campanha BP, Gallina C, Geremia T, Loro RC, Valiati R, Hubler R,* et al. Low-level laser therapy for implants without initial stability. Photomed Laser Surg 2010; 28(3): 365−9.
27. *Owen TA, Aronow M, Shalhoub V, Barone LM, Wilming L, Tassinari MS,* et al. Progressive development of the rat osteoblast phenotype in vitro: reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. J Cell Physiol 1990; 143(3): 420−30.
28. *Berglundh T, Abrahamsson I, Lang NP, Lindhe J*. De novo alveolar bone formation adjacent to endosseous implants. Clin Oral Implants Res 2003; 14(3): 251−62.
29. *Plagnat D, Giannopoulou C, Carrel A, Bernard J, Mombelli A, Belser UC*. Elastase, alpha2-macroglobulin and alkaline phosphatase in crevicular fluid from implants with and without periimplantitis. Clin Oral Implants Res 2002; 13(3): 227−33.
30. *Groeneveld MC, van den Bos T, Everts V, Beertsen W*. Cell-bound and extracellular matrix-associated alkaline phosphatase activity in rat periodontal ligament. Experimental Oral Biology Group. J Periodont Res 1996; 31(1): 73−9.

Received on December 2, 2013.
Accepted on February 6, 2014.
OnLine-First November, 2014.

ORIGINAL ARTICLE

UDC: 616.62-006-08
DOI: 10.2298/VSP131030072M

Difference in recurrence frequencies of non-muscle-invasive-bladder tumors depending on optimal usage of intravesical immunotherapy of bacillus Calmette-Guérin

Razlika u učestalosti recidiviranja mišićno-neinvazivnih tumora mokraćne bešike zavisno od optimalne primene intravezikalne imunoterapije bacilom *Calmette-Guérin*

Radovan Milošević*†, Novak Milović*†, Predrag Aleksić*†, Miodrag Lazić‡, Snežana Cerović*§, Vladimir Bančević*†, Branko Košević†, Predrag Marić†, Aleksandar Spasić†, Dejan Simić†, Božidar Kovačević§

***Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; †Clinic of Urology, §Institute for Pathology, Military Medical Academy, Belgrade, Serbia; ‡Department of Urology, Hospital Dr Dragiša Mišović, Belgrade, Serbia**

Abstract

Background/Aim. The therapy with intravesical instillation of bacillus Calmette-Guérin (BCG) after transurethral resection (TUR) of the tumor is the gold standard of treatment of non-muscle invasive bladder cancer (NMIBC). The aim of this study was to compare the frequencies of reccurence between a group of patients submitted to TUR + BCG therapy (group I) and a group of patients submitted only to TUR (group II). **Methods.** The patients with NMIBC, a total of 899, treated in our Institution from January 1, 2007 to March, 2013, were included in this study and divided into two groups: group I and group II. These two groups were divided into three subgroups: solitary first diagnosed tumor ≤ 3 cm (SFDGT), solitary first diagnosed tumor > 3 cm and multiple first diagnosed tumors (MFDGT), and recedive tumors (RCT). Statistical analysis was performed by using χ^2-test and Kolmogorov-Smirnov test. **Results:** In the group I a total of 133 cases had reccurence contrary to 75 in the group II, making a statistically highly significant difference. Analysis of recurrences through the subgroups revealed: in the group I SFDGT recurrence occured in 27 of the cases *vs* 9 cases in the group II; in the group I MFDGT recurrence occured in 49 of the cases *vs* 31 in the group II ($p < 0.001$), and finally, in the group I RCT recurrence occured in 57 cases *vs* 35 cases in the group II ($p < 0.001$). **Conclusion.** The obtained results indicate no difference in the frequency of reccurence between the group I and group II regarding SFDGT, but a very high significant difference regarding those with MFDGT and RCT. These results should be taken into consideration in everyday clinical practise.

Key words:
urinary bladder, neoplasms; carcinoma in situ; immunotherapy; mycobacterium bovis; recurrence.

Apstrakt

Uvod/Cilj. Intravezikalna imunoterapija bacilom Calmette-Guérin (BCG) smatra se zlatnim standardom u lečenju mišićnoneinvazivnih tumora mokraćne bešike (NIMBC) nakon transuretralne resekcije (TUR) tumora. Cilj istraživanja bio je uporediti učestalost recidiviranja tumora između bolesnika podvrgnutih terapiji TUR + BCG (grupa I) i samo terapiji TUR (grupa II). **Metode.** Bolesnici sa NIMBC lečeni u našoj instituciji od 1. 1. 2007. do 3. 3. 2013. (n = 899), bili su uključeni u istraživanje. Dve grupe bolesnika podeljene su u tri podgrupe: bolesnike sa solitarnim novootkrivenim tumorima ≤ 3 cm (SFDGT), bolesnike sa solitarnim novootkrivenim tumorima > 3 cm i multiplim novootkrivenim tumorima (MFDGT), i bolesnike sa recidivnim tumorima (RCT). Statistička analiza obavljena je primenom χ^2-testa i Kolmogorov-Smirnov testa. **Rezultati.** U grupi I došlo je do recidiva kod 133 bolesnika, nasuprot 75 u grupi II, što je statistički visokoznačajna razlika. Ako se analizira učestalost recidiviranja uzimajući u obzir formirane podgrupe nađeno je da se u grupi I SFDGT recidiv javio kod 27 bolesnika, nasuprot 9 bolesnika u grupi II ($p > 0,05$) u grupi I MFDGT recidiv se desio kod 49 bolesnika nasuprot 31 u grupi II ($p < 0,001$) kao i da se u grupi I RCT recidiv javio kod 57 bolesnika nasuprot 35 u grupi II ($p < 0,001$). **Zaključak.** Dobijeni rezultati ukazuju da ne postoji statistički značajna razlika u učestalosti recidiviranja kod podgrupe SFDGT, ali je prisutna kod podgrupa MFDGT i RCT. Ovo može biti od značaja za svakodnevnu kliničku praksu.

Ključne reči:
mokraćna bešika, neoplazme; karcinom in situ; imunoterapija; bacillus calmetteguerin; recidiv.

Correspondence to: Radovan Milošević, Clinic of Urology, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.

Introduction

Urinary bladder cancer, transitional cell carcinoma (TCC) is one of the most common malignancies in the USA and Europe. Most bladder tumors are non-muscle invasive tumors (NMIBC) at the moment when they were diagnosed (75–85%) [1, 2]. After more than 30 years of research, intravesical instillation of bacillus Calmette-Guérin (BCG) after transurethral resection of bladder tumor (TUR BT) remains the most effective intravesical treatment in NMIBC, but there steal exists a room for improvement [3]. The key element of BCG antitumor activity resides in its ability to switch on a robust cellular immune response, although the precise mechanism of action is not yet fully understood. The complex immunologic cascade starts with the initial adherence of mycobacteria to the urothelial lining and proceeds through the secretion of cytokines from urothelial cells, a process that attracts a large array of inflammatory cells (neutrophils, monocytes). BCG immunotherapy requires robust immune system [4, 5]. BCG has currently become the most commonly used intravesical agent and is known to be superior to other intravesical agents for prevention of tumor recurrence [2, 6]. Standard BCG induction treatment consists of six weekly bladder instillations. Many institutions give three to twenty one additional instillations during the first three years to improve results [7]. Although this therapy has been proven to reduce significantly the incidence of stage progression and recurrence in NMIBC [8, 9] there was also registered that it has minor side effects occurring in 35–71% of patients and significant morbidity in 5–23% of patients due to systematic sepsis [10]. In our institution in compliance with international standards this therapy was applied. Regarding recurrence within one year of monitoring, frequency was consistent with published data, namely 15–20%, depending on the period of follow-up. The frequency and severity of adverse effects of the treatment were also in line with literature data. According to our experience, the most common side effects were chills, fever, micro and macrohematuria. Significantly less common were severe complications such as the development of tuberculosis (TBC) of urinary tract, miliary TBC of lung, bladder contracture, reduced bladder capacity, urethral stenosis. Most rare were complications such as TBC encephalitis and hepatitis. This therapy was applied in our institution regularly until the start of 2012 and after that due to the discontinuance of production of this medication (ImmuCyst®, Sanofi-Aventis) and as no similar product has been registered so far for the Serbian market, TUR BT was the only treatment for patients suffering from NMIBC. Current situation, including side effects and costs of this therapy has imposed an idea to investigate is the usage of this therapy necessary in all cases of this stadium of the illness. The aim of our investigation was to compare the frequency of recurrence between a group of patients submitted to therapy TUR + BCG (group I) and a group of patients submitted only to TUR (group II).

Methods

The study included patients with NMIBC treated and controlled in our institution in the period January, 2007 – March 3, 2013. The study included 899 participants of both sexes [male 660 (73,4%), female 239 (26.6%)], of various ages, average 61.05 ± 10.52 years and different occupations. Whether respondents belonged to a risk group of developing bladder cancer and recurrence of the disease did not affect the possibility to be included in the study. The respondents, depending on the applied treatment, were divided into two groups: patients who underwent BCG intravesical therapy after TUR (TUR + BCG), the group I, 674 subjects, and a group in which TUR was the only treatment, the group II, 225 subjects. The patients with intravesical BCG therapy, received a single dose *per* week following the therapy, a total of six weeks. These two groups were divided into three subgroups: the solitary first diagnosed tumor ≤ 3 cm (SFDGT), the low risk of recurrence group according to the recommendations of the Guidelines of the European Association of Urologists (EAU) [2]; solitary first diagnosed tumor > 3 cm and multiple first diagnosed tumors (MFDGT) subgroups; the recidive tumours (RCT) subgroups. The group I subgroup SFDGT included 363 subjects, the group I subgroup MFDGT 152 subjects, and the group I subgroup RCT 159 subjects. The group II subgroup SFDGT included 128 subjects, the group II subgroup MFDGT 51 subjects, and the group II subgroup RCT 46 subjects. All the formed groups and subgroups were homogeneous in terms of age and gender. After the therapy was conducted, all of the respondents were in regular quarterly controls that involved basic laboratory tests, ultrasonic examination and uretrocystoscopy. If and when there was a recurrence of the disease, progresion in the grade and stage of the disease was estimated. The disease progress in terms of grade and stage was determined after the new TUR.

All the results in the text, tables and graphs are presented as the mean value ± standard deviation (SD). The significance of the differences in frequency distributions of individual parameters was checked using $\chi 2$-test or the Kolmogorov-Smirnov test. The correlation of various parameters was investigated using parametric or nonparametric correlation analysis (Pearson). The three levels of statistical significance were determined: $p < 0.05$; $p < 0.01$ and $p < 0.001$. Data processing was performed using a commercial statistical software for PCs (Stat for Windows, R.4.5, Stat Soft, Inc., USA, 1993).

Results

The results obtained in this study suggest a highly statistically significant difference in incidence of recurrence between the groups I and II. Table 1 shows the incidence of the disease recurrence in the groups I and II.

Table 1

The incidence of recurrence in relation to the therapy

Therapy	Patients (n)	Recurrences n (%)
TUR + BCG	674	133 (19.73)
TUR	225	75 (33.33)*
Total	899	208 (23.14)

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin *$\chi^2 = 16.78$, $p < 0.001$ (χ^2 test).

The results shown in Table 1 clearly indicate the difference in the frequency of recurrence between the groups I and II confirming the importance of the application of intravesical BCG therapy in treatment of patients suffering from NMIBC.

Between the subgroups SFDGT in the group I and the group II, there was no statistically significant difference in the incidence of recurrence within one year of follow-up, while there was a statistically significant difference in the frequency of recurrence between the subgroups RCT and subgroups MFDGT.

Table 2 shows the frequency of the desease recurrence depending on the forms of cancer and the applied therapy.

As it is shown in Table 2, it is clear that in addition to the applied therapy a significant role in the incidence of recurrence is played by the shape of the tumor. So in the SFDGT subgroup which according to the Guidelines of EAU included low-risk tumors, no statistically significant differences in the frequency of recurrence was observed, while this significance was present in patients with high risk NIMBC.

Between the subgroups SFDGT there was no statistically significant difference during the period of the disease relapse.

Table 3 shows the period of the disease relapse in the SFDGT subgroups of both groups.

As shown in Table 3 in the patients with low-risk NIMBC there was no statistically significant difference in the relapse period depending on the applied therapy, while in all other shapes of NMIBC there was a statistically significant difference in relapse period due to the applied therapy (Tables 4 and 5). Kolmogorov - Smirnov test was used for statistical analysis of the results.

Between the MFDGT subgroups there was a statistically significant difference in the incidence of recurrence after 9 and 12 months of follow-up and between the subgroups RCT a statistical significance was present after 6 and 9 months of follow-up. Tables 4 and 5 show the period of relapse for these two subgroups of patients.

Disease progression in grade between the SFDGT subgroups was not present in a statistically significant extent,

Table 2

Frequency of recurrence depending on the forms of cancer and the applied therapy

Applied therapy	SFDGT		MFDGT		RCT	
	Number of patients	Number (%) of recurrences	Number (%) of patients	Number (%) of recurrences	Number (%) of patients	Number (%) of recurrences
TUR + BCG	363	27 (7.4)	152	49 (32.2)	159	57 (35.8)
TUR	128	9 (7.0)	51	31 (60.8)	46	35 (76.1)
χ^2 test		n.s.		$p < 0.001$		$p < 0.001$

SFDGT – solitary first diagnosed tumors; MFDGT – multiple first diagnosed tumors;RCT – recedive tumors;TUR – transurethal resection; BCG – Bacillus Calmette-Guérin.

Table 3

Period of disease relapse in the subgroups of solitary first diagnosed tumors (SFDGT) of both groups of patients

Applied therapy	Number of patients	Number (%) of recurrences			
		months			
		3	6	9	12
TUR + BCG	363	1 (0.3)	11 (3.0)	2 (0.5)	13 (3.6)
TUR	128	1 (0.8)	3 (2.3)	1 (0.8)	4 (3.1)
Kolmogorov-Smirnov test		n.s.	n.s.	n.s.	n.s.

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin.

Table 4

Period of disease relapse in the solitary first diagnosed tumors > 3 cm and multiple first diagnosed tumors (MFDGT) subgroups of both groups of patients

Applied therapy	Number of patients	Number (%) of recurrences			
		months			
		3	6	9	12
TUR + BCG	152	7 (4.6)	11 (7.2)	13 (8.6)	18 (11.8)
TUR	51	2 (3.9)	5 (9.8)	11 (21.6)	13 (25.5)
		n.s.*	n.s.†	$p < 0.05^{\dagger}$	$p < 0.05^{\dagger}$

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin.
*** Kolmogorov-Smirnov test; $^{\dagger}\chi^2$ test**

Table 5

Period of disease relapse in RCT subgroups of both groups of patients

Applied therapy	Number of patients	Number (%) of recurrences			
		months			
		3	6	9	12
TUR + BCG	159	1 (0.6)	12 (7.5)	17 (10.7)	27 (17.0)
TUR	46	2 (4.3)	9 (19.6)	12 (26.1)	12 (26.1)
		n.s.*	$p < 0.05^{\dagger}$	$p < 0.05^{\dagger}$	n.s. †

RCT – recedive tumors; TUR – transurethal resection; BCG – Bacillus Calmette-Guérin;
*** Kolmogorov-Smirnov test was performed; $^{\dagger}\chi^2$ test.**

whereas the difference in grade progression between the MFDGT and RCT subgroups was at the level of statistical significance, as shown in Table 6.

The results in this Table indicate that BCG therapy, in the patients belonging to the low-risk group did not affect the progression of grade, whereas a significant difference in the progression of grade was present in the MFDGT and RCT subgroups, depending on the application of BCG therapy.

There was no significant statistical differences in the frequency of progression of the stadium of the disease between the SFDGT subgroups, while a statistically significant difference in the frequency of the stadium progression was present between the MFDGT and RCT subgroups, as shown in Table 7.

The most important parameter in monitoring and treating NIMBC is certainly the progression of NIMBC in infiltrative

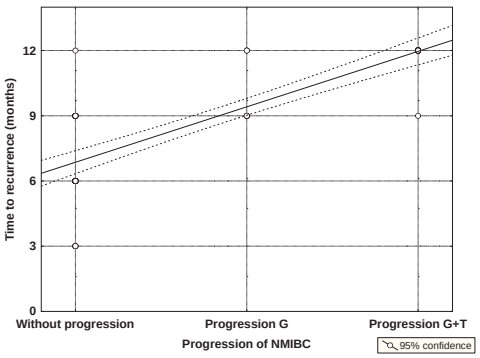

Fig. 1 – Correlation of time to recurrence and disease progression

G – grade of the disease
T – stadium of the disease
NMIBC – non-muscle invasive bladder cancer.

Table 6

Tumor progression from the grade 2 to the grade 3 in both groups of patients

Applied therapy	SFDGT		MFDGT		RCT	
	Number of patients	Number (%) of recurrences with grade progression	Number of patients	Number (%) of recurrences with grade progression	Number of patients	Number (%) of recurrences with grade progression
TUR + BCG	363	7 (1.9)	152	18 (11.8)	159	25 (15.7)
TUR	128	3 (2.3)	51	19 (37.2)	46	16 (34.8)
χ^2 test		n.s.*		$p < 0.001^{\dagger}$		$p < 0.01^{\dagger}$

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin;
SFDGT – solitary first diagnosed tumors ≤ 3 cm; MFDGT – solitary first diagnosed tumors > 3 cm and multiple first diagnosed tumors; RCT – recidive tumors;
*** Kolmogorov-Smirnov test; $^{\dagger}\chi^2$ test.**

Table 7

Progression from the stadium 1 to a higher stadium of the disease in both groups of patients

Applied therapy	SFDGT		MFDGT		RCT	
	Number of patients	Number (%) of recurrences with stadium progression	Number of patients	Number (%) of recurrences with stadium progression	Number of patients	Number (%) of recurrences with stadium progression
TUR + BCG	363	6 (1.6)	152	16 (10.5)	159	22 (13.8)
TUR	128	2 (1.6)	51	12 (23.5)	46	13 (28.3)
		n.s.*		$^{\dagger}p < 0.05$		$^{\dagger}p < 0.05$

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin; SFDGT – solitary first diagnosed tumors ≤ 3 cm; MFDGT – solitary first diagnosed tumors > 3 cm and multiple first diagnosed tumors; RCT – recidive tumors;
*** Kolmogorov-Smirnov test; $^{\dagger}\chi^2$ test.**

tumor of the bladder. The results of this study, shown in Table 7, suggest that in the low-risk of NIMBC recurrence there is no significant difference in the progression of the disease, depending on the application of BCG therapy.

Table 8 shows the correlation of NIMBC progression and the time of recurrence according to the applied therapy.

A statistically highly significant correlation between NIMBC progression and the time of recurrence was present in the patients of the group II treated with TUR, as opposed to the group I in which no statistically significant correlation was established. This correlation is graphically shown in Figure 1.

Discussion

The role and significance of intravesical BCG immunotherapy after TUR BT in reducing the rate of recurrence was confirmed by numerous publications. In the study of Gontero et al. [9] it was concluded that intravesical BCG therapy should be considered as the most effective form of intravesical therapy, but the role of this therapy in the progression of the disease in papillary tumors remains to be elucidated. Morales et al. [11] in their work from 1976, which initially included 10 patients, later reduced to 7 patients (1 died of other disease and

Table 8

Correlation of NIMBC progression and the time of recurrence according to the applied therapy

Applied therapy	Coefficient of correlation (r)	*t*-value	*p*
TUR + BCG	-0,1419	1.64	0.10
TUR	0.8131	11,93	0.001

TUR – transurethal resection; BCG – Bacillus Calmette-Guérin.

two had inadequate follow-up), announced that in 7 of these patients, in whom the intravesical BCG therapy was conducted, during 47 months of follow-up, no recurrence observed. Lamm et al. [12] published in 1980 results of a randomized prospective study on comparison, as in our study, the effects of two types of therapy: TUR and TUR + BCG. Their study included 37 patients, the follow-up period was, as with us, a year after completion of the therapy. Out of 19 patients treated with only TUR therapy in 8 (42%) recurrence developed within the follow-up period, and out of 18 patients treated with TUR + BCG therapy, patients, recurrence was registered in 3 (17%) patients. This difference is more pronounced than in our study, but our study included a much larger number of patients. Brandau et al. [3] showed the effectiveness of this therapy based on 30 years of experience, and pointed out that there is still room for improvement in the application of this therapy, indicating thereby that although numerous studies have confirmed the superiority of BCG as adjuvant therapy yet are always possible improvements in the mode of application of this therapy. Vázquez-Lavista et al. [4] showed the importance of this therapy and emphasized the role of BCG as an immunomodulator in patients with NMIBC. A history of the application of this therapy was described by Herr and Morales [8]. About treatment strategy of high risk NMIBC wrote Sharma et al. [5]. Tne incidence and the treatment of complications of BCG therapy were described by Lamm et al. [10] in their research in 1992. Ríos et al. [13] in their work amounts to conclusion that BCG is the most effective adjuvant for patients with NMIBC, especially in high risk patients. Our research verified the importance of this therapy in the same group of patients, MFDGT and RCT. The extent to which medicine has advanced in the application of this therapy, in patients with NMIBC was described by Jacobs et al. [14]. Shelley et al. [15] published a systematic overview of randomized trials and meta-analyzes that confirm the importance and place of this therapy in treatment of patients with NMIBC. An affirmative answer to the question of whether intravesical BCG instillation may reduce the rate of NMIBC recurrence, provided Han and Pan [16] in their study. Recommendations for the treatment of patients with NMBIC in clinical practice were given by Lamm et al. [17]. In spite the fact that BCG has become the most widespread and widely applied intravesical therapy in treating NMIBC [2], recommended by the guidelines of the European Association of Urologists, due to the side effects of this therapy, the critiques and questions whether this therapy is really necessary for all cases with NMBIC are inevitable.

On attempts to modify BCG therapy using a reduced dose of BCG reported Ojea et al. [18] and Martinez-Piñeiro et al. [19]. The results of our research showed that recurrence 3 months after the therapy, depending on the form of the tumor and the applied therapy, occurred in 1–7 of the patients. Such an early occurance of recurrence may open the question of whether it really is a recurrence of illnes or possibly overlooked tumor during the TUR. Brausi et al. [20] in 2002 published the analysis that included 2,410 patients from seven European Organisation for Research and Treatment af Cancer (EORTC) studies that were in the third phase of testing, in which they dealt with the phenomenon of recurrence at the first control cystoscopy. They emphasized that the rate of recurrence depended very much on the institutions, and ranged, in patients with solitary tumor in patients who did not receive adjuvant intravesical therapy, from 3.4% to 20.6%, while for patients with adjuvant intravesical treatment this percentage was from 0% to 15.4%. When it came to patients with multiple tumors with adjuvant intravesical therapy, this percentage ranged from 7.4% to 45.8%.

In our study we found no statistically significant difference in the incidence of recurrence in the patients with low risk NIMBC. Also, when it comes to this subgroup of patients there was no statistically significant difference between any of recurrences, as well as in the incidence of the disease grade progression or the stage of the disease progression. In patients with MFDGT or RCT (patients with high-risk NIMBC) there were significant differences, depending on the applied therapy (BCG + TUR or TUR the only therapy) in terms of the frequency of recurrence, as well as the progression of the disease.

Conclusion

This study indicates the importance of applying BCG intravesical immune therapy after transurethal resection of the tumor, that was also confirmed by the other authors.

However, our research indicates that it is necessary to be selective in the intravesical instillation of Bracillus Calmette-Guérin after transurethal resection of the tumor, because each non-muscle invasive tumor does not require the application of this therapy, which was confirmed by comparing the frequency of recurrence of solitary first diagnosed tumors ≤ 3. We believe that this fact, in addition to the side effects of this therapy, as well as high costs of this treatment, leaves a question whether the use of this therapy is really necessary for each patient with non-muscle invasive tumor.

REFERENCES

1. *Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes J, Bouffioux C, Denis L,* et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006; 49(3): 466−75.
2. *Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J.* EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder. Eur Urol 2008; 54(2): 303−14.
3. *Brandau S, Suttmann H.* Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: a success story with room for improvement. Biomed Pharmacother 2007; 61(6): 299−305.
4. *Vázquez-Lavista LG, Flores-Balcázar CH, Llorente L.* The bacillus Calmette-Guérin as immunomodulator in bladder cancer. Rev Invest Clin 2007; 59(2): 146−52.
5. *Sharma P, Old LJ, Allison JP.* Immunotherapeutic strategies for high-risk bladder cancer. Semin Oncol 2007; 34(2): 165−72.
6. *Bohle A, Jocham D, Bock PR.* Intravesical bacillus Calmette-Guerin versus mitomycin C for superficial bladder cancer: A

formal meta-analysis of comparative studies on recurrence and toxicity. J Urol 2003; 169(1): 90−5.
7. *Ströck V, Dotevall L, Sandberg T, Gustafsson CK, Holmäng S*. Late bacille Calmette-Guérin infection with a large focal urinary bladder ulceration as a complication of bladder cancer treatment. BJU Int 2011; 107(10): 1592−7.
8. *Herr HW, Morales A*. Hystory of bacillus Calmette-Guérin and bladder cancer: an immunotherapy succes story. J Urol 2008; 179(1): 53−6.
9. *Gontero P, Bohle A, Malmstrom PU, O'Donnell MA, Oderda M, Sylvester R*, et al. The role of bacillus Calmette-Guérin in the treatment of non-muscle-invasive bladder cancer. Eur Urol 2010; 57(3): 410−29.
10. *Lamm DL, van der Meijden PM, Morales A, Brosman SA, Catalona WJ, Herr HW,* et al. Incidence and treatment of complications of bacillus Calmette-Guerin intravesical therapy in superficial bladder cancer. J Urol 1992; 147(3): 596−600.
11. *Morales A, Eidinger D, Bruce AW*. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 1976; 116(2): 180−3.
12. *Lamm DL, Thor DE, Harris SC, Reyna JA, Stogdill VD, Radwin HM*. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol 1980; 124(1): 38−40.
13. *Ríos GE, Martínez-Piñeiro LL, Martínez-Piñeiro CJ, de la Peña BJ*. Immunotherapy in superficial bladder carcinoma. Arch Esp Urol 2000; 53(10): 879−92.
14. *Jacobs BL, Lee CT, Montie JE*. Bladder cancer in 2010: how far have we come. CA Cancer J Clin 2010; 60(4): 244−72.
15. *Shelley MD, Mason MD, Kynaston H*. Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses. Cancer Treat Rev 2010; 36(3): 195−205.
16. *Han RF, Pan JG*. Can intravesical bacillus Calmette-Guérin reduce recurrence in patients with superficial bladder cancer? A meta-analysis of randomized trials. Urology 2006; 67(6): 1216−23.
17. *Lamm DL, Colombel M, Persad R, Solowayd M, Böhlee A, Palouf J*. Clinical practise recommendations for the menagment of non-muscle invasive bladder cancer. Eur Urol Suppl 2008; 7(10): 651−66.
18. *Ojea A, Nogueira JL, Solsona E, Flores N, Gómez JM, Molina JR*, et al. A multicentre, randomised prospective trial comparing three intravesical adjuvant therapies for intermediate-risk superficial bladder cancer: low-dose bacillus Calmette-Guerin (27 mg) versus very low-dose bacillus Calmette-Guerin (13.5 mg) versus mitomycin C. Eur Urol 2007; 52(5): 1398−406.
19. *Martínez-Piñeiro JA, Martínez-Piñeiro L, Solsona E, Rodríguez RH, Gómez JM, Martín MG,* et al. Has a 3-fold decreased dose of bacillus Calmette-Guerin the same efficacy against recurrences and progression of T1G3 and Tis bladder tumors than the standard dose? Results of a prospective randomized trial. J Urol 2005; 174(4 Pt 1): 1242−7.
20. *Brausi M, Collette L, Kurth K, van der Meijden AP, Oosterlinck W, Witjes JA,* et al. Variability in the recurrence rate at first follow-up cystoscopy after TUR in stage Ta T1 transitional cell carcinoma of the bladder: a combined analysis of seven EORTC studies. Eur Urol 2002; 41(5): 523−31.

Received on October 30, 2013.
Revised on December 31, 2013.
Accepted on January 28, 2014.
OnLine-First November, 2014.

ORIGINAL ARTICLE

UDC: 616.833::616.727.4
DOI: 10.2298/VSP130417079M

Concoradance of clinical and neurophysiologic diagnoses of carpal tunnel syndrome

Podudarnost kliničke i neurofizološke dijagnoze sindroma karpalnog kanala

Vesna Martić

Clinic for Neurology, Military Medical Academy, Belgrade, Serbia; Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia

Abstract

Introduction/Aim. Clinical presentation and neurophysiological examination are crucial in diagnosing carpal tunnel syndrome (CTS). The aim of this study was to determine sensitivity and specificity of clinical examination for diagnosing of CTS in relation to neurophysiological evaluation. **Methods.** The sample included 181 patients referred to the neurologist for further diagnosis of pain and parestesias in the arm (81 women and 100 men mean age 42 ± 14 years and 52 ± 16 years, respectively). All the patients were neurophysiologicly tested. **Results.** Out of 181 patients, clinical findings were considered positive for CTS in 37 patients. The neurophysiological findings for CTS were positive in 60 patients. Both clinical and neurophysiological findings were positive in 31 patients and both findings were negative in 115 patients (sensitivity 0.51; specificity 0.95). **Conclusion.** Low sensitivity and high specificity suggest that it is easier to exclude rather than to accurately diagnose CTS based on clinical examination alone. Thus, there is the need for neurophysiological evaluation of patients with complains in the arm.

Key words:
carpal tunnel syndrome; diagnosis; signs and symptoms; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Klinička slika i neurofiziološko ispitivanje veoma su značajni za postavljanje dijagnoze sindroma karpalnog tunela (KTS). Cilj ovog istraživanja bio je da se odredi senzitivnost i specifičnost kliničke dijagnoze sindroma karpalnog tunela (KTS) u odnosu na neurofiziološki nalaz. **Metode.** Ispitivanjem je bio obuhvaćen 181 bolesnik (81 žena, prosečne starosti 42 ± 14 godina, i 100 muškaraca, prosečne starosti 52 ± 16 godina). Bolesnici su bili upućeni na neurološki pregled za dalju dijagnostiku tegoba u vezi sa bolovima i parestezijama u ruci. Svi bolesnici su potom neurofiziološki ispitani. **Rezultati.** Od ukupno 181 bolesnika, klinički nalaz za KTS bio je pozitivan kod 37, dok je neurofiziološki nalaz za KTS bio pozitivan kod 60 bolesnika. Kod 31 bolesnika bili su pozitivni i klinički i neurofiziološki nalaz, a oba nalaza su bila negativna kod 115 bolesnika (senzitivnost 0,51, specifičnost 0,95). **Zaključak.** Niska senzitivnost i visoka specifičnost ukazuju na to da je samo na osnovu kliničkog pregleda pouzdanije isključiti, nego potvrditi dijagnozu KTS. Ovo upućuje na potrebu za neurofiziološkom procenom bolesnika sa tegobama u ruci.

Ključne reči:
karpusni tunel, sindrom; dijagnoza; znaci i simptomi; senzitivnost i specifičnost.

Introduction

Common causes of pain in the arm are musculoskeletal disorders and neurological disorders, such as polyneuropathies and compressive mononeuropathies. The most prevalent compressive mononeuropathy is an entrapment of the median nerve as it runs from the forearm through the carpal tunnel into the palm of the hand [1]. This is known as the carpal tunnel syndrome (CTS).

Differentiating CTS from the causes of pain in the arm is complicated by the fact that patients with CTS, in addition to classic symptoms of tingling and pain in the fingers and hand, often complain of pain in other areas (forearm, 21%; elbow 14%; shoulder 8%; cervical spine 0.6%) [2].

Most investigators agree that there is no perfect test for diagnosing CTS. It is believed, however, that CTS is highly probable when typical symptoms are associated with specific objective findings and positive provocative tests. Neurophysiological assessment of propagation of electrical impulses along the median nerve as it passes through the carpal tunnel is considered the "gold standard" for diagnosis of CTS [2].

Since pain in the arm may be of different origin, and CTS may have more or less specific clinical presentation, it is important for clinical practice to ascertain whether the di-

Correspondence to: Vesna Martić, Clinic for Neurology, Military Medical Academy, Crnotravska 17, 11000 Begrade, Serbia.
E-mail: Vesnamartic.bgd@gmail.com

agnosis of CTS can be reliably established on clinical grounds [3]. Therefore, the objective of this study was to evaluate to what extent clinical diagnosis agrees with neurophysiological diagnosis of CTS. The specific aim was to determine sensitivity and specificity of clinical diagnosis of CTS in comparison to neurophysiological findings as the "gold standard".

Methods

Over a 4-year period (2007–2011) 181 patients were referred to the neurologist at the Military Medical Academy in Belgrade for further diagnosis of pain and parestesias in the arm. The sample consisted of 81 women (mean age 42 ± 14 years) and 100 men (52 ± 16 years).

Clinical diagnosis was based on the history and clinical examination. The mandatory symptoms considered specific for CTS [1] were tingling in the first three fingers and along inside of the fourth finger mainly present in the evening or morning hours or accompanied by waking up to shake the hand (Flick's sign) followed by relief. Clinical examination considered muscle strength and trophic changes, reflex activity and impaired sensation. The provocative tests used were Bickeles and Tinnel sign [4].

Neurophysiological evaluation was performed on the median, ulnar, and radial nerves according to the standards of the American Association of Electrodiagnostic Medicine [5]. Motor nerve studies included measurements of the latency and amplitude of the motor nerve action potential, conduction velocity, and latency of the F-wave. In sensory nerve studies, we measured the latency and amplitude of the sensory nerve action potential and conduction velocity.

For motor studies, recording electrodes were placed over the thenar (median) nerve and hypotenar (ulnar) nerve with the stimulation electrode at the wrist, 8 cm proximally. For sensory studies, recording electrodes were placed on the second finger (median nerve) and the fifth finger (ulnar nerve) with stimulation electrodes at the wrist, 14 cm proximally [6].

The most sensitive neurophysiological parameter for the diagnosis of CTS is considered the difference between terminal latencies of the sensory responses recorded from the fourth finger after stimulation of the median and ulnar nerves at the wrist 14 cm proximally. CTS is considered present if the median response is at least 0.5 ms longer than the ulnar response.

Needle electromyography was used for the muscles innervated by C5-Th1 roots (*deltoid*, *biceps brachii*, *extensor digitorum communis*, *abductor policis brevis*, and *abductor digiti minimi*). Based on features of motor unit potential (shape, duration), the findings were classified as normal or neurogenic [7].

The data were tabulated in a 2 × 2 table where rows and columns included the frequecies of positive and negative clinical and neurophysiological findings (Clinical +, Clinical -, EMG +, EMG -). Based on the frequency distribution, we calculated sensitivity, specificity, positive predictive value, and negative predictive value using standard formulas.

Sensitivity is the ratio between the true positive findings (EMG CTS +) and the sum of true positive (EMG CTS +) and false negative (Clinical CTS -) findings. Specificity is the ratio between the true negative findings (EMG CTS -) and the sum of true negative (EMG CTS -) and false positive (Clinical CTS +) findings. The positive predictive value is the ratio between the true positive findings (EMG CTS +) and the sum of true positive (EMG CTS +) and false positive (Clinical CTS -) findings. Lastly, the negative predictive value is the ratio between the true negative findings (EMG CTS -) and the sum of true negative (EMG CTS -) and false negative (Clinical CTS -) findings. The statistical software Prism 5 (GraphPad Software Inc., La Jolla, CA) was used for statistical analysis.

The results were compared with the literature data.

Results

In 181 patients clinical findings were considered positive for CTS in 37 patients and negative in 144 patients. The neurophysiological findings for CTS were positive in 60 patients and negative in 121 patients. Both clinical and neurophysiological findings were positive in 31 patients and both findings were negative in 115 patients (Table 1).

Table 1

Distribution of patients (n, %) with positive (+) and negative (-) clinical and neurophysiological diagnoses of carpal tunnel syndrome (CTS)

Clinical diagnosis	Neurophysiological diagnosis		Total
	CTS +	CTS -	
CTS +	31 (17)	6 (3)	37 (20)
CTS -	29 (16)	115 (64)	144 (87)
Total	60 (33)	121 (67)	181 (100)

Sensitivity = 31/(31 + 29) = 51%;
Specificity = 115/(115 + 6) = 95%;
Positive predictive value = 31/(31 + 6) = 84%;
Negative predictive value = 115/(115 + 29) = 80%.

The results indicated moderate sensitivity (51%) and high specificity (95%) of clinical evaluation (Table 2).

One patient diagnosed with CTS on both clinical and neurophysiological grounds also had polyneuropathy. Among 114 with clinical diagnosis negative for CTS, symptoms and signs suggested cervicobrachialgia in 85 patients and polyneuropathy in the remaining 29 patients (Figure 1).

In cases of disagreement between clinical and neurophysiological diagnoses, clinical diagnosis of CTS was made

Table 2

Diagnostic utility of the clinical diagnosis of carpal tunnel syndrome (CTS) with the corresponding 95% confidence intervals (CI) and the definition of each diagnostic parameter

Parameters	Value	95% CI	Definition
Sensitivity	0.51	0.38–0.65	Fraction of patients with CTS correctly diagnosed based on clinical findings.
Specificity	0.95	0.90–0.98	Fraction of patients without CTS correctly diagnosed based on clinical findings.
Positive prognostic value	0.84	0.68–0.94	Fraction of patients with positive clinical findings who have CTS.
Negative prognostic value	0.80	0.72–0.86	Fraction of patients with negative clinical findings who do not have CTS.

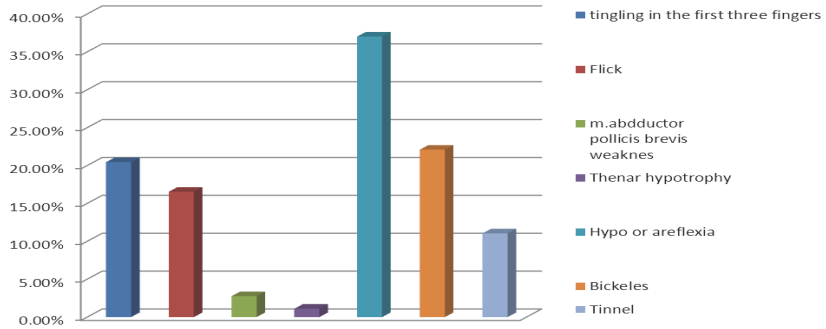

Fig. 1 – Clinical symptoms in patients population

in 6 of the patients where as neurophysiological findings indicated cervicobrachialgia in 4 of the patients (Table 3), ulnar nerve compression in 1 of the patient, and normal results in 1 of the patients.

trodiagnosis of neuromuscular diseases. The results indicated moderate sensitivitly (51%) and high specificty (95%) of clinical evaluation. In other words, only about half of the patients with positive neurophysiological findings for CTS are

Table 3

Clinical symptoms in the patients with the clinical diagnosis of carpal tunnel syndrom (CTS) and neurophysiological findings of cervicobrachialgia

Clinical symptoms	Number of patients		Total
	with	without	
Tingling in the first three fingers	4	0	4
Flick sign	0	4	4
M. abdductor pollicis brevis weaknes	0	4	4
Thenar hypotrophy	0	4	4
Hypo or areflexia	2	2	4
Bickeles sign	1	3	4
Neck pain	1	3	4
Hypo-esthesia	1	3	4

The patient with ulnar nerve compression in Guyon canal complained about tingling of the fourth finger of the hand; in his case Tinnel and Flick sings were positive, but reflexes were symmetrical with normal muscle strength in his hand.

Among the 29 patients with neurophysiologically verified CTS that was misdiagnosed on clinical grounds, the assigned clinical diagnoses were cervicobrachialgia in 17, polyneuropathy in 5, paresthesias in 2, polymialgia in 1 and undetermined in 3 of the patients.

Among the patients with cervicobrachialgia, 13 had pain in their forearm, 3 in the shoulder and 1 in the cervical spine.

In all 5 patients with clinically diagnosed polyneuropathia, CTS were present on both sides.

Discussion

The aim of this study was to determine to what extent the diagnosis of CTS can be reliably established on clinical grounds by the neurologist with extensive experience in electrodiagnosis of neuromuscular diseases. The results indicated moderate sensitivitly (51%) and high specificty (95%) of clinical evaluation. In other words, only about half of the patients with positive neurophysiological findings for CTS are likely to be correctly identified on clinical grounds, whereas almost all patients without CTS are likely to be assigned other diagnosis than CTS. This indicates a higher likelihood to exclude rather than to ascertain the diagnosis of CTS based on clinical evaluation alone.

Several factors must be taken into account when interpreting our results. Electrophysiological findings may be false positive (asymptomatic median neuropathy) in almost 18% of the general population, mostly among people with diabetes [3] of whom 25% are expected to eventually develop symptomatic CTS after 6 to 11 years [8, 9]. Although some patients in this study had diabetes, none were asymptomatic. Because all patients complained of pain in the arm, for which they were sent for further work-up, the likehood of false positive neurophysiological findings of CTS confounding the results is rather low.

Differential diagnosis of CTS *versus* other median nerve neuropathy is complicated by the fact that 10% of patients with CTS may show slowing of motor conduction ve-

locity proximally to the wrist, leading to the degeneration of the fastest axons (retrograde axonal degeneration). Still their neurophysiological findings may be within the normative range. To account for this, some authors consider CTS present only if the latency of the median motor response is 1.8 ms as longer as the ulnar nerve response [10].

An alternative criterion is a 1 ms longer latency of the median nerve motor response in the symptomatic hand compared to the opposite hand [11]. This approach, however, is rarely useful because of high prevalence of bilateral CTS.

In this study, only 5 patients with neurophysiological findings consistent with CTS were clinically misdiagnosed as having other type of neuropathy.

Conversely, pain and other symptoms of CTS may be present even when neurophysiological results are normal or minimally abnormal. It is recognized that the degree of motor and sensory nerve involvement is not necessarily proportional to the duration or severity of symptoms [9]. In this study, however, neurophysiological findings were within the normative limits only in 1 patient who was ascribed the diagnosis of CTS on clinical examination.

Conclusion

A relatively low sensitivity (51%) but high specificity (95%) of clinical diagnosis suggests that it is easier to exclude rather than to accurately diagnose carpal tunnel syndrome based on clinical examination alone. Therefore, there is the need for routine and comprehensive neurophysiological evaluation of patients who complain of pain in the arm.

REFERENCES

1. American Association of Electrodiagnostic Medicine, American Academy of Neurology, and American Academy of Physical Medicine and Rehabilitation. Practice parameter for electrodiagnostic studies in carpal tunnel syndrome: summary statement. Muscle Nerve 2002; 25(6): 918−22.
2. American Association of Electrodiagnostic Medicine. Literature review of the usefulness of nerve conduction studies and needle electromyography for the evaluation of patients with carpal tunnel syndrome. Muscle Nerve Suppl 1999; 22: S145−67.
3. *Mackinnon SE, Novak CB, Landau WM*. Clinical diagnosis of carpal tunnel syndrome. JAMA 2000; 284(15): 1924−5; author reply 1925−6.
4. *Nathan P, Takigawa K, Keniston R, Meadows K, Lockwood R*. Slowing of sensory conduction of the median nerve and carpal tunnel syndrome in Japanese and American industrial workers. J Hand Surg 1994; 19(1): 30−4.
5. *Neligan A, O'Sullivan SS, Mullins GM, McCarthy A, Kowalski RG, Kinsella J*, et al. A review of nerve conduction studies in cases of suspected compression neuropathies of the upper limb. Eur Neurol 2010; 63(1): 11−6.
6. *Pease WS, Cannell CD, Johnson EW*. Median to radial latency difference test in mild carpal tunnel syndrome. Muscle Nerve 1989; 12(11): 905−9.
7. *Sander HW, Quinto C, Saadeh PB, Chokroverty S*. Sensitive median-ulnar motor comparative techniques in carpal tunnel syndrome. Muscle Nerve 1999; 22(1): 88−98. Seror P. Tinel's sign in the diagnosis of carpal tunnel syndrome. J Hand Surg 1987; 12(3): 364−5.
8. *Stevens JC, Smith BE, Weaver AL, Bosch EP, Deen HG, Wilkens JA*. Symptoms of 100 patients with electromyographically verified carpal tunnel syndrome. Muscle Nerve 1999; 22(10): 1448−56.
9. *Werner RA, Franzblau A, Albers JW, Buchele H, Armstrong TJ*. Use of screening nerve conduction studies for predicting future carpal tunnel syndrome. Occup Environ Med 1997; 54(2): 96−100.
10. *Werner RA, Andary M*. Carpal tunnel syndrome: pathophysiology and clinical neurophysiology. Clin Neurophysiol 2002; 113(9): 1373−81.
11. *You H, Simmons Z, Freivalds A, Kothari MJ, Naidu SH*. Relationships between clinical symptom severity scales and nerve conduction measures in carpal tunnel syndrome. Muscle Nerve 1999; 22(4): 497−501.

Received on April 17, 2013.
Revised on February 24, 2014.
Accepted on April 1, 2014.
OnLine-First December, 2014.

ORIGINAL ARTICLE

UDC: 33:657.478]::[616-07/-08::616.6-07/-08
DOI: 10.2298/VSP131108078B

Comparative analysis of the current payment system for hospital services in Serbia and projected payments under diagnostic related groups system in urology

Komparativna analiza aktuelnog načina plaćanja bolničkih usluga u Srbiji i projektovanog plaćanja po sistemu dijagnostički srodnih grupa u urologiji

Uroš Babić*, Ivan Soldatović[†‡], Dejana Vuković[‡§], Milena Šantrić Milićević[†‡], Mihailo Stjepanović[‖], Dejan Kojić*, Aleksandar Argirović[¶], Vinka Vukotić*[‡]

***Clinical Hospital Center „Dr Dragiša Mišović" – Dedinje, Belgrade, Serbia; [†]Institute for Medical Statistics and Informatics; [§]Institute for Social Medicine, Belgrade, Serbia;[‡]Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [‖]Clinic for Pulmology, Clinical Center of Serbia, Belgrade, Serbia; [¶]Clinical Hospital Center Zemun, Belgrade, Serbia**

Abstract

Background/Aim. Global budget *per* calendar year is a traditional method of funding hospitals in Serbia. Diagnose related groups (DGR) is a method of hospital payment based on classification of patients into groups with clinically similar problems and similar utilization of hospital resources. The aim of this study was to compare current methods of hospital services payment with the projected costs by DRG payment method in urology. **Methods.** The data were obtained from the information system used in the Clinical Hospital Center "Dr. Dragiša Mišović" – Dedinje in Belgrade, Serbia. The implemented hospital information system was the main criterion for selection of healthcare institutions. The study included 994 randomly selected patients treated surgically and conservatively in 2012. **Results.** Average costs under the current payment method were slightly higher than those projected by DRG, however, the variability was twice as high (54,111 ± 69,789 compared to 53,434 ± 32,509, $p < 0{,}001$) respectively. The univariate analysis showed that the highest correlation with the current payment method as well as with the projected one by DRG was observed in relation to the number of days of hospitalization ($\varrho = 0.842$, $p < 0.001$, and $\varrho = 0.637$, $p < 0.001$, respectively). Multivariate regression models confirmed the influence of the number of hospitalization days to costs under the current payment system ($\beta = 0.843$, $p < 0.001$) as well as under the projected DRG payment system ($\beta = 0.737$, $p < 0.001$). The same predictor was crucial for the difference in the current payment method and the projected DRG payment methods ($\beta = 0.501$, $p < 0.001$). **Conclusion.** Payment under the DRG system is administratively more complex because it requires detailed and standardized coding of diagnoses and procedures, as well as the information on the average consumption of resources (costs) *per* DRG. Given that aggregate costs of treatment under two hospital payment methods compared in the study are not significantly different, the focus on minor surgeries both under the current hospital payment method and under the introduced DRG system would be far more cost-effective for a hospital as great variations in treatment performance (reduction of days of hospitalization and complications), and consequently invoiced amounts would be reduced.

Key words:
hospitals; financial management; urology; serbia.

Apstrakt

Uvod/Cilj. Ukupni određeni budžet za kalendarsku godinu je tradicionalni način finansiranja bolnica u Srbiji. Dijagnostički srodne grupe (DSG) je način plaćanja u bolnicama koji je baziran na klasifikaciji bolesnika po grupama sa sličnim dijagnozama, gde se očekuje slična iskorišćenost bolničkih resursa. Cilj ovog rada bilo je poređenje aktuelnog načina plaćanja bolnice i projektovanih troškova pomoću metode plaćanja prema DSG u urologiji. **Metode.** Podaci su dobijeni iz informacionog sistema koji se koristi u Kliničko-bolničkom centru (KBC) „Dr Dragiša Mišović" – Dedinje u Beogradu, Srbija. Glavni kriterijum izbora ustanova bio je uspostavljen bolnički informacioni sistem. U studiju je uključeno nasumično izabranih 994 bolesnika lečenih operativno i konzervativno u 2012. godini. **Rezultati.** Prosečni troškovi po aktuelnom načinu plaćanja bili su malo veći od projektovanog DSG, ali je varijabilitet bio dva puta veći (54 111 ± 69 789 prema 53 434 ± 32 509; $p < 0{,}001$). Univarijantnom analizom utvrđeno je da najveću korelaciju sa

Correspondence to: Uroš Babić, Strahinjića bana 66, 11 000 Belgrade, Serbia. Phone: +381 63 305 600. E-mail: urosb2001@yahoo.com

aktuelnim načinom plaćanja, kao i sa projektovanim pomoću DSG, ima broj dana hostpitalizacije (ϱ = 0,842; p < 0,001 i ϱ = 0,637; p < 0,001). Multivarijantnim regresionim modelima potvrđen je uticaj broja dana hospitalizacije na troškokove prema aktuelnom sistemu plaćanja (β = 0,843; p < 0,001), kao i prema projektovanom DSG sistemu plaćanja (β = 0,737; p < 0,001). Isti prediktor bio je i ključni za razliku aktuelnog načina plaćanja i projektovanog DSG načina plaćanja (β = 0,501; p < 0,001). **Zaključak.** Plaćanje prema DSG administrativno je složenije jer zahteva detaljno i standardizovano kodiranje dijagnoza i procedura, kao i informacije o prosečnoj potrošnji resursa (troškova) prema DSG. S obzirom na to da zbirni troškovi lečenja koji se plaćaju na dva načina, poređena u istraživanju, nisu bitno različiti, fokus na manje hirurške intervencije i u aktuelnom načinu plaćanja bolnica i po uvođenju plaćanja po DSG bio bi daleko isplatljiviji za bolnicu jer bi se smanjile velike varijacije u performansama lečenja (smanjenje dana hospitalizacije i komplikacija), a posledično i računa.

Ključne reči:
bolnice; finansije, upravljanje; urologija; srbija.

Introduction

Hospital services costs account for a large share of total spending on health care, regardless of whether the global budget, payment-for-service, case-based payment of diagnose-related groups (DRG) are used as hospital services funding mechanism [1]. The problem of financing secondary and tertiary level healthcare institutions as the greatest "consumers" in the health care system has been analyzed for more than 100 years including recently published national assessments [2]. The methods of hospital services payment are numerous, but neither of them is perfect enough in terms of ensuring both fair remuneration for performed work and medical supplies, and the control of costs of hospital treatment and care [3].

In view of developing programs to assess the utilization and quality of health insurance in a local hospital, in 1960s, Robert Vettel and his colleagues from the Yale University calculated all possible costs of optimal treatment of patients classified in DRG based on certain characteristics and severity of illness [4]. Thirty years later, a case-based payment and DRG-based payment are the main methods of hospital services payment for patients with acute conditions in most high income countries [5–8]. Measuring of costs *per* DRG creates opportunities for improving efficiency, because patients with certain characteristics and within clinically similar problems are classified into groups with similar costs of treatment and care under this approach [9]. By categorizing patients into groups with similar utilization of hospital resources, DRGs describe hospital activities in standardized units and enable the analysis, which otherwise would not be easy because hospitals treat many patients, each of whom seem to have unique health condition. The basic idea of DRG-based payment is the payment *per* episode, where the episode is deemed a period from admission to discharge, while all the costs incurred during that period are included in the price. This method of payment is commonly called "case-based payment" [10].

Under the DRG system, hospital managers and policy makers can compare hospitals or different hospital departments by length of stay, costs and quality [11]. DRG-based payment method in developed countries has clearly demonstrated to bring better quality of work and resource savings [12]. In countries in which global budget is used as a model for hospital payment, hospital management has little information on what types of services have been provided to patients and at what price in hospital wards or departments. Theoretically, the DRG-based payment provides a strong incentive for increasing the number of cases of treatment (as opposed to the global budget) and for rationalizing the number of services provided per case (as opposed to the payment-for-service system).

The global budget is a traditional method of funding hospitals in Serbia [13]. By purchasing the healthcare plan of certain healthcare institutions by Health Insurance Fund (HIF) for a calendar year, the budget of the institution is "prospectively" defined, and a fixed payment for a specified level of activity (usually determined by the number of cases or the number of hospital days) narrows the scope for improving technical and allocative efficiency and performance quality [14, 15]. By adopting the healthcare development plan by 2015, the Ministry of Health has envisaged the implementation of the DRG system for reimbursing costs of hospitalized patients with acute conditions. The induction program should be implemented in stages; originally it would be used as an analytical tool for coding, then for obtaining statistical data on hospital treatment performance, and in the final stage it would be introduced as a system for the collection of payment for rendered hospital services.

In order to identify comparative strengths and weaknesses of two methods of hospital payment and formulate recommendations to decision makers, the objective of this paper was to compare current hospital payment methods and DRG-based payment methods at the Department of Urology at the Clinical Hospital Center (CHC) "Dr. Dragiša Mišović" – Dedinje, Belgrade, Serbia.

Methods

For the purposes of this study, the data obtained from the information system used in the CHC "Dr Dragiša Mišović" – Dedinje, were processed under the pilot project of implementation of the Australian DRG model in acute patients hospital care in four healthcare institutions and the CHC "Dr Dragiša Mišović" – Dedinje was one of such institutions (the main criterion of healthcare institution selection was the implementation of the hospital information system).

The study included 994 randomly selected patients treated surgically and conservatively at the Department of Urology, CHC "Dr Dragiša Mišović" – Dedinje, in 2012. The following variables were monitored: age categorized in-

to ten-year intervals, sex, place of residence, hospital, diagnose under the International Classification of Diseases – ICD-10, medical procedures performed during hospitalization, co-morbidities accompanying the main cause of hospitalization [(based on the ASA score created by American Society of Anesthesiologists (ASA) for the assessment of physical status of patients before surgery [16]], postoperative complications (according to the Clavien scale which is a global score that assesses postoperative course [17]) and the final report on the treatment (electronic report on electronic invoices issued to the Serbian Health Insurance Fund). All the patients were determined the ASA score on admission, while the complications were monitored against the Clavien scale. The hypothetical price *per* DRG model was calculated by using the Croatian DRG grouper and the coding rules available at the website of the Croatian Institute for Health Insurance in force since 2007 [18]. The price obtained by using the Croatian grouper for a particular DRG group was converted in RSD based on the real exchange rate between two countries and divided by two as the spending on healthcare in Croatia was by twice higher than in Serbia in absolute figures in Euro.

Descriptive and analytical statistical methods were used in this study. As regards the descriptive methods, absolute and relative numbers, measures of central tendency (arithmetic mean, median) and the measures of dispersion [statistical deviation (SD) and interval of variation] were used. As regards analytical methods, difference tests (*t*-test and Kruskal-Wallis test) and correlation analysis (Spearman's and Pearson's correlation analysis, linear regression analysis) were used. All the data were processed by SPSS 15.0 (Chicago, Illinois, USA) statistical software.

Results

The study covered 994 patients in total. Of the total number of patients, 781 (78.6%) patients were male, while 213 (21.4%) patients were female. The average age of patients was 63.9 ± 14.7 years, with the median age of 66 years. Of the total number of patients who entered the study, 835 (84%) patients came from urban, while 159 (16%) patients came from rural areas. The patients with the diagnosed bladder cancer (C67) (32.3%) accounted for the highest percentage, followed by patients with benign prostate enlargement (N40) (16.8%), urinary tract calculosis (N20) (9.9%), prostate cancer (C61) (7.7%), urethral stenosis N35 (6.4%), while the remaining 23 diagnoses accounted for the percentage less than 5%. One-third of patients, i.e. 310 (31.2%) patients respectively had the ASA score of 0, while 278 (28%) of them had the ASA score of 1, 309 (31.1 %) patients had the ASA score of 2, while only 67 (6.7%) patients had the ASA score of 3. Age correlated with ASA score and among older patients those with the highest ASA score were the most numerous ($\tau = 0.433$; $p < 0.001$).

The majority of patients were treated surgically, 961 (96.7%) patients, while 33 (3.3%) patients were treated conservatively. The procedures of transurethral resection of bladder tumors, 230 (23.9%) and prostate cancer 129 (13.4%), accounted for the highest number, followed by 83 (8.6%) ureteroscopic lithotripsy (URS) and lithotripsy procedures and 82 (8.5%) cystoscopy procedures. According to the Clavien scale, the majority of patients, 817 (82.2%), had no complications, while less than one-fifth of patients had surgery complications: 62 (6.2%) patients had the Clavien scale score of 1 48 (4.8%) patients had the Clavien score of 3, and 34 (3.4%) patients had the Clavien score of 2. The highest rate of complications was observed in radical cystectomy, followed by transvesical prostatectomy, ureterorenoscopy, pyelolithotomy.

The average number of days of hospitalization was 4.9 ± 4.4 days, with the median of 4 days. The minimum number of days of hospitalization was 1, while the highest number of hospitalization days was 37. The highest average number of days was observed in patients who underwent radical cystectomy (18.6), followed by patients who underwent pyelolithotomy (15.0). The highest total number of hospital days was observed in patients with transuretheral resection (TUR) of bladder tumors (731) and transureteral resection of the prostate (TURP) (598). There is a weak positive correlation between the age and the number of days of hospitalization ($\rho = 0.197$; $p < 0.001$). Further analysis identified a positive correlation between the average number of days of hospitalization and the ASA score ($\rho = 0.301$; $p < 0.001$). Analogously to the ASA score, the average number of days of hospitalization grew in parallel with the Clavien score growth ($\rho = 0.457$; $p < 0.001$).

All of the factors (age, ASA score, and Clavien scale) were statistically significant predictors of the average number of days of hospitalization, but on the basis of the standardized beta coefficient it was established that the Clavien was the most important predictor in terms of extending the number of days of hospitalization (Table 1). The explained variability of the number of days of hospitalization with these three predictors was $r^2 = 0.260$.

The total treatment costs under the current payment system and projected DRG for patients treated at the Depart-

Table 1

Regression models with days of hospitalization, the method of payment, and the difference between the models of payment as dependents

Predictor	Method of payment		Difference between actual and DRG	Days of hospitalization
	Actual	DRG		
Age	-0.040 (0.035)	-0.044 (0.092)	-0.053 (0.068)	0.092 (0.004)
ASA score	0.023 (0.243)	-0.003 (0.924)	0.053 (0.075)	0.171 (< 0.001)
Clavien	0.036 (0.057)	-0.046 (0.074)	0.208 (< 0.001)	0.418 (< 0.001)
Days of hospitaization	0.843 (< 0.001)	0.737 (< 0.001)	0.501 (< 0.001)	

The results are presented as std. β (*p* - value); DRG – diagnostic-related groups; ASA – American Society of Anesthesiologists.

ment of Urology, though they seemed to be similar, were statistically significantly different (t = -15 516; $p < 0.001$) as the variability of costs was twice as higher under the current payment method (Table 2). The highest average costs under the current payment model were observed in case of radical cystectomy, pyelolitomy, nephrectomy and nephroureterectomy, and also the highest total cost of cystectomy; however, great costs were incurred in case of prostatectomy, nephrectomy. The average costs of certain procedures *per* DRG model were quite different from the costs incurred under the current payment model, while certain costs were similar. Also, there are differences and similarities in the amounts, depending on the type of surgery.

Table 2

Costs of therapy in RS dinar by the current method and the projected by DRG

Method of payment	$\bar{x} \pm SD$	Median (min-max)	Sum
Actual	54,111 ± 69,789	30,533.5 (1,594–858,882)	53,786,466
DRG	53,434 ±,32,509	41,152.0 (14,524–201,764)	53,060,346

DRG – diagnostic-related groups.

Further analysis revealed a high statistical correlation between the number of days of hospitalization and the costs under the current payment system ($\rho = 0.842$; $p < 0.001$), as well as between the number of days of hospitalization and DRG ($\rho = 0.637$; $p < 0.001$).

Regression models showed that the number of days of hospitalization was the most important predictor of the amount of invoice for hospitalization based on the current calculation method and based on the DRG projected model (Table 1). The explained variability was rather high ($r^2_{\text{current method}} = 0.739$; $r^2_{DSG} = 0.502$).

Complicated and expensive surgeries are more favorable for the clinic if the costs are calculated under the DRG model, rather than on the current payment system (Table 3). Average differences and amounts for certain diagnoses were attributable to DRG model, while some were attributable to the current payment system.

A new variable has been created and it represents the difference in invoiced costs based on current payment method and DRG model (Table 1). The correlation analysis revealed a highly significant statistical correlation between the Clavien scale and the difference in invoiced amounts and DRG ($\rho = 0.381$; $p < 0.001$). This correlation was by far greater than in case of ASA score ($\rho = 0.225$; $p < 0.001$).

In view of identifying the correlation between the two methods of cost calculation for hospitalization and the number of days of hospitalization, it was established that this correlation is weak in the first ten days but that it became stronger by the increase of the number of days of hospitalization ($\rho = 0.685$; $p < 0.001$). Finally, the number of days of hospitalization and complications in the form of the Clavien score were the most important predictors of the difference between the current payment method and DRG model (Table 1).

Discussion

The main reason for the popularity of the hospital payment system based on DRG is that it is considered to have the most desirable effect on the efficiency and quality as it encourages hospitals to reduce costs and increase revenue

Table 3

Differences in the costs of procedures between the actual method of payment and the projected DRG in RS dinar

Procedure	n	$\bar{x}$	Sum	Procedure	n	$\bar{x}$	Sum
Circumcision	10	-6,417.3	-6,4173	Transvesical prostatectomy	39	10,031.3	391,222
Placement of renal stent	13	-2,537.1	-32,983	Radical cystectomy with ileal conduit	22	193,795.4	4,263,499
Transuretral resection of the bladder tumor	230	-15,364.8	-3,533,925	Radical prostatectomy	41	32,575.6	1,335,601
Radical orchiectomy	9	770.0	6,930	Radical nephrectomy	27	4,224.2	114,054
Transurethral resection of the prostate (TURP)	129	-4,066.8	-524,622	Partial nephrectomy	21	-4,116.7	-86,451
Bilateral orchiectomy	30	-1,994.2	-59,826	Ureterolithotomy	5	28,460.6	142,303
Ureterorenoscopy lithotripsy	83	-5,210.6	-432,487	Percutaneous nephrolithotomy	4	16,243.5	64,974
Transurethral resection of bladder neck	12	-7,596.6	-91,160	Nephrectomy	10	62,385.4	623,854
Cystoscopy	82	-13,779.1	-1,129,886	Ureterocystoneostomy	5	9,776.2	48,881
TURP with uretrotomy	10	-10,885.6	-108,856	Pyelolithotomy	3	147,930.6	443,792
Testicular biopsy	1	-133,62.0	-13,362	Nephroureterectomy	8	28,182.6	225,461
Ligation of spermatic vein	16	-16,964.4	-271,430	Explorative laparotomy	3	14,444.3	43,333
Punction of renal cyst	7	-39,434.7	-276,043	Cystolithotomy	2	25,533.5	51,067
Internal uretrotomy	38	-41.2	-1,566	Pyeloplasty	4	16,823.0	67,292
Marsupielisation	21	-17,996.6	-377,929	Laparoscopic nephrectomy	1	10,726.0	10,726
Orchiectomy	11	13,070.8	143,779	Hydrocele operation	34	-2,046.4	-69,578
Transobturator tape	8	28,268.0	226,144	Penile surgery	9	-3,171.2	-28,541
Percutaneous nephrostomy	7	-18,688.3	-130,818	Prostate biopsy	5	-19,569.2	-97,846

DRG – diagnostic-related groups.

per treated patient and to increase the number of patients [18]. Case-based costs in hospitals can be reduced by shortening the length of stay, reducing the intensity of service and selecting patients to whom hospitals may provide treatment at costs below the payment rates under DRG [19–20]. The results of this study confirmed that the total amount of a hospital invoice was really affected mostly by the number of treatment days and treatment complications measured by Clavien scale. It has been established that as the age increases, the larger the proportion of patients with co-morbidities and complications, and that the incidence of complications is higher in patients with higher ASA score.

By replacing the system of retrospectively determined fee-for-service with the DRG payment model, hospitals in the USA and some European countries have received strong incentives to reduce costs since DRG contributed to increased transparency in the provision of healthcare services and hospitals are encouraged to invest in quality improvement which leads to cost reduction (for example, by infection control measures and improving surgical technology) [21]. However, in Europe the replacement of the global hospital budgeting was supposed to increase hospital efficiency [1], and since by the introduction of DRG system hospitals have received an incentive to reduce costs, the effects of the DRG payment system on the quality of healthcare services should be indicated [22]. The hospital performance efficiency increase is contributed by the shortened length of stay, optimized treatment and care, and reduced intensity of providing unnecessary and duplicated services [23]. However, shortening of the stay can lead to inappropriate early ("bloody") discharge, and the intensity of services may be reduced to ensuring minimum services, resulting in poor quality services [24]. Hospitals can be more efficient and ensure better quality by specializing in treatments available only to patients with whom they can achieve a competitive advantage (as they have more qualified staff or ensure better quality services). However, there is a risk that hospitals will focus only to those patients whose treatment costs are expected to be lower than DRG group costs (so-called "cream skimming"), for example, by selecting patients without adverse effects if they are not adequately included in the DRG system, or that they "will" reject unprofitable patients transferring them to other hospitals, or just avoid them [25–26]. The abovementioned is demonstrated by this study, according to which in case of introduction of DRG system it would be more cost effective for a hospital to carry out less costly interventions than expensive surgical procedures. Therefore, the application of the DRG-based payment method must be continuously improved and revised dynamically through mutual cooperation of healthcare professionals and health insurance organizations [5]. For example, in Germany and Netherlands, the DRG payment system operates within the global budget and the incentives to hospital productivity are lower than in England, where hospital activity is not limited by the global budget.

The introduction of DRG-based payment method should facilitate monitoring and comparison of hospital service quality since hospitals are encouraged to improve the coding of diagnoses and procedures which improves the quality of data on hospital activity and costs are reduced if measures to improve the quality of work are introduced, such as better coordination between hospitals, providers of outpatient services, and facilities providing long-term care. However, hospitals may be tempted to "make savings" on the quality by avoiding to conduct certain diagnostic tests, neglecting hygiene standards and reducing the number of staff per bed, since DRG does not specify which services should be provided during the treatment of a particular patient. In the famous article from 2012, Volkmer et al. [22] came to the conclusion that physician and anesthetic skills and practices influenced the results of treatments in urology and stressed the need to adapt, amend and adjust the German DRG hospital payment method at least once a year, and even more frequently as appropriate. Every year since 2007, German urologist German Wenke et al. [27] have analyzed the effects of coefficients, comorbidities and the introduction of new treatment options to the DRG payment method, and propose necessary amendments to the content and adaptations to the National Centre for DRG Monitoring.

DRGs are different with respect to the criteria for defining patient groups: health problems similarity (diagnosis, condition, need for healthcare), treatment outcome (real health condition), the treatment method (intervention, procedure, etc.), usefulness of treatment (value, counter-value in money, health benefits), prognosis (expected health condition) and the treatment costs (resources utilization). Some methods combine several criteria, such as clinical attributes of patients and the treatment costs, widely known as the "case-mix". However, both methods can not accurately classify each patient into a particular category, and therefore we have the episodes of treatment with costs higher than average costs of DRG to which the patient belongs, called "episodes of extreme outliers" [28]. In our research, the episodes of extreme outliers are the maximum values observed in patients who have underwent radical cystectomy. "Episodes of high outliers" are usually additionally paid for each day above a certain threshold, which is called "a trim day". A trim day is usually three times longer than the average stay for a particular DRG. So the trim day for a DRG with an average length of stay of five days would be the day 15 which means that the hospital is entitled to additional payment for each day after the day 16 onwards [29–30].

The DRG payment method for hospital services ensures "benchmark competition" because DRG prices are set against the level of average costs of all hospitals [31]. If a hospital defines a DRG at the price below average costs compared to other hospitals, it has a direct benefit and retains the generated financial surplus; if the hospital does not perform as expected it generates a deficit, and it will be ultimately exposed to the risk of bankruptcy. All hospitals, including the most effective are motivated to continually reduce costs. If a DRG does not control the differences between the patient groups or differences in services provided (within the DRG) sufficiently, the amounts due for very complicated cases may be too low, while the amounts due for less complex cases may be too high. Accordingly, hospitals may try to avoid the risks of treating complex patients.

Along with the implementation of the DRG-based payment method, there are a number of options in practice for hospitals to increase (technical and financial) efficiency, as well as to avoid duplication and unnecessary tests, replace costly hospitalization with less expensive alternative treatments with similar efficiency, and improve treatment technologies (for example, using the Protocol for evaluating the reasons for hospitalization, clinical protocols and clinical guidelines), thus reducing the length of stay, as some studies have shown that about 20% of hospital days are completely unjustified, and that clinical guidelines are not used in the daily work to an extent in which they should be used [32–36]. In the field of urology, Serbian hospitals can apply clinical practice guidelines, which are published annually by the European Association of Urologists and the American Association of Urologists.

Conclusion

Payment under the DRG system is administratively more complex because it requires detailed and standardized coding of diagnoses and procedures as well as the information on the average consumption of resources (costs) per DRG. Given that aggregate costs of treatment under the two hospital payment methods compared in the study are not significantly different, the focus on minor surgeries both under the current hospital payment method and under the introduced DRG system would be far more cost-effective for a hospital as great variations in treatment performance (reduction of days of hospitalization and complications), and consequently invoiced amounts, would be reduced.

DRG can be a good tool for measuring the efficiency and performance of each hospital units and departments in the field of urology, as well as surgical branches and can show how much revenue is generated by the work of health professionals, and how much money is paid because the "installed capacities" are in place, i.e., services and the staff in place. It is important that the hospital payment method based on DRG be always carefully monitored and adjusted to the advances made in medical science and the profession. Therefore, it would be useful to establish a kind of a center, or at least a department in the Ministry of Health or in the Insurance Fond responsible for monitoring the implementation and continuous modification of DRG.

REFERENCES

1. *Street A, Vitikainen K, Bjorvatn A, Hvenegaard A.*. Introducing Activity-Based Financing: A Review of Experience in Australia, Denmark, Norway and Sweden. York, UK: Centre for Health Economics, University of York; 2007.
2. *Jovanovic M, Lazic Z, Jakovljevic V, Djukic A, Velickovic R, Antunovic M.* Current efforts and proposals to reduce healthcare costs in Serbia. Ser J Exp Clin Res 2011; 12(4): 161−3.
3. *Roger France FH.* Casemix use in 25 countries: a migration success but international comparisons failure. Int J Med Inform 2003; 70(2−3): 215−9.
4. *Jacobs R, Smith P, Street A.* Measuring efficiency in health care: analytic techniques and health policy. Cambridge: Cambridge University Press; 2006.
5. *Fetter R, Shin Y, Freeman JL., Averill RF, Thompson JD.* Case Mix Definition by Diagnosis-Related Groups. Medical Care 1980; 18(2 Suppl 3): 1−53.
6. *Busse R, Geissler A, Quentin W, Wiley M.* Diagnosis related groups in Europe: moving towards transparency, efficiency and quality in hospitals. Berkshire, England: Open University Press, Mcgraw-Hill; 2011.
7. *Legido-Quigley H, McKee M, Nolte E, Glinos IA.* Assuring the Quality of Health Care in the European Union: A Case for Action. Copenhagen, Denmark: WHO Regional Office for Europe; 2008.
8. *Paris V, Devaux M, Wei L.* Health Systems Institutional C haracteristics: a Survey of 29 OECD Countries. Paris: OECD Health Working Paper; 2010.
9. *Fischer W.* Die DRG-Familie. Stand 2007. Wolfertswil: Zentrum für Informatik und Wirtschaftliche Medizin (ZIM); 2008.
10. *Kimberly J.* The Globalization of Managerial Innovation in Health Care. Cambridge: Cambridge University Press; 2008.
11. *Farrar S, Yi D, Sutton M, Chalkley M, Sussex J, Scott A.* Has payment by results affected the way that English hospitals provide care? Difference-in-differences analysis. Br Med J 2009; 339: b3047.
12. *Freitas A, Silva-Costa T, Lopes F, Garcia-Lema I, Teixeira-Pinto A, Brazdil P*, et al. Factors influencing hospital high length of stay outliers. BMC Health Serv Res 2012; 20(12): 265.
13. *Ranković A, Rančić N, Jovanović M, Ivanović M, Gajović O, Lazić Z,* et al. Impact of imaging diagnostics on the budget – are we spending too much. Vojnosanit Pregl 2013; 70(7): 709−11.
14. *Simić S.* Social medicine. Beograd: Medicinski fakultet; 2012. (Serbian)
15. *Jakovljevic MB.* Resource allocation strategies in Southeastern European health policy. Eur J Health Econ 2013; 14(2): 153−9.
16. *Owens WD, Felts JA, Spitznagel EL.* ASA physical status classifications: a study of consistency of ratings. Anesthesiology 1978; 49(4): 239−43.
17. *Dindo D, Demartines N, Clavien P.* Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004; 240(2): 205−13.
18. Croatian Institute for Health Insurance. Available from: http://www.hzzo-net.hr/ (Croatian)
19. *Wiley M.* From the origins of DRGs to their implementation in Europe. In: *Busse R, Geissler A, Quentin W, Wiley M,* editors. Diagnosis-Related. Groups in Europe. Moving towards transparency, efficiency and quality in hospitals. Berkshire (UK): World Health Organization on behalf of European Observatory on Health Systems and Policies, Open University Press, McGraw-Hill; 2011.
20. *Lave JR.* The effect of the Medicare prospective payment system. Annu Rev Public Health 1989; 10: 141–61.
21. *Berki SE.* DRGs, incentives, hospitals, and physicians. Health Affairs 1985; 4(4): 70−6.
22. *Volkmer BG, Stredele R, Petervari M, Petschl S, Pühse G.* Amendments to the German diagnosis-related groups (G-DRG) system for urology in 2012. Urologe A 2012; 51(8): 1109−16. (German)
23. *Kahn KL., Keeler EB, Sherwood MJ, Rogers WH, Draper D, Bentow SS*, et al. Comparing outcomes of care before and after implementation of the DRG-based prospective payment system. JAMA 1990; 264(15): 1984−8.
24. *Ellis RP.* Creaming, skimping and dumping: provider competition on the intensive and extensive margins. J Health Econ 1998; 17(5): 537−55.

25. *Martinussen PE, Hagen TP*. Reimbursement systems, organisational forms and patient selection: evidence from day surgery in Norway. Health Econ Policy Law 2009; 4(2): 139−58.
26. *Newhouse JP, Byrne DJ*. Did Medicare's prospective payment system cause length of stay to fall. J Health Econ 1988; 7(4): 413−6.
27. *Wenke A, Gaber A, Hertle L, Roeder N, Pühse G*. Complexity level simulation in the German diagnosis-related groups system: the financial effect of coding of comorbidity diagnostics in urology. Urologe A 2012; 51(7): 975−81. (German)
28. *Schreyögg J, Stargardt T, Tiemann O, Busse R*. Methods to determine reimbursement rates for diagnosis related groups (DRG): a comparison of nine European countries. Health Care Manag Sci 2006; 9(3): 215−23.
29. *Shleifer A*. A theory of yard stick competition. Rand J Econ 1985; 16(3): 319−27.
30. *Gertman PM, Restuccia JD*. The appropriateness evaluation protocol: a technique for assessing unnecessary days of hospital care. Med Care 1981; 19(8): 855−71.
31. *Payne SM, Ash A, Restuccia JD*. The Role of Feedback in Reducing Medically Unnecessary Days of Hospital Care. Med Care 1991; 29(8 Suppl): AS91–105.
32. *Bentes M, Gonsalves ML, Santos M, Pina E*. Design and development of a utilization review program in Portugal. Int J Qual Health Care 1995; 7(3): 201−12.
33. *Burgers JS, Grol R, Klazinga NS, Mäkelä M, Zaat J*. Towards evidence-based clinical practice: an international survey of 18 clinical guideline program. Int J Qual Health Care 2003; 15(18): 31−45.
34. *Cheah J*. Development and implementation of a clinical pathway programme in an acute care general hospital in Singapore. Int J Qual Health Care 2000; 12(5): 403−12.
35. *Apolone G, Alfieri V, Braga A, Caimi V, Cestari C, Crespi V*, et al. A survey of the necessity of the hospitalization day in an Italian teaching hospital. Qual Assur Health Care 1991; 3(1): 1−9.
36. *Aruldas V*. Appropriateness Evaluation Protocol: An Application in a Multi-speciality Hospital. Vikalpa 1999; 24(3): 19−28.

Received on November 8, 2013.
Revised on December 9, 2013.
Accepted on February 25, 2014.
OnLine-First December, 2014.

ORIGINAL ARTICLE

UDC: 616.75-002.4-07-089
DOI: 10.2298/VSP131223081K

A 5-year retrospective analysis of necrotizing fasciitis – A single center experiences

Petogodišnja retrospektivna studija nekrotizujućeg fasciitisa – iskustvo jednog centra

Aleksandar I Kiralj*, Zlata Janjić†, Jelena Nikolić†, Borislav Markov*, Marija Marinković†

***Clinic for Maxillofacial Surgery, †Clinic for Plastic and Reconstructive Surgery, Clinical Center of Vojvodina, Novi Sad, Serbia**

Abstract

Background/Aim. Necrotizing fasciitis (NF) is usually an acute infection of superficial fascia with rapid progression in around soft tissue. If not promptly recognized and aggressively treated NF usualy leads to sepsis and multiorgan failure with fatal outcome, thus early diagnosis and prompt surgical treatment are crucial for healing of these patients. The aim of this article was to evaluate the clinical presentation of all patients with acute NF diagnosed and treated in surgical clinics of Clinical Center of Vojvodina, Novi Sad, Serbia. **Methods.** The medical records of patients treated for acute NF localized on a different parts of the body in Clinical Center of Vojvodina, Novi Sad, Serbia, during a 5-year period (from January 2008 to December 2012) were retrospectively evaluated. This study enrolled patients admitted *via* Emergency Center of Vojvodina with the diagnosis of acute NF either as the primary diagnosis or with the diagnosis at discharge after surgical treatment. **Results.** During a 5-year period there were 216 patients with final diagnosis of acute NF. Most of our patients (140 – 64.81%) were admitted with the initial diagnosis of cellulitis, abscesses, phlegmons or sepsis. Unfortunately, the clinical symptoms of acute NF were atypical at time of initial examination. Pain and swelling of the affected localization were the most presented bias of symptoms (183 – 84.72%). The majority of our patients were male (164 – 75.92%). Among the 216 patients, the most common pre-existing single factor was drug abuse (39 – 18.05%), followed by obesity (38 – 17.59%) and diabetes mellitus (31 – 14.35%). Trauma was most common etiological factor (22 – 10.8%) in infected wounds, followed by abdominal (15 – 6.94%) and orthopedic (11 – 5.09%) surgical intervention. In the present study idiopathic acute NF was diagnosed in 22 (10.18%) patients and more than one etiological factor were diagnosed in 20 (9.25%) patients. The majority of our patients had type I acute NF (172 – 79.62%) with Streptococcal species as the most common microorganism (125 – 71.02%). The most common localization was an extremity (151 – 69.90%). The minority of our patients had head and neck localization of infection (7 – 3.24%). Surgical treatment was performed in all the patients and most of them (183 – 84.72%) received the first surgery within 24 h. Other patients (23 – 10.64%) received operation after stabilization of general status or after getting the diagnosis of acute NF (unclear diagnosis on admission). During hospitalization, the most common complication among our patients was sepsis (156 – 72.22%). The mortality rate was 14.35%. **Conclusion.** Acute NF is a rare but very difficult and sometimes life-threatening disease of superficial fascia and around soft tissue. If acute NF is suspected, early radical excision of all the affected tissue with exploration and excision of superficial fascia with pathological and microbiological assessment are most significant for treatment. Appropriate antibiotics and intensive care setting to manage other organ failure of NF are recommended at the same time with surgery.

Key words:
fasciitis, necrotizing; risk factors; surgical procedures, operative; serbia.

Apstrakt

Uvod/Cilj. Nekrotizujući fasciitis (NF) obično je akutna infekcija površne fascije sa rapidnom progresijom u okolno tkivo, koja često dovodi do sepse i multiorganskog oštećenja sa fatalnim završetkom ukoliko se ne prepozna brzo i ne leči agresivno. Rana dijagnoza i što brže hirurško lečenje presudni su za izlečenje ovih bolesnika. Cilj ovog rada bio je da proceni kliničku sliku svih bolesnika sa akutnim NF koji su dijagnostikovani i lečeni na hirurškim klinikama Kliničkog centra Vojvodine u Novom Sadu. **Metode.** Retrospektivno je ocenjena medicinska dokumentacija bolesnika lečenih od akutnog NF, lokalizovanog na različitim delovima tela, u periodu od januara 2008. do decembra 2012. godine u Kliničkom centru Vojvodine u Novom Sadu. Ova studija je obuhvatila sve bolesnike koji su primljeni preko Urgentnog centra Vojvodine, kojima je postavljena početna dijagnoza akutnog NF i bolesnike kod kojih je dijagnoza postavljena

Correspondence to: Clinic for Maxillofacial Surgery, Clinical Center of Vojvodina, Novi Sad, Serbia.
E-mail: kiralj.aleksandar@gmail.com

nakon hirurške intervencije. Dokumentovane su razlike u godinama i polu, komorbiditetu ili predispoziciji, lokalizaciji infekcije, biogramima rane i krvi, tipu i broju hirurških intervencija, te stopi mortaliteta. **Rezultati.** Tokom 5-godišnjeg perioda imali smo 216 bolesnika sa završnom dijagnozom akutnog NF. Većina naših bolesnika (140 – 64.81%) primljena je sa dijagnozom celulitisa, abscesa, flegmone ili sepse. Nažalost, klinički simptomi akutnog NF bili su atipični u vreme inicijalnog ispitivanja. Bol i otok zahvaćene lokalizacije bili su najčešći bias simptoma (183 – 84.72%). Većina naših bolesnika bili su muškog pola (164 –75.92%). Među našim bolesnicima najčešća predispozicija bila je uživanje droge (39 – 18.05%), zatim gojaznost (38 – 17.59%) i šećerna bolest (31 – 14.35%). Trauma je bila najćešći etiološki faktor (22 – 10.80%) inficiranih rana, slede ih abdominalne (15 – 6.94% i ortopedske (11 – 5.09%) intervencije. U ovoj studiji idiopatski oblik akutnog NF bio je dijagnostikovan kod 20 (9.25%) bolesnika. Većina njih imala je akutni NF tipa I (172 – 79.62%) sa *Streptococcus* species kao najčešćim mikroorganizmom (125 – 71.02%). Najčešća lokalizacija oboljenja bili su ekstremiteti (151 –69.90%). Najmanji broj bolesnika imao je infekciju lokalizovanu na glavi i vratu (7 – 3.24%). Hirurško lečenje primenjeno je kod svih bolesnika i većina njih (183 – 84.72%) imali su prvu operaciju tokom 24 h. Ostali bolesnici (23 – 10.64%) imali su operaciju nakon stabilizacije opšteg stanja i nakon postavljanja dijagnoze akutnog NF (nejasna dijagnoza pri prijemu). Ekscizioni debridman svih zahvaćenih tkiva, uvek sa fasciektomiom urađen je kod svih bolesnika. Većina naših pacijenata je dva puta operisana (74 – 34.25%). Kod 15 bolesnika (6.94%) urađena je amputacija. U toku hospitalizacije najčešća komplikacija bila je sepsa (156 – 72.22%). Stopa mortaliteta iznosila je 14.34%. **Zaključak.** Akutni NF retko je, ali veoma teško i, ponekad po život opasno oboljenje supeficijalne fascije i okolnog tkiva. Ukoliko postoji sumnja na akutni NF imperativ lečenja je rana radikalna ekscizija zahvaćenog tkiva sa eksploracijom i ekscizijom superficijalne fascije, te patohistološkim i mikrobiološkim ispitivanjem. Istovremeno sa hirurškim lečenjem preporučuju se odgovarajući antibiotici, te intenzivna terapija koja će regulisati rad organa oštećenih ovom bolešću.

Ključne reči:
fasciitis, nekrotizujući; faktori rizika; hirurgija, operativne procedure; srbija.

Introduction

Acute necrotizing fasciitis (NF) is an uncommon surgical emergency disease. Most articles suggest that the incidence and mortality of NF are increasing in the whole world [1–4]. This acute and devastating infection of superficial fascia easily spreads across the subcutaneous tissue and skin or toward deep fascia and muscles [5–7]. The reason for acute course of infection and fast extension of necrosis is thrombosis of microvessels in and around the affected fascia. Acute NF was uncovered and described by Hippocrates about 500 BC. After that description a few crucial articles were published and reported on the detail description of NF but under different names of the disease. British surgeon Joseph Jones described this disease as "hospital gangrene" in 1871. French physician Jean Alfred Fournier described the same disease "to spread over perineum in male patients". In the recent time this localization of NF has begun to bear his name. The other name for NF such as suppurative fasciitis, streptococcal gangrene, necrotizing erysipelas, gas gangrene, hemolytic or clostridial gangrene, Meleney's gangrene, Fournier's gangrene, ("flesh-eating bacteria" or "killer bugs" in newspaper articles) have begun also to be used in the literature. In 1951 Wilson proposed the term "necrotizing fasciitis" for all difficult forms of soft tissue infection with superficial fascia necrosis [7, 8]. Nowadays, as was usually in the past time, the diagnosis of NF is often delayed because the symptoms mimic some of similar conditions in the early stage of the disease [9–11]. If physicians miss the diagnosis in the early course of the disease, morbidity and mortality rate is high. Immediate and aggressive excision of the affected tissue is recommended after the diagnosis [10–12]. The cornerstone of surgical treatment is excision of all the affected tissues and repeating the same procedure every day until the newly infected tissue removal. The lack of purulent collection during surgical treatment usually leads to insufficient excision and to the infection progression [10, 12, 13]. That leads to long hospital stay, repeated surgical excisions, residual deformity and a high rate of mortality. The final outcome of the treatment of those with acute NF depends mainly on the prompt and aggressive surgical treatment [9–11, 14]. For all patients presented with acute infection, differentiation between other soft tissue infection and acute NF is a matter of great importance. Unfortunately, presentation of acute NF has no specific signs and symptoms. Usually, patients may be presented with general signs of infection (systemic toxicity and organ dysfunction). Local signs are erythema, edema, cellulitis, bullae or crepitus. Patients with acute NF usually complain of severe pain usually out of proportion to clinical findings. In the advanced stages of acute NF, pain as a sign of infection may decrease [13–15]. In surgical practice distinguishing acute NF from the other common but not severe skin infection is often challenging. Sometimes, imaging studies are useful before immediate surgical treatment. On the plane radiographs findings may include only the for real signs: thickening and hyperdensity of subcutaneous soft tissue due to fluid accumulation and the presence of gas was visible. Ultrasonography as an imaging study was not recommended specially in adults because swelling and thickening of soft tissue blocks ultrasound transmission. Magnetic resonance imaging is a useful method for confirming diagnosis of acute NF and evaluating their localization, thickness and distribution but magnetic resonance images must not delay a life-saving surgical treatment [15–18]. The final diagnosis of NF should be based on the following operative findings: lack of bleeding from the fascia during resection, lack of resistance of muscular fascia during dissection, the presence of fetid-smelling "dish water" pus, the presence of grey necrotic fascia. A full thickness skin and affected tissue biopsy and taking swabs for bacteriological examination are required [5, 7, 11, 13]. Frozen section biopsy of the affected tis-

sue, as a histological technique is useful in differential diagnosis NF and could be life-saving procedure in patients with lack of specific skin signs of this difficult disease. Histological evaluation reveals obliterative endarteritis with thrombosis of the subcutaneous vessels, leukocytic infiltration and microabscess formation. Histological examination of specimen showed that the whole microscopic view is filled with granulocytes (granulocytes counts as stages 3.) [19–21]. Since some laboratory findings were typical in acute NF, in 2004. Wong et al. [22] introduced the laboratory risk indicator (LRINEC) score for early recognition and prognostic outcome of acute NF. Laboratory data including in LRINEC score are hemoglobin, creatinine, glucose, sodium, C-reactive protein (CRP) level and white blood cell count. According to the investigation of some authors the LRINEC score was impressive diagnostic method and they advised them to distinguish NF from the other soft tissue infection [23, 24]. Several microorganisms cause acute NF but no single microorganism or combination of them could not be appointed as a specific cause of NF. There were too many aerobic, anaerobic and mixed bacterial floras as a cause of infection of acute NF. The most important individual cause of infection was the group A *Streptococcus* classified as monomicrobial infection (or type II NF with l0% of all acute NF). Frequently, acute NF is polymicrobial infection cause by different anaerobic and aerobic organisms (or type I NF with 90% of all acute NF) [2, 9, 16]. Some etiological or predisposing factors are very important as a cause of acute NF (trauma, diabetes mellitus, obesity or poor nutritional state, alcohol or drug abuse, chronic or oncologic disease, immunosuppression) but sometimes acute NF is idiopathic [1, 3, 8].

The head and neck region has an excellent blood supply and acute NF in that localization is rare.

Many authors founded that infrequent localization in the head and neck region was the reason for the delayed diagnosis and insufficient surgical excision [4, 8, 12, 25]. In numerous group of soft tissue infections, acute NF is perhaps the most serious disease with the possibility of mortality rate from 6% to 76% and differentiating this uncommon infection is often challenging for all surgical specialties [2, 3, 7].

Methods

The medical records of patients treated for acute NF, localized on different parts of the body, in the Clinical Center of Vojvodina, Novi Sad, Serbia, during a 5-year period (from January 2008 to December 2012) were retrospectively evaluated. The following diagnostic criteria for acute NF (International Classification of Diseases, Ninth Revision, code 72886) were used: clinical evidence of rapid superficial fascial necrosis either by direct clinical inspection or by surgical exploration; the diagnosis was made indirectly by laboratory findings (microbiological or histological examination). According to our criteria, nobody of our patients had complete laboratory findings on admission, so we analyzed only leucocytes formula and serum sodium level. Frozen section biopsy was not required because most of our patients were admitted after working hours of the pathologist but the patients had the results of excisional biopsy (definitive pathological examination). Swab, tissue or pus culture was performed at initial wound exploration in our patients. Native radiographic examination of the affected area and chest were performed in all the patients, but magnetic resonance imaging was not performed because of the critical general condition of most of the patients. We also examined and analyzed comorbidities at admission. All the patients had aggressive surgical excisional debridement and most of them repeated surgical procedures. This study enrolled patients admitted *via* the Emergency Center of Vojvodina with the diagnosis of acute NF either as the primary diagnosis or with discharged diagnosis after surgical treatment. The differences in age, sex, comorbidities or predisposing factors, symptoms and localization of infection, wound and blood culture, laboratory findings, the type and the number of surgical treatment and mortality rate were documented and retrospectively analyzed.

Results

Within a 5-year period there were 216 patients with the final diagnosis of acute NF, the majority of whom (140 – 64.81%) were admitted with the initial diagnosis of cellulitis, abscesses, phlegmons or sepsis (Table 1).

Table 1

Clinical diagnosis at admission

Diagnosis	Patients	
	n	%
Cellulitis	91	42.1
Acute necrotizing fasciitis	76	35.2
Phlegmone	19	8.8
Sepsis	19	8.8
Abscess	11	5.1
Total	216	100.0

The majority of our patients were male (164 – 75.92%) and 52 (24.07%) female, with the mean age 52 years (range 18 to 85 years).

Unfortunately, according to our experience, the clinical symptoms of acute NF were atypical at time of initial examination. Pain and swelling of the affected localization (183 – 84.72%) were the most presented bias of symptoms in our patients (Table 2).

Table 2

Local clinical symptoms on admission

Symptoms	Patients	
	n	%
Pain	183	84.7
Sweling	183	84.7
Erytema	121	56.0
Skin necrosis	31	14.4
Bullae	164	75.9
Crepitation	5	2.3

Among the 216 patients, the most common pre-existing single factor was drug abuse (39 –18.05%), followed by obesity (38 – 17.59%) and diabetes mellitus (31 – 14.35%). Trauma was the most common etiologic factor (22 – 10.18%) between infected wounds, followed by abdominal (15 – 6.94%) and orthopedic surgical intervention (11 – 5.09%). Deep soft tissue penetration and retention of a foreign body lead to serious infection of the affected region. In the present study idiopathic acute NF was diagnosed in 22 (10.18%) patients and more than one etiological factor was diagnosed in 20 (9.25%) patients (Table 3).

Table 3

Etiological factors and the pre-existing disease

Etiological Factor	Patients	
	n	%
Idiopatic	22	10.2
Obesity	38	17.6
Drug abuse	39	18.1
Trauma	22	10.2
Diabetes mell	31	14.4
Chronic disease	5	2.3
Malignancy	6	2.8
Orthopaedic patients	11	5.1
Abdominal patients	15	6.9
Head and neck infections	7	3.2
More than one factors	21	9.3

The majority of our patients had type I acute NF (172 – 79.62%) with streptococcal species as the most common microorganism (125 – 71.02%) (Table 4).

Table 4

Type of acute necrotizing fasciitis (NS) and the results of wound culture

Parameters	Patients	
	n	%
Type I NS	172	79.6
Type II NS	44	20.4
Microorganisms		
Staphylococcus aureus	111	51.4
Group A *Streptococcus*	125	71.0
Escherichia coli	93	43.01
Pseudomonous aerugenosum	48	22.2
Acinetobacter spp	62	28.7
Proteus spp	98	44.4

At admission all the patients had increased band cell count in peripheral blood and hyponatremia in laboratory findings. Localizations of acute NF were as listed in Table 5.

Table 5

Body sites of infection

Body sites of infection	Patients	
	n	%
Head and neck	7	3.2
Trunk	58	26.9
abdominal wall	23	10.6
perineum	35	16.2
Extremity	151	69.9
upper extremity	53	24.5
lower extremity	98	45.4

The most common localizations were extremities (151 – 69.90%) (Figure 1). The minority of our patients had head and neck (7 – 3.2%) localization of infection and abdominal wall (23 – 10.6) (Figure 2). Surgical treatment was performed in all the patients and most of them (183 – 84.72%) received the first surgery within 24 hours. The other patients (23 – 10.64%) received surgical treatment after stabilization of general condition or after making the diagnosis of acute NF (unclear diagnosis on admission). Table 6 shows the number and the type of surgical treatment.

Table 6

Number and type of operation

Variables	Patients	
	n	%
Number of operation		
1	0	0
2	101	46.75
3	64	29.62
4	51	23.61
Type of operation		
excision fasciectomy	216	100
suture adaptations	14	6.48
plastica sec. Thiersch	187	86.57
amputation	15	6.94

Excision of all the affected tissues often with fasciectomy was performed in all our patients. During operation, the infected tissues were excised and sent for histological examination. All histological reports confirmed the diagnosis of acute NF. After excision the wounds were left open. The patients were taken to the operative theatre every 24 to 48 hour to continue with surgical reexamination of infected and necrotic tissue. When the wound infection was under control wound reconstruction was finished (suture adaptationes, skin grafts or amputations). Split thickness skin grafting was required for most of the patients (187 – 86.57%). Most of our patients required two operations (74 – 34.25%). Unfortunately, despite our efforts 15 (6.94%) patients received amputation. Complications were listed in Table 7.

Table 7

Complications of acute necrotizing fasciitis

Complication	Patients	
	n	%
Skin lost	64	29.6
Sepsis	156	72.2
Septic shock	29	13.4
Multiorgan failure	45	20.8
Extremity lost	15	6.9
Death	31	14.4

During hospitalization, the most common complication among our patients was sepsis (156 – 72.22%), followed by skin loss (64 –29.62%) and multiple organ failure (55 – 20.83%). Mortality rate among our patients was 14.35%. All patients were treated with broad spectrum antibiotics (started empirically and followed with target antibiotic therapy in according with the results of wound or blood cultures). Intensive care (intravenous fluids, parenteral nutritions, and other substitutional therapy) and monitoring of all vital parameters

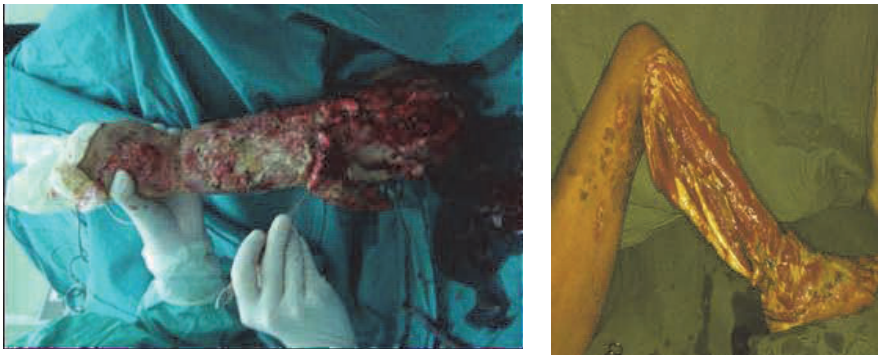

Fig. 1 – Necrotizing fasciitis localization in the upper and lower extremities.

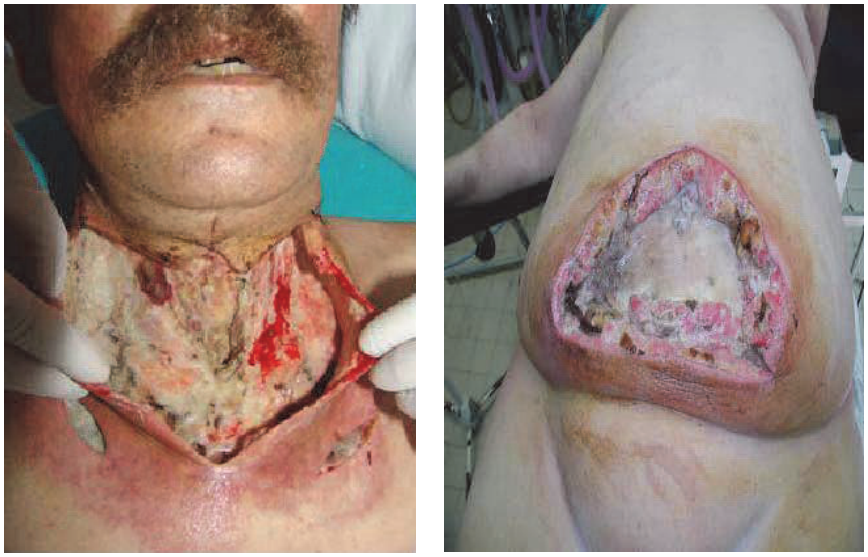

Fig. 2 – Necrotizing fasciitis in the neck (left) and abdominal wall region (right).

immediately after admission were required in all our patients. Hyperbaric oxygen therapy was not used in the treatment of acute NF because this modality may delay appropriate early excisional debridement.

Discussion

Acute NF is a very serious disease in all the parts of the world with the increasing incidence [2, 9]. On the basis of our previous study, acute NF was highly increasing in annual incidence from 16 to 43 patients [26]. Acute NF is a rapidly progressing and life treating infection of superficial fascia which may be difficult to differentiate from the other soft tissue infection [14–16, 27]. In the present study, most of the patients (140 – 64.81%) were admitted with the initial diagnosis of cellulitis, abscesses, phlegmons or sepsis, because as a matter of fact, only in a small group of patients there were "hard" clinical signs of acute NF, like in other clinical studied [1, 3, 9, 27]. In our previous studies we reported the similar results [26, 28]. Unfortunately, presentation of acute NF in our patients showed no pathognomonic or specific signs; pain and swelling of affected localization (183 – 84.72%) were the most presented bias of symptoms. Based on the current literature, the results of signs examintion were similar [2, 5, 6]. Usually, patients may present with general signs of infection (systemic toxicity and organ dysfunction) and with severe pain, out of proportion to the clinical findings. But, confusion arose in the advanced stages of acute NF, because pain as a sign of infection may decrease [14–16]. This fact was the main reasons for delay in making the diagnosis and surgical treatment, leading to complications [8, 9, 11, 26]. Given the heterogeneity in acute NF characteristics, comparison between studies from the whole world is sometimes difficult [2, 3, 5, 9]. Other differences in studies outcome may be explained by the preexisting factors [1, 8, 12, 16]. In our previous study drug and alcohol abuse and diabetes mellitus were similar pre-existing factors and no patient had obesity [26]. In our new study the most common pre-existing single factor was drug abuse (39 – 18.05%), followed by obesity (38 – 17.59%) and diabetes mellitus (31 – 14.35%). Recent world studies have reported an increasing incidence of NF among injection drug users [1, 2, 29]. Most of these patients had HIV infection because of the lack of local immune response. Contrary to expectations, we found that obesity was the most increased single risk factor among our patients in the last ten years. One possible reason may be a generally higher body mass index among the citizens of Vojvodina in the last three decades. Some studies examined obesity as a risk factor associated with repeated acute NF [6]. According to Wong et al. [30] patients with diabetes mellitus were over 70% of all cases in theirs study. Lin et al. [17]

showed that the most prevalent comorbidity in NF was also diabetes [17]. In our previous and the new study the most common localization was an extremity (151 – 69.90%) no matter what the microbiology results were. In order to define the etiology of acute NF localized in extremities, the number of infected chronic wounds associated with trauma was the most common factor in our study, like in the other study with the same localization [5, 6].

Fortunately, only a few of our patients had infection of surgical wounds (3 after abdominal and 2 after orthopedic operation). We know that NF occurs after minor trauma, penetrating wounds or insect bites, but surgical procedures were most dramatic. One of the limitations of this study was uncompleted information of impact of trauma and his detail analyzes in our patients with acute NF. In our series the majority of patients had type I acute NF (172 –79.62%) with Streptococcal species as the most common microorganism (125 – 71.02%). There may be multiple reasons for the variability between different studies. According to the report of Giuliano et al. [31], type I acute NF was commonly associated with trauma and abdominal wound and type II was more typically localized in extremities. Liu et al. [29] showed that type II NF was more serious presentation than type I. The others authors show no significant difference among clinical presentations of type I and type II NF [1, 12]. According to Tsai et al. [32], *Staphylococcus aureus* and *Streptococcus spp* were the most common microorganisms in the USA and Europe and monomicrobial Gram-negative microorganisms were most common in Asia. Some authors conclude that clinical characteristics of Gram-negative infections are more fulminant than of Gram-positive infections. The minority of our patients had head and neck localization of infection (7 –34.24%), like in the other studies [12, 15, 16]. This localization was associated with diabetes mellitus as the risk factor and with the other similar infection such as Ludwig's angina [13]. According to several authors, cervicofacial acute NF was well-described but potentially fatal infection [8, 13, 33]. Postoperative infection following dental extractions was common and sometimes followed by acute NF as the most serious infection of this localization [4]. Infection spreads in the superficial fascia very fast at the beginning. After that, blood vessels are thrombosed, and the lymphatics disrupted and the skin of face or neck becomes ishemic and necrotic. Same of the most important characteristics of acute NF on the head and neck localization are extreme systemic toxicity and rapid spread of disease, associated with high morbidity, mortality rate [4, 8, 13]. The second limitation of this study are uncompleted laboratory and radiographic findings. All our patients were critically ill and we evaluated them in the Emergency Center as soon as possible. Fluid resuscitation, antibiotic and the other substitutional therapy was started after admission, like in other articles [1, 4, 6, 7]. After that most of our patients (83 – 84.72%) were transported to the operative theatre because of aggressive surgical excision of infected tissue and wound care. Laboratory evaluation always includes complete blood counts, blood glycemia and electrolyte and arterial blood gases. All our patients had hyponatremia and leucocytosis. In the literature one of the proposed models for predicting outcomes from acute NF was laboratory risk indicator score [23]. Using the current literature, we found that a score was unsatisfactorily examined and remained invalidated in lager studies [1, 6, 34, 35]. Some authors recommended fresh frozen section biopsy for the fast diagnosis of acute NF [20–22]. The third limitation of this study is impossibility to make an accurate pathological diagnosis of acute NF, because the pathologist does not work on alert. Early detection and excisional debridement of all the infected tissue, whatever its size, was the key to save the patient's life. Several study recognized a very high mortality rate for patients who did not receive surgery [9, 10, 17, 36]. High mortality and morbidity are often associated with lately diagnosed acute NF and with delayed surgical treatment [7, 10, 27]. Surgical treatment was performed in all our patients and in most of them (183 – 84.72%) within 24 hours. Like in other infections, the aim of surgical debridement in acute NF is to completely remove infected tissue, if possible in a single operation [7, 10, 16]. Like in the management of other infected wounds, we always leave them open and use moist wound dressing. Not all patients with acute NF could be cured in one surgical procedure. In most patients secondary wound infection may occur due to increased tissue ischemia [10, 34, 35]. Most of our patients required two operations (74 –34.25%). Repeated excisional debridement was a cornerstone of surgical procedure in treatment of acute NF [1, 6, 7, 10, 14]. After excisional debridement and when the wound infection was under control, wound reconstruction was finished (suture situationes, skin grafts or amputations). The majority of our patients underwent reconstruction with split thickness skin grafts (187 – 86.57%). Unfortunately, in our study 15 (6.94%) patients received amputation. The other authors showed that amputation was the most common in diabetic patients or in drug users [1, 10, 20]. The rate of amputation varied from 0 to 22% according to other studies [1, 7, 34, 36]. The mortality rate in our present study is 14.35% (31 of 216 patients). Even with up to date knowledge of acute NF, all studies showed a very high mortality rate in any anatomic site ranging from 10 to 76% [1, 3, 7, 33]. According to most studies, predictive variables of mortality are age > 75 years, male gender, obesity and diabetes mellitus [6, 9, 12, 36].

Conclusion

Acute necrotizing fasciitis is a rare but very difficult and sometimes life-threatening disease of the superficial fascia and around soft tissue. We highlight the possibility that acute NF is not an expected presentation of soft tissue infection yet, and usually not recognized in the early course of the disease. Acute necrotizing fasciitis usually used to be mistaken as cellulitis, phlegmons or abscess. We suggested a high index of suspicion for all patients with unexplained severe pain of any part of the body with general signs of infection and unexplained multiorgan failure. If acute necrotizing fasciitis is suspected, early radical excision of all affected tissue with exploration and excision of superficialis fascia with pathological and microbiological assessment must be the treatment of choice. Appropriate antibiotics and intensive care setting in order to manage other organ failure due to necrotizing fasciitis are recommended at the same time with surgery.

REFERENCES

1. *Hutchison R, Bharania P, Lam F.* Necrotizing soft tissue infection for the orthopaedic surgeon. Infection 2010; 24(5): 355−62.
2. *Das DK, Baker MG, Venugopal K.* Increasing incidence of necrotizing fasciitis in New Zealand: a nationwide study over the period 1990 to 2006. J Infect 2011; 63(6): 429−33.
3. *Kao LS, Lew DF, Arab SN, Todd S, Awad SS, Carrick MM*, et al. Local variations in the epidemiology, microbiology, and outcome of necrotizing soft-tissue infections: a multicenter study. Am J Surg 2011; 202(2): 139−45.
4. *Sahoo NK, Tomar K.* Necrotizing fasciitis of the cervico-facial region due to odontogenic infection. J Oral Maxillofac Surg Med Pathol 2014; 26(1): 39−44.
5. *Lombardi C, Silver LM, Lau KK, Silhanek AD, Connolly FG.* Necrotizing fasciitis in the lower extremity: A review and case presentation. J Foot Ankle Surg 2000; 39(4): 244−8.
6. *Bernal NP, Latenser BA, Born JM, Liao J.* Trends in 393 necrotizing acute soft tissue infection patients 2000-2008. Burns 2012; 38(2): 252−60.
7. *Sarani B, Strong M, Pascual J, Schwab WC.* Necrotizing fasciitis: current concepts and review of the literature. J Am Coll Surg 2009; 208(2): 279−88.
8. *Sarna T, Sengupta T, Miloro M, Kolokythas A.* Cervical necrotizing fasciitis with descending mediastinitis: literature review and case report. J Oral Maxillofac Surg 2012; 70(6): 1342−50.
9. *Rangaswamy M.* Necrotizing fasciitis: a 10-year retrospective study of cases in a single university hospital in Oman. Acta Trop 2001; 80(2): 169−75.
10. *Wong C, Yam AK, Tan AB, Song C.* Approach to debridement in necrotizing fasciitis. Am J Surg 2008;196:19-24.
11. *Chao WN, Tsai CF, Chang HR, Chan KS, Su CH, Lee YT,* et al. Impact of timing of surgery on outcome of Vibrio vulnificus-related necrotizing fasciitis. Am J Surg 2013; 206(1): 32−9.
12. *Hsiao C, Weng H, Yuan Y, Chen C, Chen I.* Predictors of mortality in patients with necrotizing fasciitis. Am J Emerg Med 2008; 26(2): 170−5.
13. *Kavarodi AM.* Necrotizing fasciitis in association with Ludwig's angina-a case report. Saudi Dent J 2011; 23(3): 157−60.
14. *Edlich RF, Cross CL, Dahlstrom JJ, Long WB 3rd.* Modern concepts of the diagnosis and treatment of necrotizing fasciitis. J Emerg Med 2010; 39(2): 261−5.
15. *Afifi RY, El-Hindawi AA.* Acute necrotizing fasciitis in Egyptian patients: a case series. Int J Surg 2002; 6(1): 7−14.
16. *Malghem J, Lecouvet FE, Omoumi P, Maldague BE, Vande BB.* Necrotizing fasciitis: contribution and limitations of diagnostic imaging. Joint Bone Spine 2013; 80(2): 146−54.
17. *Lin JN, Chang LL, Lai CH, Lin HH, Chen YH.* Group A streptococcal necrotizing fasciitis in the emergency department. J Emerg Med 2013; 45(5): 781−8.
18. *Turecki MB, Taljanovic MS, Stubbs AY, Graham AR, Holden DA, Hunter TB,* et al. Imaging of musculoskeletal soft tissue infections. Skeletal Radiol 2010; 39(10): 957−71.
19. *Yu JS, Habib P.* MR imaging of urgent inflammatory and infectious conditions affecting the soft tissues of the musculoskeletal system. Emerg Radiol 2009; 16(4): 267−76.
20. *Stamenkovic I, Lew PD.* Early recognition of potentially fatal necrotizing fasciitis. The use of frozen-section biopsy. N Engl J Med 1984; 310(26): 1689−93.
21. *Majeski J, Majeski E.* Necrotizing fasciitis: improved survival with early recognition by tissue biopsy and aggressive surgical treatment. South Med J 1997; 90(11): 1065−8.
22. *Wong C, Khin L, Heng K, Tan K, Low C.* The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med 2004; 32(7): 1535−41.
23. *Stegeman SA, Nijhuis I, van Leeuwen GA, Bonsing BA, Steenvoorde P.* The value of frozen section biopsy in diagnosing necrotizing fasciitis: proposal of a new grading system. J Tissue Viability 2012; 21(1): 13−6.
24. *Holland MJ.* Application of the Laboratory Risk Indicator in Necrotising Fasciitis (LRINEC) score to patients in a tropical tertiary referral centre. Anaesth Intensive Care 2009; 37(4): 588−92.
25. *Liao CH, Lee KY, Su Y, Chuang C, Wong C.* Validation of the laboratory risk indicator for necrotizing fasciitis (LRINEC) score for early diagnosis of necrotizing fasciitis. Tzu Chi Med J 2012; 24(2): 73−6.
26. *Janjic Z, Momcilovic D, Skrbic S, Kiralj A, Nikolic J,* Necrotizing fasciitis/our experience. VI Croatian Congress of Plastic, Recontructive and Aesthetic Surgery. Opatija; 2006 October 6−11; Opatija: Abstract book; 2006; 41: M64.
27. *Wall DB, Klein SR, Black S, de Virgilio C.* A simple model to help distinguish necrotizing fasciitis from nonnecrotizing soft tissue infection. J Am Coll Surg 2000; 191(3): 227−31.
28. *Janjic Z, Nikolic J, Marinkovic M, Djermanov N.* Necrotizing fasciitis acute disease of contemporary man. Wounds J 2012: 1(Suppl 1):12.
29. *Liu G, Zhing X, Fu W, Zhao L, Zhang C.* Early treatment of necrotizing fasciitis secondary to perineal abscess. Eur Surg 2012; 44(2): 120−3.
30. *Wong CH, Chang HC, Pasupathy S, Khin LW, Tan JL, Low CO.* Necrotizing fasciitis: clinical presentation, microbiology, and determinants to mortality. J Bone Joint Surg Am 2003; 85−A(8): 1454−60.
31. *Giuliano A, Lewis F, Hadley K, Blaisdell FW.* Bacteriology of necrotizing fasciitis. Am J Surg 1977; 134(1): 52−7.
32. *Tsai YH, Huang KC, Shen SH, Hsu WH, Peng KT, Huang TJ.* Microbiology and surgical indicators of necrotizing fasciitis in a tertiary hospital of southwest Taiwan. Int J Infect Dis 2012; 16(3): e159−65.
33. *Lee JH, Choi H, Kim CH, Sohn JH, Kim H.* Fulminant Cerebral Infarction of anterior and posterior cerebral circulation after ascending type of facial necrotizing fasciitis. J Stroke Cerebrovasc Dis 2014: 23(1): 173−5.
34. *Bellapianta JM, Ljungquist K, Tobin E, Uhl R.* Necrotizing fasciitis. J Am Acad Orthop Surg 2009; 17(3): 174−82.
35. *Morgan MS.* Diagnosis and management of necrotising fasciitis: a multiparametric approach. J Hosp Infect 2010; 75(4): 249−57.
36. *Aragón-Sánchez J, Quintana-Marrero Y, Lázaro-Martínez JL, Hernández-Herrero MJ, García-Morales E, Beneit-Montesinos JV,* et al. Necrotizing soft-tissue infections in the feet of patients with diabetes: outcome of surgical treatment and factors associated with limb loss and mortality. Int J Low Extrem Wounds 2009; 8(3): 141−6.

Received on December 23, 2013.
Revised on March 27, 2014.
Accepted on April 2, 2014.
OnLine-First December, 2014.

GENERAL REVIEW

UDC: 616.31-053.2::616.379-008.64-053.2
DOI: 10.2298/VSP131212050D

Gingivitis and periodontitis in children and adolescents suffering from type 1 diabetes mellitus

Gingivitis i parodontopatija kod dece i adolescenata obolelih od dijabetesa melitusa tipa 1

Dragana Daković*†, Ivan Mileusnić‡, Zoran Hajduković†§, Saša Čakić¶, Miloš Hadži-Mihajlović¶

***Dental Clinic, §Clinic of Endocrinology, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; ‡Institute of Dentistry, Faculty of Stomatology, University Business Academy in Novi Sad, Pančevo, Serbia; ¶Clinic of Periodontology, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia**

Key words:
diabetes mellitus, type 1; gingivitis; periodontitis; child; adolescent.

Ključne reči:
dijabetes melitus, tip 1; gingivitis; periodontitis; deca; adolescenti.

Introduction

Type 1 diabetes mellitus (DM) is a systemic disease which causes a number of complications which reduce the quality of life of the affected individuals. Gingivitis and periodontitis are local inflamatory diseases of the supporting tooth structures (periodontium) which can have an influence on other organs and organic systems. The Expert Group Report on Diagnosis and Classification of Diabetes [1] included periodontitis as one of the pathological conditions which often occur in adults with DM. However, it has not yet been defined whether patients with DM are likely to develop periodontitis, or if periodontitis leads to the exacerbation of DM. Although, there are many data indicating the correlation between periodontitis and type 1 DM in children and adults, the influence of the level of glycaemic control on the condition of periodontium is still not entirely clear. Additionally, the quantitative status of immunological markers in saliva in these two diseases is unknown.

The aim of this paper was to present clinical studies dealing with the biochemical processes in saliva and other bodily fluids, which could elucidate the relationship between gingivitis and periodontitis, on the one hand, and type 1 DM, on the other.

The literature data on the correlation between gingivitis/periodontitis and type 1 DM were collected in the period from 1968 to September 2011 using the PubMed and Medline data bases and 59 studies were selected for this paper. The analysis encompassed original papers, review articles and scientific and expert meetings reports. All selected studies were published in English language. The analysis did not include: case reports, letters to the editors, short announcements, studies with inadequate methodology and incomplete or unrelated articles. The following key words were used for the electronic search in the MeSH browser: periodontitis, gingivitis, type 1 diabetes mellitus, cytokines, interleukin-8 and saliva.

A summary table was created with data including the authors' name, the year of publication of the study, the duration of type 1 DM, the monitored biochemical and clinical parameters and the conclusion based on the examined articles (Addendum 1).

Type 1 diabetes mellitus

Type 1 DM is primarily found in children and adolescents and makes up 5–10% of the total number of patients with both types of diabetes [2]. The pathological basis of type 1 DM is the autoimmune destruction of pancreatic islets' β-cells resulting in a marked or complete loss of insuline secretion resulting in hyperglycemia. Various types of molecular markers of autoimmune β-cells destruction can be detected in 85–90% of patients with hyperglycemia [2]. Some of them, such as autoantibodies against insulin, glutamic acid decarboxylase and tyrosine phosphatases, are used for the diagnosis and risk assessment of developing type 1 DM [3]. Compli-

Correspondence to: Ivan Mileusnić, Institute of Dentistry, Faculty of Stomatology in Pančevo, Žarka Zrenjanina 179, 26 000 Pančevo, Serbia. Phone: +381 64 433 4203; E-mail: imileusnic@gmail.com

cations of type 1 DM are retinopathy with a possible loss of sight, nephropathy which leads to renal failure, peripheral neuropathy with a risk of ulcerations and amputation of the lower extremities and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular diseases. Atherosclerosis, cardiovascular, peripheral arterial and cerebrovascular diseases are more often present in patients with diabetes mellitus [2]. In addition to these, accompanying skin lesions were examined in children with type 1 DM [4]. Furthermore, in 1993, periodontitis was identified as one of the classic complications of DM [5]. An important indicator of general condition and possible diabetes complications is metabolic control, which is monitored through the blood glucose level (BGL) and glycosylated hemoglobin level (HbA1c). In healthy individuals, the proportion of HbA1c ranges between 4% and 6% of total hemoglobin, while in the hyperglycaemic it can reach 15%. Since HbA1c remains stable for several months after the occurrence of hyperglycaemia, it serves as a marker of the severity of DM or the efficacy of metabolic control during a certain period of time. The treatment of DM is aimed at normalising the blood glucose levels and reducing the HbA1c level to less than 7.5% [6].

Gingivitis and periodontitis

Gingivitis and periodontitis are localised infections of the periodontium, which consists of the gingiva, the periodontal membrane, the root cementum and the alveolar bone. Gingivitis is an inflammation which affects only the gingiva, whereas periodontitis is an inflammation of the deeper periodontal tissues. Both diseases result from the interaction between periodontal pathogenic microorganisms and host tissues. Periodontal pathogens consist the bulk of the soft (dental biofilm) and solid (dental calculus) deposits on teeth. Dental biofilm is a colony of microorganisms which spontaneously and progressively accumulate on all solid surfaces in the mouth, primarely on teeth. Although this accumulation is spontaneous, many local factors can facilitate it. Gingivitis is initiated by the presence of periodontal pathogenic microorganisms which cause the disease, but the host response to the infection is critical for disease progression. Various systemic factors can influence this response. The clinical course of the advanced periodontitis does not differ in relation to the type of periodontal bacteria which induce the immune-mediated destructive processes [7]. When dental biofilm is present gingiva becomes red, enlarged, and bleeds easily when probed. Further expansion of the inflammatory process into the deeper periodontal tissues leads to the apical migration of the epithelial attachment resulting in the periodontal pocket, which is a pathognomonic sign of periodontitis. Unless the process is arrested by treatment, it will lead to alveolar bone resorption, which ultimately causes tooth loss. It was previously thought that gingivitis, if left untreated, will progress into periodontitis. However, gingivitis and periodontitis are two different pathological entities and although periodontitis is preceded by gingivitis, not all cases of gingivitis will progress to periodontitis. So far, it is not completely clear why and how gingivitis progresses to periodontitis.

Besides oral implications, periodontitis has an influence on systemic health. Systemic inflammation was more pronounced in patients with periodontitis as detected by the increased level of various serum inflammatory markers compared to the healthy controls [8]. Also, depending on the degree of the host response during periodontits, various inflammatory biomarkers can be detected in samples of gingival crevicular fluid and saliva. Some of these molecules can influence gene expression in individuals with genetic polymorphism with the tendency to develop periodontitis [9]. These molecules can be used as diagnostic markers and tests and methods of assessing the risk of periodontitis by measuring the level of biomarkers are constantly being improved.

The correlation between diabetes mellitus and gingivitis/periodontitis

Diabetes and gingivitis/periodontitis are widespread chronic diseases. Their pathogenetic mechanisms are believed to be interrelated and many authors have proposed the mechanisms to explain their correlation. These common mechanisms can be attributed to the following factors: micro vascular disease, changes in the composition of the gingival crevicular fluid, changes in collagen metabolism, altered host response, increased presence of periodontal pathogenic microorganisms, genetic predisposition and non-enzymatic glycosylation. Generally, in order to confirm the relationship between DM and gingivitis/periodontitis biologically plausible mechanisms must exist underlying the pathological interactions. These mechanisms are very similar to those of classic diabetic complications such as retinopathy, nephropathy, microvascular and macrovascular diseases and impaired wound healing.

Many studies indicate a higher incidence and severity of gingivitis and periodontitis in children and adolescents with type 1 DM compared to healthy children. According to Cianciola et al. [10], severity and incidence of periodontal involvement was statistically more prominent in patients with type 1 DM and accompanying periodontitis compared to non-diabetic individuals of that age. In support to the above data, the results of Lalla et al. [11] and Daković and Pavlović [12] showed that gingival inflammation was significantly more common in children and adolescents with type 1 DM compared to the systemically healthy subjects of the same age group. Jenkins and Papapanou [13] demonstrated that children with type 1 DM were in high risk of developing gingivitis, while the incidence of gingivitis in children and adolescents was almost double compared to adults. Some studies showed that severe forms of gingivitis were more frequent in children with type 1 DM compared to healthy children [10, 14], whereas other studies did not find this difference [15].

The available literature data on the exclusive influence of dental biofilm on the development of gingivitis in patients with type 1 DM are contraversial. In one study, the presence of gingivitis in children with type 1 DM was not related to higher dental biofilm accumulation, because a significant increase in plaque index (PI) was not found in this population [16]. Contrary to this, another study showed a significant

correlation between the presence of dental biofilm and gingivitis in children with type 1 DM compared to a healthy population [13]. In the research of Daković and Pavlović [12] the percentage of tooth sites with a gingival index (GI) higher than 2 was 18.3% in children with type 1 DM compared to 9.7% in healthy children, which is almost twice as high and with a high statistical significance ($p < 0.001$). This is in accordance with most authors who examined GI in this category of patients [11, 14, 17, 18]. Additionally, the percentage of tooth sites where PI score was higher than 2 was significantly higher in children with type 1 DM compared to healthy children [12], which was in accordance with most authors [11, 14, 16, 17]. Daković and Pavlović [12] also investigated the relationship between gingival bleeding and dental biofilm (the number of bleeding tooth sites compared to the number of biofilm covered tooth sites). The multivariate logistic regression analysis showed that periodontitis was in a positive correlation with GI and, more importantly, with bleeding/dental biofilm [12]. Additionally, the increase in the relationship between bleeding and dental biofilm can identify the individuals inclined to develop periodontitis and be of great importance as a prognostic indicator of potential periodontitis [19].

Several studies showed a positive correlation between glucose level and gingival inflammation. Hyperglycemia in children with a newly diagnosed DM was associated with a more severe form of gingival bleeding which is reduced by the improvement in glucose metabolism after the insulin therapy training. Children with poor metabolic control (average HbA1 = 15%) had a higher level of gingival bleeding compared to those with good or mild metabolic control [20]. These children had a higher risk of developing a more severe form of periodontitis [12, 17, 21]. Such findings in patients with type 1 DM can be explained by the fact that the excessive blood glucose, which enters the oral cavity through saliva and gingival crevicular fluid, soaks the biofilm and causes an increase in total biofilm accumulation. In children with poor metabolic control this mechanism can overwhealm the host defence against the dental biofilm and increase the risk of developing gingivitis. However, the reduction of hyperglycemia did not affect the clinical attachment level (CAL), probing pocket depth (PPD), gingival bleeding index (GBI) and PI [12]. This can be explained by the fact that the improvement of glycaemic control in patients with type 1 DM can improve the periodontal parameters, but only if the patients adhere to proper oral hygiene measures.

It was found that pathological morphologic characteristics of capillaries in the gingiva and labial mucosa in patients with DM were more prominent compared to the healthy individuals [22]. Long-term hyperglycemia causes thinning of the basal membrane of vessel walls lining leading to the deterioration of tissue nourishment and leukocyte migration. This contributed to the concept of importance of the morphological and functional diabetic microvascular changes in the periodontium in patients with type 1 DM.

The values of clinical periodontal parameters in the subjects with type 1 DM [12] showed a better condition of their periodontium compared to the values of other authors in the same category [11, 14, 17]. However, the incidence of periodontitis was statistically higher in children suffering from DM, compared to healthy children. The ratio of periodontitis in healthy children was 7.9%, while in children with type 1 DM periodontitis was three times more common, i.e. 21.5%. Additionaly, there was a higher presence of a more severe form of periodontits, albeit statistically insignificant [12]. The analysis of the effects of type 1 DM on the destruction of periodontal tissues showed some interesting results. Comparing CAL in diseased and healthy children a stastistically significant difference was not found, but there was a significant difference in these two groups when the number of teeth and the number of tooth sites affected by periodontitis were compared [12]. Type 1 DM was significantly correlated with the level of destruction of the periodontium regardless of the cut-off clinical parameter values used to define periodontitis [12]. This information, as well as the results of other authors [17], show that type 1 DM is a significant systemic factor which influences the development of periodontitis.

Numerous studies show that patients with DM and periodontitis have a higher risk of deterioration of glycaemic control over time compared to patients with DM alone [23]. This is in accordance with the results of other authors who have found that children with type 1 DM and poor metabolic control had a higher risk of developing a more severe form of periodontitis [12, 17, 21]. The influence of periodontal infection on the glycaemic control can be explained in several ways. The systemic inflammation following systemic infections, stimulates insulin resistance and affects the dynamics of glucose in the body [24]. There is evidence that periodontitis, although a local disease, can stimulate or lead to a continuous increase in the systemic chronic inflammatory condition, reflected in the elevated level of serum C-reactive protein (CRP), interleukin-6 (IL-6) and fibrinogen, especially in those harbouring Gram-negative periodontal pathogens (*Porphyromonas gingivalis, Tannerella forsythensis* and *Prevotella intermedia*) [5]. Bacteremia and endotoxemia, a result of the systemic dissemination of periodontal pathogens or their products, induce a systemic imflammatory disorder with an increase in the levels of serum inflammatory markers [25]. One study showed that the level of endotoxin in blood was five times higher in patients with periodontitis compared to healthy individuals [26]. The presence of periodontitis enables oral microorganisms and their products to reach systemic circulation [27].

Conversely, the mechanisms influencing the development of other complications of type 1 DM can also affect the pathogenesis of periodontitis [28]. Some studies examining the correlation between type 1 DM and periodontitis are shown in Addendum 1. The resuts of these studies are difficult to compare due to a variaty of clinical and laboratory protocols used. Besides, there is a certain number of studies which are not shown in Table 1 due to the obsolescence or inadequate presentation of clinical parameters. Namely, the parameters of gingival and periodontal health have recently been examined in more detail, whereas the percentage of the affected sites and the ratio between bleeding and dental biofilm were also examined in adition to GI and PI. Furthermore, special attention is paid to the parameters of periodontal destruction,

particularly to CAL, so that the term 'periodontal destruction' implies that there is at least one site with epithelial attachment loss larger than 1.5 mm (or 2 mm depending on the author) on two non-adjacent teeth. The duration of DM is an important risk factor for developing periodontitis. In young patients with type 1 DM periodontal tissue destruction starts relatively early, in pre-adolescent and adolescent period, depending on the duration of the underlying disease [12]. Loss of epithelial attachment was more pronounced in patients suffering from type 1 DM longer than 10 years [29]. On the same lines, the results of Lalla et al. [11] are important since they show that type 1 DM have a strong role in the development of periodontitis in early childhood. In contrast, previous studies showed that there were no significant differences in the values of periodontal parameters in children with type 1 DM, compared to the control group [13, 14]. These findings can be explained by the differences in the susceptibility to periodontitis between different subpopulations with type 1 DM, such as race, gender, etc. One of the important reasons supporting these findings can be the fact that the authors of the previous studies measured CAL, but not the total periodontitis-affected teeth and sites. A significant correlation between the duration of the disease, its metabolic control and gingival inflammation presented by the relationship between bleeding and dental biofilm was found by analysing the relation of the clinical parameters of diabetes with the number of teeth affected by periodontal infection (defined as any loss of epithelial attachment level visible on minimum of two non-adjacent teeth) [12]. It indicates once again that in young patients with type 1 DM the duration of the disease and metabolic control have an impact on the prevalence of gingival inflammation and progression toward periodontitis and that there is a higher risk of developing more severe forms of periodontitis [11, 12, 17, 30]. Besides, the study of Foia et al. [31] clearly showed that children and adolescents are susceptible to destructive types of periodontitis, especially when the external etiological factors (microbial flora) are associated with diabetes. These data can be interpreted by the lack of metabolic control which increases the risk of developing gingival inflammation and hinders the healing of the incurred damage.

Diagnostic biomarkers of periodontitis and type 1 diabetes mellitus

An early detection of the disease has an important role in the success of therapy. If the disease is diagnosed earlier, the chances for a successful treatment are increased, or, if impossible, a better control of the disease course is enabled. In most cases, the diagnosis is established only after the development of the first symptoms of the disease. Therefore, in order to increase the rate of early detection of diseases, researchers are focused on identifying biomarkers which could indicate the presence of the disease prior to the manifestation of clinical symptoms [32].

One of the trends in the development of early diagnosis of periodontitis is the development of disease monitoring mechanisms. Basic and regular clinical and radiolographic measurements are useful for the detection and the diagnosis of the disease, but nowadays disease markers in saliva are increasingly used as diagnostic tests for periodontitis [33]. Saliva is used primarely beacause it is an ultrafiltrate of plasma and a body fluid which can be collected easily and non-invasively making it suitable for investigating biochemical parameters such as free oxygen radicals, lipid peroxidation products, such as malondialdehyde (MDA) and cytokines. Even so, the examinations of the composition of saliva in children with Type 1 DM are scarce. Aren et al. [18] were one of the few who examined the composition saliva and found that the saliva of children with Type 1 DM had a lower pH and buffering capacity, whereas the peroxidase activity and the glycose, magnesium and calcium levels were increased compared to healthy children. Besides this, a positive correlation was demonstrated between the peroxidase activity of gingival exudates and the severity of gingival inflammation, as well as the periodontal pocket depth. Additionally, MDA level in saliva and plasma [34, 35] and cytokine level in the gingival crevicular fluid [36] and in the serum [37] were statistically significantly increased in patients with DM and gingivitis or periodontitis compared to healthy individuals, so more attention has recently been directed to the analysis of saliva in diabetic patients. Various studies have demonstrated that the biological activity of cytokines can directly influence the degree of periodontal destruction (epithelial attachment loss, destruction of collagen and alveolar bone resorption) [38, 39]. The individual course of periodontitis indicates the importance of conducting complex researches of cytokines responsible for the beginning, progression and/or suppression of the immune response [40]. However, only a few studies have investigated local periodontal inflammation at the biochemical and immunological level in patients with DM. The latest findings suggest that hyperglycaemia can lead to the increased production of inflammatory mediators, e.g. Engebretson et al. [41] demonstrated that inadequate metabolic control was associated with an elevated level of interleukin-1β (IL-1β) in the gingival crevicular fluid. These data also indicate the mechanism which explains the relationship between poor metabolic control and periodontal destruction. Hypertriglyceridemia, which is accompanied by DM, leads to an increased production of pro-inflammatory mediators in monocytes, such as tumor necrosis factor-α (TNF-α) and IL-1β, while neutrophils produce more IL-1β accompanied by the disorders of chemotaxis and phagocytosis. The increased level of pro-inflammatory mediators, which can be used for diagnostic purposes, was found not only in serum, but also in the gingival crevicular fluid of hyperlipidemic patients with type 2 DM [42, 43].

It is assumed that patients with periodontitis can have increased levels of some circulating inflammatory markers. Periodontal monocytes, macrophages, fibroblasts and endothelial cells respond to the microorganisms, lipopolysaccharides and other antigens of the dental biofilm, and secrete numerous chemokines and inflammatory cytokines, primarely TNF-α, prostaglandin E2 (PGE2) and interleukins (IL-1β and IL-6), in the systemic circulation. Advanced glycation end products (AGE), accumulated in monocytes due to hyperglycemia, and their binding to their dedicated receptors

(RAGE) increases the oxidative stress in cells and activates the transcription nuclear factor-kappa B (NF-κB) which influences the phenotype of monocytes/macrophages and leads to the increased production of proinflammatory cytokines, such as IL-8 and TNF-α [44]. This increased production of proinflammatory cytokines is critical for the chronic inflammatory processes in the formation of atheromatous lesions in large blood vessels [45, 46]. Through the oxidative stress, DM affects the inflammatory processes in the gingiva by increasing the accumulation of AGE-modified protein [47]. The levels of IL-8, TNF-α and PGE2 in the gingival crevicular fluid are increased in patients with DM compared to healthy individuals due to the interaction between the AGE-modified protein and RAGE receptor in the periodontal tissues.

IL-8 is a proinflammatory cytokine that plays a significant role in the pathogenesis of periodontitis in patients with DM, especially in those with poor metabolic control. The study of Erbağci et al. [48] showed that the level of serum IL-8 was significantly higher in children and adolescents with type 1 DM compared to the systemicaly healthy children while maintaining a correlation between age, weight, lipid status, apolipoproteins and glycaemic control. In the same group of subjects, the concentration of IL-8 in saliva was also statistically much higher in children with type 1 DM [49]. However, a statistically significant difference was not found when the level of IL-8 in the saliva of children with type 1 DM and concomitant periodontitis was compared to children with only type 1 DM. This was the first examination of salivary inflammatory mediators in children and adolescents with type 1 DM accompanied by periodontitis. Having in mind that the advanced periodontitis can cause an additional increase in the level of IL-8 in the saliva, the authors of this study determined the severity of periodontitis. The average PPD in children with type 1 DM and periodontitis was 2.05 mm, while the average value of CAL was 1.31 mm, demonstrating that periodontitis was in its initial phase. Considering that there was no correlation between the periodontal parameters and the level of IL-8 in saliva, the authors concluded that the increased level of salivary IL-8 was caused by the presence of metabolic changes related to DM and not by periodontitis. The source of the elevated IL-8 level could be hyperglycaemia which can induce the transcription of IL-8 gene in human endothelial cells [50]. In addition, it was shown that hyperglycaemia and ketosis regulate the production of IL-8 in cultivated monocytes [51].

Besides IL-8, according to the results of Karlsson et al. [52], the level of interferon-γ (IFN-γ) in peripheral mononuclear cells in children and adolescents with type 1 DM with good and poor metabolic control was statistically much higher compared to the control group of healthy children and adolescents. According to the same authors' data, the elevated level of IFN-γ caused adaptation or improvement of the immune response to infection. Contrary to Karlsson et al. [52], a statistically significant increase in the level of IFN-γ was not found in the serum [48] and saliva [49] of patients with type 1 DM and periodontitis, although the level of IFN-γ in children with type 1 DM was 17% higher compared to healthy subjects. Considering that saliva is an ultrafiltrate of plasma, an increased serum level of IFN-γ was not reflected in the saliva, because it is a large molecule that probably could not pass easily through the capillary endothelium of the oral mucosa. Taking into account that IL-5 and IL-4 have some common characteristics in relation to the reduced infection sensitivity, it is important that Karlsson et al. [52] found that patients with type 1 DM had a statistically significant increase in the relationship of both markers to the IFN-γ level in blood. Accordingly, the results of Karlsson et al. [52] and Foss Freitas et al. [53] show that the favorable relationship between the increased levels of IFN-γ and IL-5 represents an adequate response of the immune system to an inflammatory process in the oral cavity (i.e. periodontitis). It should be stressed that another study showed that the large difference in the increase of the level of IL-5 compared to IFN-γ (4.8 times) in the saliva was statistically much higher, and that the level of IL-5 in the saliva of children with type 1 DM was increased by 81.8% compared to the results of the control group [54]. The increasing tendency draws attention supported by the assumption that the determination of the IL-5 level may serve as one of the markers of the degree of inflammation.

The development of molecular biology in clarifying insulin resistance and dysfunction of β-cells has enabled the identification of the increased role of inflammatory mediators, especially cytokines and elements of the innate immune system in the pathogenesis of type 2 DM [55]. The production of cytokines as a result of infection could potentially contribute to the insulin resistance in numerous ways. In contrast, children and adolescents with type 1 DM depended solely on the exogenous insulin, whose rhythmic intake effects glucose metabolism in an uneven way. This is exactly the reason why the general condition of children and adolescents was disrupted in the following years, and therefore at greater risk of developing a severe form of periodontitis. Thus, similar to adults, the parallelism can be expected between the severe form of periodontitis and a significant increase in the levels of cytokines, which would hamper their dental care.

Conclusion

Type 1 diabetes mellitus is a significant etiopathogenic factor responsible for the development of diabetic periodontitis. This is confirmed by a statistically significant increase in the incidence and proinflammatory condition of the entire organism. Hyperglycaemia damages periodontal tissues through several mechanisms, primarily due to the damages of the mechanism of immune response, non-enzymatic glycosylation and increased oxidative stress.

In terms of a very high incidence of both diseases and their potentially serious repercussions, other medical specialists should have an important role in encouraging patients to visit their dentists regularly in order to control the etiological factors, especially the dental biofilm. Dentists should also bear in mind that the deterioration of glycolic metabolism and diabetes may deteriorate the condition of periodontitis. Contemporary literature on the treatment of children and

adolescents suffering from the aforementioned diseases emphasises the need for the regular application of the following analysis and procedures: glycemic values, HbA1C and the most significant biochemical parameters of DM, because hyperglycaemia has a negative impact on the anti-inflammatory response and increases the oxidative stress of microvascular disorder in periodontal tissues. It is essential to have a protocol for the treatment of periodontitis, which involves the local periodontal mechanical treatment as well as local and systemic medication treatment. The elimination of periodontal pathogens from the periodontal, pockets reduces the intensity of local inflammation, thus improving the overall condition of the body, because of the positive effect on the overall condition of glucose regulation.

Besides that periodontitis has already been defined as the sixth complication of diabetes mellitus, the results of numerous studies imply that periodontitis is an inflammation with the possible systemic response and subsequent disorder of glucose regulation.

In addition to the regular procedures in monitoring the condition and prognosis of diabetes and periodontitis, the analyses of saliva and gingival crevicular fluid are also recommended as non-invasive and relatively reliable sources of information on the aforementioned diseases. Therefore, the cooperation between pediatric endocrinologists and dentists is required in order to preserve and improve the general health of children and adolescents.

Acknowledgement

This research study is a part of the project the Ministry of Education, Science and Technological Development of the Republic of Serbia No: III41008 and 175075.

REFERENCES

1. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997; 20(7): 1187−93.
2. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up Report on the Diagnosis of Diabetes Mellitus. Diabetes Care 2003; 26(11): 3160−67.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2006; 29(Suppl 1): 43−8.
4. *Pavlović MD, Milenković T, Dinić M, Misović M, Daković D, Todorović S,* et al. The prevalence of cutaneous manifestations in young patients with type 1 diabetes. Diabetes Care 2007; 30(8):1964-7.
5. *Löe H*. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care 1993; 16(1): 329−34.
6. *Alberti KG, Zimmet PZ*. Definition, diagnosis and classification of diabetes mellitus and its complications, Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15(7): 539−53.
7. *Rakić M, Zelić K, Pavlica D, Hadžimihajlović M, Milašin J, Miličić B*, et al.. Association between clinical parameters and the presence of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis in patients with progressive periodontal lesions. Vojnosanit Pregl 2010; 67(11): 898−902.
8. *Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, de Nardin E*. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol 2001; 72(9): 1221−7.
9. *Ebersole JL, Machen RL, Steffen MJ, Willmann DE*. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. Clin Exp Immunol 1997; 107(2): 347−52.
10. *Cianciola LJ, Park BH, Bruck E, Mosovich L, Genco RJ*. Prevalence of periodontal disease in insulin-dependent diabetes mellitus (juvenile diabetes). J Am Dent Assoc 1982; 104(5): 653−60.
11. *Lalla E, Cheng B, Lal S, Tucker S, Greenberg E, Goland R,* et al. Periodontal changes in children and adolescents with diabetes: a case-control study. Diabetes Care 2006; 29(2): 295−9.
12. *Daković D, Pavlović MD*. Periodontal disease in children and adolescents with type 1 diabetes in Serbia. J. Periodontol 2008; 79(6): 987−92.
13. *Jenkins WM, Papapanou PN*. Epidemiology of periodontal disease in children and adolescents. Periodontology 2000 2001; 26: 16−32.
14. *Pinson M, Hoffman WH, Garnick JJ, Litaker MS*. Periodontal disease and type I diabetes mellitus in children and adolescents. J Clin Periodontol 1995; 22(2): 118−23.
15. *Goteiner D, Vogel R, Deasy M, Goteiner C*. Periodontal and caries experience in children with insulin-dependent diabetes mellitus. J Am Dent Assoc 1986; 113(2): 277−9.
16. *Firatli E*. The relationship between clinical periodontal status and insulin-dependent diabetes mellitus. Results after 5 years. J Periodontol 1997; 68(2): 136−40.
17. *Lalla E, Cheng B, Lal S, Kaplan S, Softness B, Greenberg E*, et al. Diabetes mellitus promotes periodontal destruction in children. J Clin Periodontol 2007; 34(4): 294−8.
18. *Aren G, Sepet E, Ozdemir D, Dinççağ N, Güvener B, Firatli E*. Periodontal health, salivary status, and metabolic control in children with type 1 diabetes mellitus. J Periodontol 2003; 74(12): 1789−95.
19. *Abbas F, van der Velden U, Hart AA, Moorer WR, Vroom TM, Scholte G*. Bleeding/plaque ratio and the development of gingival inflammation. J Clin Periodontol 1986; 13(8): 774−82.
20. *Grossi SG, Skrepcinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG*, et al. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. J Periodontol 1997; 68(8): 713−9.
21. *Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC,* et al. Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. J Periodontol 1996; 67(10 Suppl): 1085−93.
22. *Manouchehr-Pour M, Spagnuolo PJ, Rodman HM, Bissada NF*. Impaired neutrophil chemotaxis in diabetic patients with severe periodontitis. J Dent Res 1981; 60(3): 729−30.
23. *de Fronzo RA, Ferrannini E*. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; 14(3): 173−94.
24. *D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D,* et al. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. J Dent Res 2004; 83(2): 156−60.
25. *Mealey BL, Oates TW*. Diabetes mellitus and periodontal diseases. J Periodontol 2006; 77(8): 1289−303.
26. *Geerts SO, Nys M, De Mol P, Charpentier J, Albert A, Legrand V,* et al. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. J Periodontol 2002; 73(1): 73−8.

27. *Southerland JH, Taylor GW, Offenbacher S*. Diabetes and Periodontal Infection: Making the Connection. Clinical Diabetes 2005; 23(4): 171−8.
28. *Lalla E, Lamster IB, Feit M, Huang L, Spessot A, Qu W*, et al. Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. J Clin Invest 2000; 105(8): 1117−24.
29. *Glavind L, Lund B, Loe H*. The relationship between periodontal state and diabetes duration, insulin dosage and retinal changes. J Periodontol 1968; 39(6): 341−7.
30. *Taylor GW*. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Ann Periodontol 2001; 6(1): 99−12.
31. *Foia L, Toma V, Surlin P*. Diabetes mellitus impact on periodontal status in children and adolescents. In: *Manakil J*, editor. Periodontal Diseases - A clinician's Guide. Rijeka, Croatia: InTech 2011. p. 179−96.
32. *Lee Y, Wong DT*. Saliva: an emerging biofluid for early detection of diseases. Am J Dent 2009; 22(4): 241−8.
33. *Patil PB, Patil BR*. Saliva: a diagnostic biomarker of periodontal diseases. J Indian Soc Periodontol 2011; 15(4): 310−7.
34. *Daković D*. Malondialdehyde as an indicator of local oxydative cell damage in patients suffering from periodontitis [mater's thesis]. Belgrade, Serbia: Military Medical Academy; 2005. (Serbian)
35. *Daković D, Brkić Z, Žunić G*. Measurement of the concentration of salivary malondialdehyde in patients suffering from periodontitis. Stomatološki informator 2005; 11(17): 7−11. (Serbian)
36. *Kamma JJ, Giannopoulou C, Vasdekis VG, Mombelli A*. Cytokine profile in gingival crevicular fluid of aggressive periodontitis: influence of smoking and stress. J Clin Periodontol 2004; 31(10): 894−902.
37. *Ide M, Jagdev D, Coward PY, Crook M, Barclay G, Wilson RF*. The short-term effects of treatment of chronic periodontitis on circulating levels of endotoxin, C-reactive protein, tumor necrosis factor-alpha, and interleukin-6. J Periodontol 2004; 75(3): 420−8.
38. *Belardelli F, Ferrantini M*. Cytokones as a link between innate and adaptive antitumor immunity. Trends Immunol 2002; 23(4): 201−8.
39. *Górska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madaliński K*. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. J Clin Periodontol 2003; 30(12): 1046−52.
40. *Graves DT, Liu R, Alikhani M, Al-Mashat H, Trackman PC*. Diabetes-enhanced inflammation and apoptosis - impact on periodontal pathology. J Dent Res 2006; 85(1): 15−21.
41. *Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, Hsu D, Celenti RS, Grbic JT,* et al. Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. J Periodontol 2004; 75(9): 1203−8.
42. *Iacopino AM, Cutler CW*. Pathophysiological relationships between periodontitis and systemic disease: Recent concepts involving serum lipids. J Periodontol 2000; 71(8): 1375−84.
43. *de Queiroz AC, Taba M, O'Connell PA, da Nóbrega PB, Costa PP, Kawata VK*, et al. Inflammation markers in healthy and periodontitis patients: a preliminary data screening. Braz Dent J 2008; 19(1): 3−8.
44. *Schmidt AM, Yan SD, Wautier JL, Stern D*. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res 1999; 84(5): 489−97.
45. *Sloop GD*. Atherosclerosis: An inflammatory disease. N Engl J Med 1999; 340(24): 115−26.
46. *Verma S*. C-reactive protein incites atherosclerosis. Can J Cardiol 2004; 20(Suppl B): 29−31.
47. *Schmidt AM, Weidman E, Lalla E, Yan S, Hori O, Cao* R, et al. Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. J Periodontol Res 1996; 31(7): 508−15.
48. *Erbağci AB, Tarakçioğlu M, Coşkun Y, Sivasli E, Sibel NE*. Mediators of inflammation in children with type I diabetes mellitus: cytokines in type I diabetic children. Clin Biochem 2001; 34(8): 645−50.
49. *Daković D, Čolić M, Čakić S, Mileusnić I, Hajduković Z, Stamatović N*. Salivary interleukin-8 levels in children suffering from type 1 diabetes mellitus. J Clinical Pediatr Dent 2013; 37(4): 377−80.
50. *Chettab K, Zibara K, Belaiba SR, Mcgregor JL*. Acute hyperglicaemia induces changes in the transcription levels of 4 major genes in human endothelial cells: macroarrays-based expression analysis. Tromb Haemost 2002; 87(1): 141−8.
51. *Jain SK, Rains JL, Croad JL*. High glucose and ketosis (acetoacetate) increases, and chromium niacinate decreases, IL-6, IL-8, and MCP-1 secretion and oxidative stress in U937 monocytes. Antioxid Redox Signal 2007; 9(10): 1581−90.
52. *Karlsson Faresjo MG, Ernerudh J, Ludvigsson J*. Cytokine profile in children during the first 3 months after the diagnosis of type 1 diabetes. Scand J Immunol 2004; 59(5): 517−26.
53. *Foss Freitas MC, Foss NT, Donadi EA, Foss MC*. Effect of metabolic control on interferon-gamma and interleukin-10 production by peripheral blood mononuclear cells from type 1 and type 2 diabetic patients. Braz J Med Biol Res 2007; 40(5): 671−7.
54. *Daković D*. The influence of type 1 diabetes mellitus on periodontal damage and salivary cytokines leves in chidlren and adolescents [dissertation]. Belgrade, Serbia: Military Medical Academy; 2010. (Serbian)
55. *Kolb H, Mandrup Poulsen T*. An immune origin of type 2 diabetes. Diabetologia 2005; 48(6): 1038−50.
56. *Rylander H, Ramberg P, Blohme G, Lindhe J*. Prevalence of periodontal disease in young diabetics. J Clin Periodontol 1987; 14(1): 38−43.
57. *Rosenthal IM, Abrams H, Kopczyk A*. The relationship of inflammatory periodontal disease to diabetic status in insulin-dependent diabetes mellitus patients. J Clin Periodontol 1988; 15(7): 425−9.
58. *Sandholm L, Swanljung O, Rytomaa I, Kaprio EA, Maenpaa J*. Periodontal status of Finnish adolescents with insulin-dependent diabetes mellitus. J Clin Periodontol 1989; 16(10): 617−20.
59. *Karjalainen KM, Knuuttila ML*. The onset of diabetes and poor metabolic control increases gingival bleeding in children and adolescents with insulin-dependent diabetes mellitus. J Clin Periodontol 1996; 23(12): 1060−7.

Received on December 12, 2013.
Revised on January 17, 2014.
Accepted on January 28, 2014.
OnLine-First August, 2014.

Addendum 1

Findings of clinical studies which investigated the correlaton beetwen type 1 diabetes mellitus (DM) and periodontal disease

Author/ year	Study population	Duration of diabetes mellitus Type 1 (years)	Glycated haemoglobin level – HbA1c (%)	Gingival parameters	Periodontal parameters	Main findings
Rylander et al. 1987. [56]	46 patients with DM1 mean age 22.1 ± 4.7 41 healthy individuals mean age 22.3 ± 2.1	10–14 years – 24 children 15–19 years – 20 children > 20 years – 2 children	< 7.0 – 2 children 7.0–8.9 – 13 children 9.0–11.9 – 17 children 12.0–13.9 – 12 children > 14.0 – 2 children	PI GI	PPD (mm) only probing depths of > 3 mm CAL (mm) measured at 4 sites around each tooth GR (mm)	Higher frequency with clinical attachment loss on buccal sites, and GR in diabetic group than in the control group. The presence of dental biofilm on these tooth surfaces was equally low in the 2 groups; in interproximal regions very low frequency of periodontal tissue breakdown
Rosenthal et al. 1988. [57]	52 patients with DM1 mean 14.5 years	NR	Patients with DM1 without periodontitis HbA1c – 12.56% DM1 patients with periodontitis HbA1c – 9.17%	PI GI SBI	PPD (mm) measured at 4 sites around each tooth	GI and SBI were significantly higher in the periodontitis group. PI was not significantly different between the groups. Diabetics with periodontitis had a significantly lower glycosylated hemoglobin than diabetics without periodontitis
Sandholm et al. 1989. [58]	85 patients with DM1 mean 15.1 ± 1.5 SV years 85 healthy adolescents mean 15.1 ± 1.6 years	mean 5.2 ± 3.5 years	mean 10.9 ± 2.2 (SD)	PI GI RC	CPITN (registrated using 16, 21, 26, 36, 41 and 46 as index teeth) PPD (mm) measured at 4 sites around each tooth	Finnish adolescents with DM1 had more gingivitis than their age- and sex-matched healthy controls. There were no differences in periodontal destruction as measured in number of pockets > 4 mm. All gingivitis does not lead to periodontitis, but periodontitis preceeded by gingivitis
Pinson et al. 1995. [14]	26 patients with DM1 mean 13.50 ± 3.36 years included one set of identical twins 24 control subjects mean 13.54 ± 3.08 years	mean 6.58 ± 3.66 years, with 24 of the patients having had DM1 for at least 5 years	3.4–6.1 – 6 children 6.2–9.0 – 9 children > 9.0 – 11 children	PI GI gingival bleeding on probing	PPD (mm) measured at 4 sites of each tooth CAL (mm) measured at 4 sites around each tooth GR (mm)	Significant association of age with pocket probing depth, clinical attachment levels, and bleeding in the diabetic group. Duration of diabetics and metabolic control level were not related to periodontal parameters. DM1 had a significant association with severity of periodontitis when tooth sites in patients groups were statistically compared
Karjalainen et Knuuttila 1996. [59]	12 patients with DM1 mean 10.6 ± 2.4 years 80 healthy subjects mean 14.5 ± 1.6 years	NR	on the 3rd day in hospital 14.9 ± 3.8% on the 12rd day in hospital 13.1 ± 2.9% on the 1st outpatient visit at the hospital 1 month later 8.4 ± 1.5%	PI % of visible plaque gingival bleeding on probing % of bleeding surfaces	NR	Hyperglycaemia and poor metabolic control of diabetes increased gingival bleeding. Not all gingivitis proceeds into a destructive periodontal disease. Lower resistance toward dental plaque in poorly-controled patients indicates that biological alterations could take place during glucose balance deterioration
Firatli 1997. [16]	77 patients with DM1 mean age 12.47 years 77 healthy volunteers mean age 12.59 years	mean 48.34 ± 23.69 months	children with DM1 9.34 ± 3.99% control group 5.96 ± 1.02%	PI GI gingival bleeding on probing	PPD (mm) measured at 4 sites of each tooth CAL (mm) measured at 4 sites around each tooth	A positive correlation between the duration of DM1 and CAL. Relationship between the duration of DM1 and severity of periodontal alterations
Aren et al. 2003. [18]	Group 1: 16 newly diagnosed children; mean age 12.8 ± 5.8 Group 2: 16 children with diabetes of long duration; mean age 12.7 ± 3.8 Group 3: 16 healthy	Children in Group 1 with newly diagnosed DM1 and Group 2 with diabetes of long duration;	Group 1 8.01 ± 1.79 % Group 2 8.43 ± 1.36 % Group 3 5.05 ± 0.36 %	PI GI gingival bleeding on probing	PPD (mm)	Glycaemic status of the diabetic subjects affects the periodontal probing depths, salivary pH, buffering capacity, and peroxidase activity.

	subjects; mean age 12.4 ± 1.9					
Lalla et al. 2006. [11]	182 patients with DM1 mean 11.9 ± 3.3 years 160 healthy children mean 10.9 ± 2.6 years	mean 4.5 ± 8.0 years Age of diagnosis DM1 mean 7.8 ± 4.0 years	< 7.5% – 55 children 7.5–9.5% – 80 children > 9.5% – 36 children	PI % of sites PI GI % of bleeding sites GI score of 2 or 3 denotes a bleeding site	PPD (mm) measured at 4 sites of each tooth CAL (mm) measured at 4 sites around each tooth Number of affected teeth (at least one site with attachment los > 2 mm on at least two teeth)	Periodontal destruction was increased in children and adolescents with DM1. Diabetes started earlier in life than formerly recognized. Duration of DM1, and especially mean A1c, were not significantly correlated with the number of affected teeth.
Lalla et al. 2007. [17]	350 patients with DM1 mean 11.33 ± 3.41 years	mean 3.96 ± 3.39 years Age of diagnosis DM1 mean 7.54 ± 4.0 years	< 7.5% – 97 children 7.5–9.5% – 170 children > 9.5% – 73 children	PI % of sites PI GI % of bleeding sites GI GI score of 2 or 3 denotes a bleeding site	PPD (mm) measured at 4 sites of each tooth CAL (mm) measured at 4 sites around each tooth Number of affected teeth (at least one site with attechment loss > 2 mm on at least two teeth)	Increased periodontal destruction in children and adolescents with DM1 was connected with increased metabolic control. If gingival bleeding and attachment loss measurements were both used, the present study revealed that hemoglobin A1c significantly correlated with periodontitis
Daković and Pavlović 2008. [12]	187 patients with DM1 mean 12.4 ± 4.2 years 178 healthy children mean 11.4 ± 4.3 years	mean 4.9 ± 3.5 years Age of diagnosis DM1 mean 7.9 ± 4.2 years	< 7.5% – 20 children 7.5–9.5% – 95 children > 9.5% – 65 children	PI % of sites PI GI % of bleeding sites GI GBI bleeding/plaque ratio	PPD (mm) measured at 4 sites of each tooth CAL (mm) measured at 4 sites around each tooth Number (%) of patients with periodontitis and any level of CAL, and CAL > 1,5 mm	Periodontal disease was more prevalent in children with DM1 and was in function of metabolic control and disease duration. The gingival inflammation in the evolution of periodontal destruction was more important in children with DM1 than in subjects without the disease
Foia et al. 2011. [31]	42 patients with DM1 and periodontitis 42 non-diabetic subjects with periodontitis The subjects were divided into subgroups, according to the age and metabolic control of DM1	NR	< 7% – 22 children > 7% – 20 children	PI GBI	CAL (mm) measured at 4 sites around each tooth	All studies on clinical parameters reffering to periodontal status in diabetic children were much higher than those in sistemically healthy group. Children were susceptible to periodontal destruction, especially when the etiologic external factors were associated with host related systemic impairment, such as insulin dependent diabetes.. Systemic diseased metabolic balance was significantly conected with Gram-negative species-mediated cytokine translocation from the periodontal space into the circulation.

PI – plaque index; PPD – pocket probing depth; GR – gingival recession; GI – gingival index; CAL – clinical attachment level; NR – not reported; SBI – sulcus bleeding index; RCI – retentive calculus index; CPITN – community periodontal index of treatment needs; GBI – gingival bleeding index.

CURRENT TOPIC

UDC:577.115:615.451.2]:[615.279:615.874.2
DOI: 10.2298/VSP130429028P

Intravenous fat emulsion in clinical practice: nutrient and antidote

Intravenska emulzija masti u kliničkoj praksi: nutrijent i antidot

Vesna Putić*, Jasmina Jović-Stošić†‡

***Sector for Pharmacy, †National Poison Control Center, Military Medical Academy, Belgrade, Serbia; ‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Key words:
fat emulsions, intravenous; clinical medicine; parenteral nutrition; antidotes; treatment outcome.

Ključne reči:
emulzije, masne, intravenske; medicina, klinička; ishrana, parenteralna; antidoti; lečenje, ishod.

Introduction

For more than 50 years intravenous fat emulsions (IVFEs), or intravenous lipid emulsions, have been used for nutritive support for patients who are unable to attain adequate nourishment *via* the gastrointestinal tract [1, 2]. This made IVFEs an important part of total parenteral nutrition regimens [3, 4]. Although used to meet the caloric requirements, their role is not only to provide fat as physiological nonprotein energy source, but also to prevent or correct essential fatty acids (FA_S) deficiency (linoleic acid and linolenic acid) [5]. These acids cannot be synthesized in the body, and they are important to maintain the normal composition of the structural body lipids, as well as in the synthesis of the various important metabolic mediators [6].

IVFEs advantages include limitation of likely side effects of infusing large amounts of glucose. Substituting some glucose calories with lipids reduces undesirable effects of high glucose such as hyperglycemia, excessive CO_2 production, and liver FA infiltration. Lipid emulsions provide different tissues with potentially liposoluble vitamins or therapeutic agents as IVFEs were found to be useful as a delivery vehicle for drugs that are poorly soluble in water. Some of these drugs include anesthetics, sedatives, cytotoxic drugs, analgesics, and anti-inflammatory agents. Those products are prepared by dissolving drugs in the vehicle, or by addition of the drug to oil phase prior to the homogenization process for the emulsion.

In recent animal studies and human case reports, IVFEs have been proven to be an effective antidote for treating useful toxicity effects from overdose of several lipid-soluble drugs including local anesthetics, calcium channel blockers, beta blockers, tricyclic antidepressants and other psychotropic agents [7].

Composition of IVFEs

IVFEs are complex pharmaceutical products consisting of one or more triglyceride-containing oils, a phospholipid emulsifier, glycerol and water for injection. The emulsifier produces a barrier to prevent coalescence of oil droplets dispensed in internal phase of emulsion and keeps the appropriate mean particle size. Their diameter generally ranges between 100 and 500 nm, with the mean value of 200−350 nm. Glycerol is used to isotonise and stabilize oil in the water emulsion.

The first well-tolerated emulsions were made of soybean or safflower oils or a mixture of both and they contained exceptionally long-chain triglycerides (LCTs). Today, a wide variety of lipid emulsions are available world-wide, differing in triglyceride and FA contents and in concentrations of certain components, such as phospholipid [8]. The differences in the proportion of phospholipid subcomponents can be found between the emulsifiers of different manufacturers, which may also influence the metabolism of lipid components. The most frequently used lipid emulsion contains LCTs, with FA chain lengths of 16 to 20 carbon atoms. For instance, one of the most commonly used commercial products (Intralipid® 20%) contains: purified soybean oil 200g/1,000 mL; purified egg phospholipids 1.2%; glycerol anhydrous. Water for injection and sodium hydroxide are added to adjust the pH so that the final product pH range is from 6 to 8.9, while simultaneously highly purified sodium oleate serves as coemulsifier. Organic phosphate presents approximately 15 mmol/1,000 mL. The product has an osmolality of approximately 350 mOsm/kg water. Energy content is 8.4 megajoules (2,000 kcal)/1,000 mL. The interior phase of this product includes purified soybean oil which predominantly includes LCT with a high amount (> 60%) of polyunsaturated FAs (PUFAs), which contain multiple double bonds.

Correspondence to: Vesna Putić, Sector for Pharmacy, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: vputic@gmail.com

Soybean oil is a reliable source of essential FAs, in the form of linoleic acid (ώ -6 FA) and α-linolenic acid (ώ -3 FA) [9].

Stability and pharmacokinetics of IVFEs

The emulsifying agent is phospholipid from fractionated egg lecithin. Phospholipid emulsifiers are important in the development and function of cell membranes as they provide stability to IVFEs by functioning as not only a mechanical but also as an electrical barrier. Phospholipid molecules have a polar (hydrophilic) and a nonpolar (lipophilic) end, and they orient themselves in order to create the oil-water interface. The polar ends toward water exist in the neutral environment mostly in the dissociated states. This results in an anionic charge that creates a repulsive force, thus preventing fat particles from coalescing. This role is very important because if it were not for these forces, the emulsion would crack, lipids would coalesce, and IVFEs, if administered, would produce fat emboli. Since the basis of the electrical barrier is the anionic charge, IVFE stability might be compromised by divalent cations (magnesium and calcium), trivalent cations (iron), or an acid pH (especially at pH < 5). In most cases, even in the presence of these agents or conditions, complete destabilization of the emulsion takes time, depending on the concentration of chemical and environmental conditions such as extreme temperatures. During this period, the particle size of emulsion may increase, and this might result in excessive uptake by the reticuloendothelial system, causing a functional impairment in this system's ability to clear bacteria [10].

The structure of all IVFEs is similar to endogenous chylomicrons (a core composed of triglycerides surrounded by a layer of phospholipids). But, there are no cholesteryl ester molecules in the core of emulsion particles and there are only small amounts of free cholesterol and no apoproteins on their surface.

They are cleared by enzyme lipoprotein lipase, which hydrolyzes triglycerides, releasing free FAs, glycerin, and phospholipids. The dosage, phospholipid content, particle size, and infusion rate all have an impact on the plasma clearance. The rate of free FAs released from IVFE depends on its component oil. Soybean oil consists mainly of LCTs which require a carnitine-dependent cotransport system in order to be taken up by mitochondria and subsequently oxidized [11]. This process involves converting the LCTs into acyl coenzyme A (CoA), which is not sufficiently water soluble to pass into mitochondria. Carnitine picks up the acyl component of acyl CoA (acylcarnitine) and transports it across the mitochondria matrix where the acylcarnitine equilibrates with CoA to form acyl CoA within the mitochondria, completing its transport in this way. Once cleared from the plasma by various tissues, not all fat is oxidized.

The IVFEs clearance is decreased when free phospholipids interfere with lipoprotein lipase activity. Due to IVFEs lower concentration of free phospholipids and its larger particle size its clearance is 20%, which is relatively fast. The third factor determining plasma clearance of IVFEs is the infusion rate. A recommended maximum dosage of IVFEs for adults, as a nutrient, is 3.0 g triglycerides/kg of body weight/day.

Metabolic effects of IVFEs

The FA composition influences the metabolic effects of IVFEs administration. The intravascular metabolism of emulsion particles includes various processes. FAs in LCTs are the main component of cell membranes, and responsible for membrane structural integrity. Not only do they influence the biophysical and biochemical properties of membranes, but also serve as precursors of potent, short-lived oxylipids that modulate signal transduction between the cell membrane and the nucleus [12]. Long chain PUFAs metabolites serve as precursors of eicosanoids, which are of great physiological importance [13]. Eicosanoids contain 20 C and include prostaglandins, leukotrienes, thromboxanes, prostacyclins and lipoxins. These substances have an impact on a variety of processes like platelet aggregation, neurotransmitter release, and vascular function. Such properties can have an influence on many other biochemical and physiologic functions related to inflammatory, immune, and protective reactions. This means that PUFAs reduce the levels of total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

Essential FA deficiency may result in clinical sequelae including disturbances in cardiopulmonary function, infection, bleeding, dermatitis, hepatic dysfunction [12].

Fatty changes in the liver are linked to a reduction in albumin synthesis and mRNA downregulation of antioxidant enzymes which may cause the production of reactive oxygen species, the promotion of oxidative stress in the hepatocyte membrane, functional damage to the liver and consequently growth delay and death.

Antidote use of IVFEs

Over the last few years many experimental and anecdotal evidence showed that IVFEs can reverse some hemodynamic, electrocardiographic and neurological parameters and potentially decrease morbidity and mortality in poisoned patients. Recently, IVFEs in the form of Intralipid® 20% have been used in clinical practice for reducing the bioavailability and toxicity of lipophilic poisonous agents in circulation. It has been suggested that several mechanisms could explain why IVFEs might work as antidote to an overdose of cardiotoxic drugs, including calcium channel blockers, beta blockers, digoxin, local anesthetics, tricyclic antidepressants, antipsychotics, atypical antidepressants, and mood stabilizers[14-16].

The "lipid sink" phenomenon is the most widely accepted mechanism of action for IVFEs. When infused into an aqueous medium such as blood, emulsified fat droplets form a lipid compartment into which lipophilic substances are theoretically partitioned. They are drawn into the "lipid sink" and a concentration gradient develops between tissue and blood. It causes poisonous agent to move away from tissue receptors in the heart or the brain (areas of high concentrations) to the "lipid sink" [17, 18]. This mechanism suggests that one of the important

parameters in the design of antidotal emulsion may be the partition coefficient of the agent in the emulsion. After trapping the poison, the emulsion will be metabolized slowly, allowing the patient's liver to chemically detoxify and excrete the toxin released from emulsion droplets.

This theory complies with animal studies on lipid rescue. They show greater efficacy, improving survival time and increasing median lethal dose with the use of IVFEs, after experimental overdoses of many lipid soluble drugs including the most liposoluble beta blockers (propranolol) [19], calcium channel blockers (verapamil) [20, 21], antidepressants (clomipramine) [22, 23] and local anesthetic [17, 24–27]. In an experimental rat model, Weinberg et al. [17] demonstrated that radiolabeled l-bupivacaine when added *in vitro* to lipid-treated rat plasma preferentially moves to the lipid phase. In further experiments on an isolated heart model, Weinberg et al. [24] showed that infusion of lipid emulsion produces faster removal of radiolabeled bupivacaine from myocardial tissue compared with controls. The follow-up experiments examined the efficacy IVFEs in anesthetized dogs after intravenous overdose of bupivacaine. All dogs receiving lipid infusion recovered normal blood pressure and ECG traces after 10 min of lipid therapy, while all control animals died [25].

It was observed that IVFEs act faster *in vivo* settings than it was anticipated based on a simple lipid sink mechanism. Stehr et al. [26] demonstrated that lipid emulsion reverses bupivacaine-induced contractile depression at concentrations that are too low to provide a significant "lipid sink" phenomenon, suggesting a metabolic explanation for the positive effect. According to the bioenergetics theory, a large bolus of FAs provides energy substrate for a failing myocardium. Theoretically, lipid emulsion could result in the increase of intracellular FA content. FAs are the main substrates for energy generation in cardiomyocyte and represent the source of more than 80% of cardiac adenosine triphosphate (ATP) under normal conditions. Eledjam et al.[27] demonstrated that pretreatment of isolated myocardial strips with ATP prevents depression of contractility caused by toxic agents. It is possible that massive lipid infusion may result in increased intracellular FA content which may contribute to the enhancement of ATP synthesis in the cardiomyocyte. Van de Velde et al. [28] suggested such mechanism by demonstrating that infusion of 20% lipid emulsion improves cardiac contractility in a dog model. Huang et al. [29] demonstrated that FAs activate calcium channels in ventricular myocytes, so except for increasing intracellular FA content and improving ATP synthesis, positive inotropic effect of lipid emulsion may be the result of calcium influx. Blood pressure elevation due to central sympathetic activation may contribute to beneficial hemodynamic effects of lipid emulsion [30]. It is possible that lipid rescue resuscitation is a combination of all of these mechanisms [18].

Although the exact mechanisms of action of lipid emulsion infusion in treating poisoning are still not clear enough, it can be supposed that the key components are the binding property of the emulsion, characteristics of a toxic agent (liposolubility and mechanism of action).

Multiple animal models were used to compare recovery after local anesthetics and other liposoluble drugs overdose treated with IVFEs *vs* standard resuscitation protocols [31, 32]. They suggest that lipid infusions are more effective than placebo or standard antidotal therapies. They show no interaction with drugs used in standard treatment of poisoning. These studies have led to numerous human case reports which demonstrated beneficial effects of lipid emulsion in treatment of lipophilic drugs overdose. However, as randomized controlled clinical trials are not possible in this field, the evidence in humans has to be limited to case reports and small case series.

The first successful clinical application of IVFEs (Intralipid® 20%), was noted in 2006. Rosenblatt at al. [33] used it to resuscitate a patient in prolonged bupivacaine−related cardiac arrest. After more than 20 min of asystole and no response to advanced cardiac life support the use of lipid emulsion reversed systemic toxicity, including seizures, electrocardiogram abnormalities, and cardiac arrest without neurological sequelae. A few months later, Litz et al. [34] reported on the rescue of a patient in asystolic cardiac arrest secondary to the accidental overdose of ropivacaine. After 10 min of unsuccessful cardiopulmonary resuscitation, they administered a bolus of 100 mL of 20% lipid emulsion followed by continuous infusion of 10 mL *per* min. After the total IVFE dose of 200 mL, spontaneous electrical activity continued and cardiac output was restored. The first successful use of lipid emulsion as an antidote for non-local anesthetic drugs was in a 17-year-old girl who had developed seizures and cardiovascular collapse after massive ingestion of bupropion and lamotrigine. The patient had cardiac arrest with ventricular fibrillation and pulseless electrical activity. After 70 min of unsuccessful standard cardiopulmonary resuscitation, a 100 mL intravenous bolus of 20% lipid emulsion was administered, as the last attempt to restore hemodynamic stability. Normal vital signs were restored within one min of IVFEs administration [35]. IVFEs have been used to reverse coma caused by sertraline and quetiapine overdose [36]. Young et al. [37] published the first human case of verapamil toxicity of a patient who was in shock that was refractory to standard resuscitation therapy, but was resolved with administration of IVFE 20% within 5 min. Several reports on successful treatment of beta blockers toxicity have been published in recent years [38–43].

Soon after the case report by Rosenblat et al. [33] was published, Weinberg [7] established a Web site www.lipidrescue.org as a widely informational resource about local anesthetic and toxicity from other drug classes. Based on this experience Weinberg [7] has recommended a dose regimen for clinical use. A bolus of Intralipid® 20% 1.5 mL/kg over a min was recommended with subsequent 0.25 mL/kg min. A bolus can be repeated every 3 or 4 min up to the dose of 3 mL/kg total dose until circulation is restored. Infusion should be continued until hemodynamic stability is achieved and the rate increased to 0.5 mL/kg/min if blood pressure drops. The total maximum dose is 8−10 mL/kg. This is the current protocol recommended by the Association of Anesthetists of Great Britain and Ireland [44], the American Society of Critical Care Anesthesiologists, the American Society of Anesthesiologists Committee on Critical Care Medicine, and the Resuscitation Council of the UK. In 2010 the

American Society of Regional Anesthesia published a practice advisory on local anesthetic toxicity, stressed the lipid's role in local anesthetic systemic toxicity [45]. The guidelines included the use of intravenous lipid emulsion as an adjunct to airway management and good cardiopulmonary resuscitation, stating: "... lipid emulsion therapy can be instrumental in facilitating resuscitation, most probably by acting as a lipid sink that draws down the content of lipid-soluble local anesthetics from within cardiac tissue, thereby improving cardiac conduction, contractility, and coronary perfusion".

Adverse events associated with the use of IVFE

The clinical events and adverse effects of IFVEs are related to their composition, characteristics, stability and sterility.

It is contraindicated in patients with severe hyperlipemia, liver insufficiency, hemophagocytic syndrome, hypersensitivity to egg-, soy- and peanut protein (risk of cross-allergy reactions) or to any of the active substances or excipients.

The manufacturers recommend laboratory studies for all lipid emulsions. These include complete blood count, serum electrolytes, blood glucose, serum proteins, parameters for liver function, serum and urinary osmolality and monitoring of triglyceride levels on daily basis. This is the major preventive measure in avoiding overfeeding and liver injury. Serum triglyceride levels can rise rapidly if the capacity to clear them is compromised as is the case in genetically caused impaired metabolism. As a consequence, overdosage and fat overload syndrome might occur. Patients with impaired lipid metabolism and liver function, uncompensated diabetes mellitus, pancreatitis, hypothyroidism and sepsis require monitoring of the serum triglyceride concentration.

It is also important to stress that lipemia can interfere with some laboratory analysis. This is why the producers recommend analysis after 5−6 h without the use of lipids.

Undesirable effects connected with IVFEs administration include a slight rise in body temperature, chills, lack of appetite and nausea/vomiting (with less than 1% incidence), the feeling of warmth or blueness, head and bones ache, and in very rarely – priapism. Other adverse effects are extremely rare, less than one *per* one million infusions. If the symptoms of overdose occur, they are usually reversible if infusion is discontinued. However, an excessive amount of FAs, particularly linoleic acid, may alter the structure and the function of the cellular membrane and increase oxidative stress. A high content of FAs and their limited content of α-tocopherol, in long-term use of soybean oil–based emulsions may reduce α-tocopherol in plasma lipoproteins and deplete antioxidant defenses [46]. The risk of lipid peroxidation may be increased. This is particularly worrying in situations when patients are often exposed to oxidative stress under intensive care conditions.

Although LCT-based lipid emulsions have been shown to be safe and clinically well−tolerated, several studies showed that they might interfere negatively with the immune system. Since 1970s studies on the effects of FAs on the inflammatory response and the immune system have indicated a potential role of lipids in modulating inflammatory and immunological functions. An increased ratio of ώ-3 to ώ-6 FAs intake inhibits the metabolism of arachidonic acid and results in decreased production of proinflammatory cytokines such as interleukin (IL) 6, IL-8, tumor necrosis factor-α, and other inflammatory mediators such as platelet activating factor and adhesion molecules [47]. A high concentration of unmetabolised FAs increases the production of immunosuppressive (E2 series) prostaglandins. Pulmonary dysfunction may result from the release of vasoactive amines produced of excessive prostaglandin E_2 levels. Moreover, a large amount of the infused LCTs is not readily oxidized, due to the relative deficiency of carnitine or inhibition of the carnitine acyltransferase system for translocating LCTs across the inner mitochondrial membrane in critically ill patients [48]. On the other hand, other studies did not confirm the negative effects of LCT-based lipid emulsions on the immunological response or even showed beneficial effects. A meta-analysis by Wirtitsch et al. [49] has not revealed any effects of the lipid regimen on the evolution of the immunological status or mortality in humans.

Future research

In many animal and human studies the efficacy and safety of IFVEs 20% as a calorie source have been demonstrated. The results obtained experimentally and clinically in the past years demonstrated the safety and efficacy of Intralipid® 20% for use in total parenteral nutrition.

Animal studies and additional human reviews in further research on efficacy and safety of IFVEs 20% as an antidote, will shed more light on lipid therapy according to the best rate and total dose of infusion that follows bolus dosing; efficacy when used in conjunction with other resuscitative measures and assessment; the risk of recurrence of toxicity once the lipid infusion is stopped; dosing parameters of lipid administration adjustment for different patient population especially hepatic and renal-impaired. They will also show whether the other available lipid emulsions have the same effects as rapid infusion of Intralipid® 20%. Until that evidence is obtained, lipid emulsion is recommended in the resuscitation of local anesthetic and highly lipophilic medication toxicity refractory to conventional models of resuscitation with significant hemodynamic, neurological or cardiovascular symptoms [50-52].

Conclusion

Beside the fact that intravenous fat emulsions have been used for decades for nutritional support, intravenous fat emulsions also can reverse some hemodynamic, electrocardiographic and neurological parameters and potentially decrease morbidity in poisoned patients. Although it is not yet considered a generic first-line treatment in cases of unknown drug overdoses, the use of intravenous fat emulsions should be strongly considered, particularly in failed resuscitations.

REFERENCES

1. *Mirtallo J, Dasta J, Kleinschmidt K, Varon J.* State of the art review: Intravenous fat emulsions: Current applications, safety profile, and clinical implications. Ann Pharmacother 2010; 44(4): 688– 700.
2. *Goulet O, Postaire M, De Potter S, Boya I, Jouniaux AM, Bereziat G*, et al. Medium-chain triglycerides and long-term parenteral nutrition in children. Nutrition 1992; 8(5): 333−7.
3. *Jeejeebhoy KN.* Total parenteral nutrition: potion or poison? Am J Clin Nutr 2001; 74(2): 160−3.
4. *Mirković D, Antunović M, Putić V, Aleksić D.* Stability investigation of total parenteral nutrition admixture prepared in a hospital pharmacy. Vojnosanit Pregl 2008; 65(4): 286−90. (Serbian)
5. *Carpentier Y, Simoens C, Siderova V, El Nakadi I, Vanweyenberg V, Eggerickx D*, et al. Recent developments in lipid emulsions: relevance to intensive care. Nutrition 1997; 13(9): 73S −8S.
6. *Puiggròs C, Sánchez J, Chacón P, Sabín P, Roselló J, Bou R*, et al. Evolution of lipid profile, liver function, and pattern of plasma fatty acids according to the type of lipid emulsion administered in parenteral nutrition in the early postoperative period after digestive surgery. JPEN J Parenter Enteral Nutr 2009; 33(5): 501−12.
7. *Weinberg G.* LipidRescue™ Resuscitation. Available from: www.lipidrescue.org
8. *Garcia-de-Lorenzo A, Lopez-Martinez J, Planas M, Chacon P, Montejo J, Bonet A,* et al. Safety and metabolic tolerance of a concentrated long-chain triglyceride lipid emulsion in critically ill septic and trauma patients. JPEN J Parenter Enteral Nutr 2003; 27(3): 208−15.
9. Intralipid 20%. Available from: www.accessdata.fda.gov/.../017643s072,084
10. *Lee E, Simmer K, Gibson RA.* Essential fatty acid deficiency in parenterally fed preterm infants. J Paediatr Child Health 1993; 29(1): 51−5.
11. *Lutz O, Lave T, Frey A, Meraihi Z, Bach AC.* Activites of lipoprotein lipase and hepatic lipase on long- and medium-chain triglyceride emulsions used in parenteral nutrition. Metabolism 1989; 38(6): 507−13.
12. *Carpentier YA, Thonnart N.* Parameters for evaluation of lipid metabolism. JPEN J Parenter Enteral Nutr 1987; 11(5 Suppl): 104S−8S.
13. *Wretlind A.* Development of fat emulsions. JPEN J Parenter Enteral Nutr 1981; 5(3): 230−5.
14. *Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, Chauny JM.* Lipid emulsions in the treatment of acute poisoning: a systematic review of human and animal studies. Clin Toxicol (Phila) 2010; 48(1): 1−27.
15. *Picard J, Harrop-Griffiths W.* Lipid emulsion to treat drug overdose: past, present and future. Anaesthesia 2009, 64(2): 119-21.
16. *Cave G, Harvey M, Graudins A.* Review article: Intravenous lipid emulsion as antidote: A summary of published human experience. Emerg Med Australas 2011; 23(2): 123−41.
17. *Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ.* Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anestesiology 1998; 88(4): 1071−5.
18. *Weinberg G.* Lipid rescue resuscitation from local anaesthetic cardiac toxicity. Toxicol Rev 2006; 25(3): 139−45.
19. *Cave G, Harvey M, Castle C.* The role of fat emulsion therapy in a rodentmodel of propranolol toxicity: a preliminary study. J Med Toxicol 2006; 2(1): 4−7.
20. *Bania T, Chu J, Perez E, Su M, Hahn IH.* Hemodynamic effects of intravenous fat emulsions in an animal model of severe verapamil toxicity resuscitated with atropine calcium, and saline. Acad Emerg Med 2006; 13(2): 134−9.
21. *Perez E, Bania T, Medlej K, Chu J, Mouravev R.* Determining the optimal dose of intravenous fat emulsions for the treatment of severe verapamil toxicity in a rodent model. Acad Emerg Med 2008; 15(5 Suppl1): S92−3.
22. *Harvey M, Cave G.* Intralipid outperforms sodium bicarbonate in a rabbit model of clomipramine toxicity. Acad Emerg Med 2007; 49(2): 178−85.
23. *Harvey M, Cave G, Hoggett K.* Correlation of plasma and peritoneal dialysate clomipramine concentration with hemodynamic recovery after intralipid infusion in rabbits. Acad Emerg Med 2009; 16(2): 151−6.
24. *Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, Strichartz G*, et al. Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. Reg Anesth Pain Med 2006, 31: 296–303.
25. *Weinberg G, Ripper R, Feinstein DL, Hoffman W.* Lipid emulsion infusion rescue dogs from bupivacaine-induced cardiac toxicity. Reg Anesth Pain Med 2003; 28(3): 198–202.
26. *Stehr SN, Ziegeler JC, Pexa A, Oertel R, Deussen A, Koch T*, et al. The effects of lipid infusion on myocardial function and bioenergetics in l-bupivacaine toxicity in the isolated rat heart. Anesth Analg 2007; 104(1): 186–92.
27. *Eledjam JJ, de La Coussaye JE, Brugada J, Bassoul B, Gagnol JP, Fabregat JR*, et al. In vitro study on mechanisms of bupivacaine-induced depression of myocardial contractility. Anesth Analg 1989; 69(6): 732−5.
28. *Van de Velde M, Wouters PF, Rolf N, Van Aken H, Flameng W, Vandermeersch E.* Long-chain triglycerides improve recovery from myocardial stunning in conscious dogs. Cardiovasc Res 1996; 32(6): 1008−15.
29. *Huang JM, Xian H, Bacaner M.* Long-chain fatty acids activate calcium channels in ventricular myocytes. Proc Natl Acad Sci USA 1992; 89(14): 6452−6.
30. *Florian JP, Pawelczyk JA.* Non-esterified fatty acids increase arterial pressure via central sympathetic activation in humans. Clin Sci (Lond) 2009; 118(1): 61−9.
31. *Di Gregorio G, Schwartz D, Ripper R, Kelly K, Feinstein DL, Minshall RD,* et al. Lipid emulsion is superior to vasopressin in a rodent model of resuscitation from toxin-induced cardiac arrest. Crit Care Med 2009; 37(3): 993−9.
32. *Ciechanowicz S, Patil V.* Lipid emulsion for local anesthetic systemic toxicity. Anesthesiol Res Pract 2012; 2012: 131784.
33. *Rosenblatt MA, Abel M, Fischer GW, Itzkovich CJ, Eisenkraft JB.* Successful use of a 20% emulsion to resuscitate a patient after a presumed bupivacaine-related cardiac arrest. Anesthesiology 2006; 105(1): 217−8.
34. *Litz RJ, Popp M, Stehr SN, Koch T.* Successful resuscitation of a patient with ropivacain-induced asystole after axillary plexus block using lipid infusion Anesthesia 2006; 61(8): 800−1.
35. *Sirianni AJ, Osterhoudt KC, Calello DP, Muller AA, Waterhouse MR, Goodkin MB.* Use of lipid emulsion in the resuscitation of a patient with prolonged cardiovascular collapse after overdose of bupropion and lamotrigine. Ann Emerg Med 2008; 51(4): 412−5.
36. *Finn SD, Uncles DR, Willers J, Sable N.* Early treatment of a quetiapine and sertraline overdose with Intralipid. Anesthesia 2009; 64(2): 191−4.
37. *Young AC, Velez LI, Kleinschmidt KC.* Intravenous fat emulsion therapy for the intentional sustained-release verapamil overdose. Resuscitation 2009; 80(5): 591−3.

38. *Harchelroad F, Palma A*. Efficacy and safety of intravenous lipid therapy in a B- blocker overdose. Clin Toxicol 2008; 46(7): 634.
39. *Dean P, Ruddy JP, Marshall S*. Intravenous lipid emulsion on propranolol [corrected] overdose. Anaesthesia 2010; 65(11): 1148−50.
40. *Jovic-Stosic J, Gligic B, Putic V, Brajkovic G, Spasic R*. Severe propranolol and ethanol overdose with wide complex tachycardia treated with intravenous lipid emulsion: A case report. Clin Toxicol (Phila) 2011; 49(5): 426–30.
41. *Dolcourt B, Aaron C*. Intravenous fat emulsion for refractory verapamil and atenolol induced shock: a human case report. Clin Toxicol 2008; 46(7): 619−20.
42. *Carr D, Boone A, Hoffman RS, Martin K, Ahluwalia N*. Successful resuscitation of carvedilol overdose using intravenous fat emulsion (IFE). Clin Toxicol 2009; 47(6): 726.
43. *Stellpflug SJ, Harris CR, Engebretsen KM, Cole JB, Holger JS*. Intentional overdose with cardiac arrest treated with intravenous fat emulsion and high-dose insulin. Clin Toxicol (Phila) 2010; 48(3): 227−9.
44. Guidelines for the Management of severe local Anaesthetic Toxicity. Association of Anaesthetists of Great Britain and Ireland. Available from: http://www.aagbi.org/publications/guidelines/docs/latoxicity07.pdf. [accessed 2012 February].
45. *Neal JM, Bernards CM, Butterworth JF 4th, Di Gregorio G, Drasner K, Hejtmanek MR,* et al. ASRA Practice Advisory on Local Anesthetic Systemic Toxicity. Reg Anesth Pain Med 2010; 35(2): 152−61.
46. *Sandstrom R, Hyltander A, Korner U, Lundholm K*. Structured triglycerides were well tolerated and induced increased whole body fat oxidation compared with long-chain triglycerides in postoperative patients. JPEN J Parenter Enteral Nutr 1995; 19(5): 381−6.
47. *Goulet O, Antébi H, Wolf C, Talbotec C, Alcindor LG, Corriol O,* et al. A new intravenous fat emulsion containing soybean oil, medium-chain triglycerides, olive oil, and fish oil: a single-center, double- blind randomized study on efficacy and safety in pediatric patients receiving home parenteral nutrition. JPEN J Parenter Enteral Nutr 2010; 34(5): 485-95.
48. *Chen FM, Wang JY, Sun LC, Juang RF, Huang TJ, Hsieh JS*. Efficacy of medium-chain triglycerides compared with long-chain triglycerides in total parenteral nutrition in patients with digestive tract cancer undergoing surgery. Kaohsiung J Med Sci 2005; 21(11): 487−94.
49. *Wirtitsch M, Wessner B, Spittler A, Roth E, Volk T, Bachman L*, et al. Effect of different lipid emulsions on the immunological function in humans: a systematic reviw with meta-analysis. Clin Nutr 2007; 26(3): 302−13.
50. *Picard J, Ward SC, Zumpe R, Meek T, Barlow J, Harrop-Griffiths W*. Guidelines and the adoption of 'lipid rescue' therapy for local anaesthetic toxicity. Anaesthesia 2009; 64(2): 122−5.
51. *Martin TG*. Intravenous lipid emulsion therapy for poisoning. Abstracts of the XXVIII International Congress of the European Association of Poison Centres and Clinical Toxicologists; 2008 May 6−9; Seville, Spain. Clin Toxicol 2008; 46(5): 403.
52. *Weinberg G*. Intravenous lipid emulsion: Why wait to save a life? Emerg Med Australas 2011; 23(2): 113−5.

Received on April 29, 2013.
Revised on February 19, 2014.
Accepted on February 24, 2014.
OnLine-First April, 2014.

CASE REPORTS

UDC: 616.529.1::616.329-07
DOI: 10.2298/VSP1503280R

Epidermolysis bullosa of the esophagus – A case report

Bulozna epidermoliza jednjaka

Maja Radić, Darka Hadnadjev

Center of Radiology, Clinical Center of Vojvodina, Novi Sad, Serbia

Abstract

Introduction. *Epidermolysis bullosa* is a rare skin disease which could be hereditary or acquired with autoimmune mechanism. Even though it is known that epidermolysis bullosa appears on various mucosa, the esophagus is seldom affected. **Case report.** We reported 19-year-old female patient who had been admitted due to dysphagia and odynophagia to solid food. Erythematous changes with bullae and excoriations could be found on the hands, feet, elbows and knees. The patient underwent barium swallow which revealed retaining of contrast in the valleculas and piriform recesses, as well as dilatation of meso- and hypopharynx – upper achalasia syndrome. The cause was stenosis at the level of upper functional sphincter of the esophagus, 10 mm in length with benign apperance. Small leakage of contrast into the trachea was visible at the later stage of examination, concomitant with volume load of the pharynx. Bullae were not detected. The whole esophagus was fairly uniformly stenotic and had fibrotic appearance. **Conclusion.** The authors emphasize that barium swallow can provide sufficient information regarding stenosis, dynamics of the disorder, as well as the stage of the disease. Furthermore, we highlight the importance of providing a complete diagnostic strategy in all dermatology patients who could simultaneously have mucous changes.

Key words:
epidermolysis bullosa; esophagus; diagnosis.

Apstrakt

Uvod. Bulozna epidermoliza (*epidermolysis bullosa* – EB) je retko kožno oboljenje koje može biti nasledno ili stečeno po autoimunom mehanizmu. Poznato je da EB zahvata i sluzokožu. Ipak, jednjak je retko zahvaćen. **Prikaz bolesnika.** U radu je prikazana devetnaestogodišnja bolesnica sa tegobama disfagije i odinofagije na čvrstu hranu. Na rukama, stopalima, laktovima i kolenima videle su se eritematozne promene sa bulama i ekskorijacijama. Urađeni su akt gutanja i pasaža jednjaka s kontrastom (BaSO4) tokom kojih je ustanovljeno njegovo zadržavanje u valekulama i piriformnim recesusima, dilatacija mezo- i hipofarinksa sa benignom stenozom od 10 mm u visini gornjeg funkcionalnog sfinktera jednjaka, što je definisano kao sindrom gornje ahalazije. Istovremeno sa volumnim opterećenjem farinksa video se linearni prodor kontrasta u disajno stablo. Bule nisu viđene, a ceo jednjak je bio sužen, fibroznog izgleda. **Zaključak.** Važno je istaći da se aktom gutanja i pasažom jednjaka dobija dovoljno informacija o stepenu stenoze, dinamici poremećaja i stadijumu bolesti. Važno je da se naglasi da je kod bolesnika sa kožnim promenama i tegobama sa digestivnim traktom potrebno uraditi radiološki pregled digestivnog trakta s obzirom na to da mogu da postoje udružene promene na sluzokoži koje su i uzrok tegoba.

Ključne reči:
epidermoliza, bulozna; jednjak; dijagnoza.

Introduction

Epidermolysis bullosa is a rare skin disease characterized by nail dystrophy, formation of vesicles and bullae as a response to minimal trauma. Most frequently it affects elbows, knees, palms and feet [1, 2].

The disease can be hereditary (there are two autosomal dominant and one autosomal recessive forms) or acquired with autoimmune mechanism. Furthermore, it can be subclassified based on histological findings – depending on the level of skin in which bullae are formed (epidermis, *lamina propria* and dermis).

Symptoms appear very early in life, usually at infant age, but sometimes the first signs appear in adolescence. This depends on the form of the disease.

Although *epidermolysis bullosa* appears on mucosa, esophagus is seldom affected.

Here, we presented a patient with the whole esophagus stenotic with prestenotic dilatation and consecutive leakage of contrast material into the respiratory tract.

Correspondence to: Maja Radić, Center of Radiology, Clinical Center of Vojvodina, Hajduk Veljkova 1–9, 21000 Novi Sad, Serbia. Phone.:+381 63 8227 415, Fax: +381 21 520 577, E-mail: maja.pilipovic@yahoo.com

Case report

A 19-year-old female patient was admitted due to dysphagia and odynophagia to solid food. The symptoms first appeared a few months before admition and aggravated. The patient denied hematemesis, vomiting and melena. The family history was negative for similar diseases. The patient noticed skin changes before but paid no attention to them because, as she stated, her skin had always been fragile. When scratching it, small ulcers appeared turning into scars.

Physical examination revealed that the patient was ill nourished, slightly anemic and dehydrated. Oral mucosa appeared normal and without pathological changes. Nails had normal appearance. Skin was pale and its turgor was decreased. Erythematous changes with bullae and excoriations could be found on the hands, feet, elbows and knees. Lung examination revealed no pathology. The abdomen was slightly tender on light and deep palpation. There were no signs of defance or rebound tenderness.

Laboratory findings (including blood count, serum electrolytes, sedimentation rate) showed a decreased level of hematocrit and hemoglobin while other parameters were within the range of normal.

The patient underwent radiological examination because of difficulties with swallowing: barrium swallow and evaluation of esophageal passage.

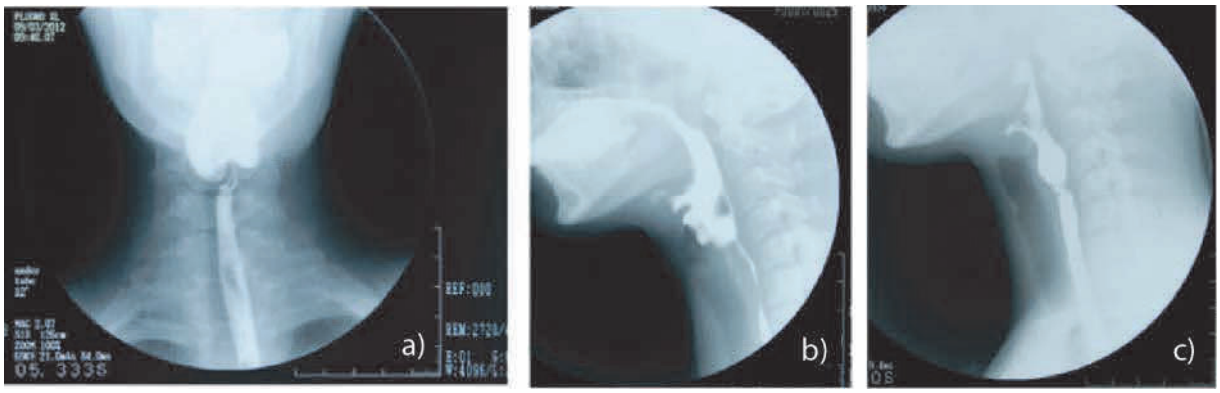

Fig. 1 – a) Anterioposterior view – retaining of contrast in the piriform recesses, dilatation of the pharynx and distal stenosis; b) Lateral view – dilatation of the hypo- and mesopharynx; c) Lateral view – level of stenosis

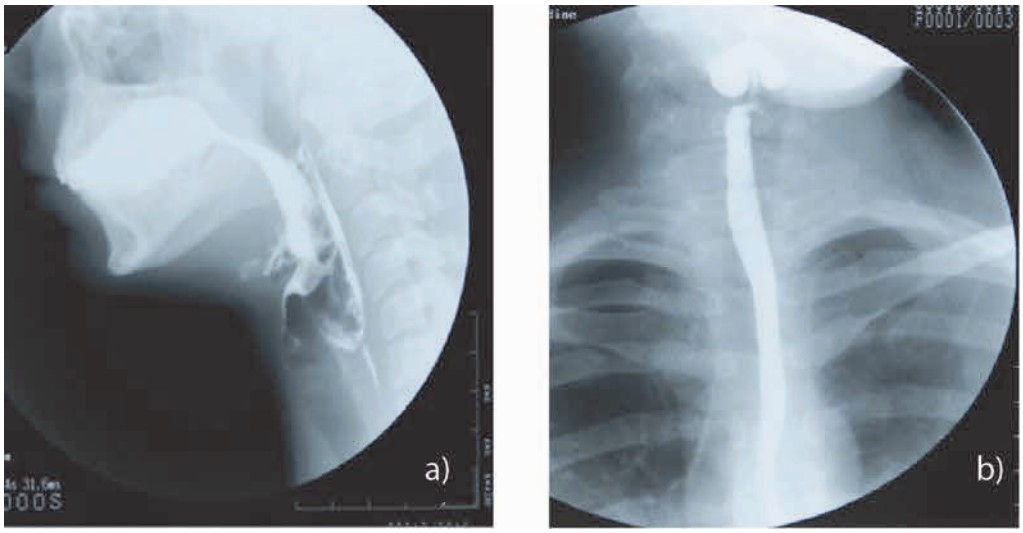

Fig. 2 – a) Lateral view – linear leakage of contrast into the trachea; b) Dynamic study – whole esophagus stenotic, with smooth margins and slow peristalsis.

The oral phase of examination showed no pathological changes. The retaining of contrast in the valleculas and piriform recesses was seen, as well as dilatation of meso- and hypopharynx at the level of the upper functional sphincter of the vel of upper the functional sphincter of the esophagus. Stenosis was 10 mm in length and slightly tortuous, had the subtotal width and reduced elasticity (Figures 1a-c).

This was defined as upper achalasia syndrome. Also, there was a thin leakage of contrast into the trachea (Figure 2a).

Dynamic study (7 frames *per* second) revealed a completely stenotic esophagus whose contours were smooth with slow peristalsis and diminished anterograde propulsion. Bullous changes were absent (Figure 2b)

Esophagoscopy was not performed. Histological findings of skin changes biopsy showed that the level of skin where changes appeared was dermis.

Discussion

Epidermolysis bullosa is a rare skin condition. Its etiology is hereditary (inherited in autosomal dominant or recessive patterns) or acquired when its etiology is autoimmune.

Further subclassification is based on histological findings. Depending on the level of skin where changes appear, it can be divided into *epidermolysis bullosa simplex* (epidermis, autosomal dominant pattern), junctional (junction of epidermis and basal membrane, also autosomal dominant), dystrophic (changes are inside dermis leading to scarring, inherited both autosomal dominant and recessive) and *epidermolysis bullosa acquisita* (acquired form, autoimmune etiology). Based on histological findings of skin changes, we concluded that our patient had dystrophic form.

Dysphagia first appears in the first decade of life in the majority of patients [3]. In the presented patient, symptoms occurred somewhat later, in the second decade of life, even though skin changes are likely to appear sometime before. It is highly unlikely that the presented patient had a recessive form of the disease because it did not appear in early childhood. Genetical mapping is under way.

Epidermolysis bullosa affects not only skin but mucosa as well. Pathophysiological mechanism is identical on both skin and mucosa – first bullae and vesicles form, then ulcers, all leading to scarring. Of course, erosions appear more often on mucosa. Trauma of the mucosa leads to formation of bullae and erosion forms after a bulla bursts. Reparative processes lead to formation of segmental stenosis [4].

Even though *epidermolysis bullosa* appears on mucosa (eye, oral, nasal, anal, genital), the esophagus is seldom affected. The cause of esophageal changes is not known in the literature, but it is suspected that the trigger is a minor but repetitive food trauma.

Most frequently, the disease affects the proximal and distal third of the esophagus [5]. A very rare form of the disease, as in the presented patient, is when the whole esophagus is affected.

In further management, patients should be referred to the gastroenterologist who could perform endoscopic examination and biopsy of changes. Some patients have oral changes which make esophagoscopy impossible to perform. In the presented patient it was not the case – no changes of oral mucosa were seen. Sometimes a very narrow esophagus could disable endoscopic examination, thus a pediatric esophagoscope is used.

The therapy for the disease is administration of steroids and for partial stenosis – dilatation, palliative gastrostoma and colon transplant [6, 7].

Barium swallow provided information about stenosis, dynamics of the disorder as well as the stage of the disease. The cause of upper achalasia (with clinically significant stasis of contrast in the valleculas and piriform recessuses) was stenosis at the level of the upper functional sphincter of the esophagus. Stenosis was 10 mm in length and slightly tortuous, had the subtotal width and reduced elasticity. It had radiological features of benign stenosis.

Small leakage of contrast into the trachea was visible at the later stage of examination, which was concomitant with stenosis and volume load of the pharynx.

Bullae were not detected. The whole esophagus was fairly uniformly stenotic and had fibrotic appearance (lumen was reduced with smooth margins, slow peristalsis and diminished anterograde propulsion).

The symptoms of this disease resemble bullous pemphigoid but bullous pemphigoid affects older population.

Conclusion

Generally, this insidious disease has to be considered in all patients who have difficulties with swallowing together with skin changes. In this way, one can improve the quality of patient's life and postpone complications. The presented patient was in the advance stage of the disease with fibrosis of the esophagus, segmental stenosis at the level of the upper esophageal sphincter, volume load of the pharynx and ensuing leakage of contrast into the respiratory tract. What was atypical in the presented case was that the entire esophagus was affected unlike proximal or distal third which are more common. Barium swallow can provide sufficient information regarding stenosis, dynamics of the disorder as well as the stage of the disease

REFERENCES

1. *Tishler JM, Han SY, Helman CA.* Esophageal involvement in epidermolysis bullosa dystrophica. AJR Am J Roentgenol 1983; 141(6): 1283−6.
2. *Mardsen F, Sambrook G, Mac Donald A. Main R.* Epidermolysis bullosa of the oesophagus with oesophageal web formation. Thorax 1974; 29(3): 287−95.
3. *Mauro MA, Parker LA, Hartley WS, Renner JB, Mauro PM.* Epidermolysis bullosa: radiographic findings in 16 cases. AJR Am J Roentgenol 1987; 149(5): 925−7.
4. *Luedtke P, Levine MS, Rubesin SE, Weinstein DS, Laufer* I. Radiologic diagnosis of benign esophageal strictures: a pattern approach. Radiographics 2003; 23(4): 897−909.
5. *Levine MS, Rubesin SE.* Diseases of the esophagus: diagnosis with esophagography. Radiology 2005; 237(2): 414−27.
6. *Warren RB, Warner TF, Gilbert EF, Pellet JR.* Acquired double-barrel oesophagus in epidermolysis bullosa dystrophica. Thorax 1980; 35(6): 472−6.
7. *Sehhat S, Amirie SA.* Oesophageal reconstruction for complete stenosis due to dystrophic epidermolysis bullosa. Thorax 1977; 32(6): 697−9.

Received on July 27, 2013.
Revised on February 16, 2014.
Accepted on February 18, 2014.

CASE REPORT

UDC: 616.329-005-02-08
DOI: 10.2298/VSP1503283A

Efficacy of long-acting somatostatin analogs in recurrent variceal bleeding in a patient with pre-hepatic portal vein thrombosis

Delotvornost leka dugotrajnog dejstva analognog somatostatina kod bolesnice sa ponovljenim varikoznim krvarenjem i trombozom prehepatične portne vene

Tamara Alempijević*†, Ana Balović†, Aleksandra Pavlović-Marković*†, Dino Tarabar‡§, Miodrag Krstić*†, Predrag Miljić*‖, Miloš Bjelović*¶

***Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Clinic for Gastroenterology and Hepatology, ‖Institute for Hematology, ¶First Surgical University Hospital Clinic, Clinical Centre of Serbia, Belgrade, Serbia; ‡Clinic for Gastroenterology, Military Medical Academy, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Bleeding from esophageal varices is a serious medical problem because of the risk of recurrent bleeding and high mortality rate (17–54%). Gastroesophageal varices develop in 50% of cirrhotic patients with portal hypertension, but can also develop in other pre- or post-hepatic causes of portal hypertension. **Case report.** We reported a 48-year-old female patient with portal hypertension caused by mesenterial vein thrombosis due to congenital thrombophilia. The patient was hospitalized several times because of recurrent gastroesophageal bleeding. Thrombosis of portal, lienal and mesenteric veins was diagnosed using multislice computed tomography (MSCT) angiography. Sclerotherapy and/or variceal ligation could not be used due to variceal size and distribution. Beta blockers were ineffective. Balloon tamponade and octreotide were used in each massive bleeding episode. Carvedilol therapy was introduced but rebleeding occured. Surgical treatment was considered a high risk procedure due to massive thrombosis of mesenterial veins, patient`s general condition and high risk of postoperative thrombotic events. Thus, long-acting somatostatin analogue – Sandostatin® LAR was initiated at a dose of 30 mg *im*/month. The patient responded to the therapy well and variceal bleeding did not occur for the following 3 months. After 3 months another episode of gastric variceal hemorrhage occurred and surgical treatment was reconsidered. Total gastrectomy was performed in order to prevent repeated bleeding from large gastric varices and the patient recovered successfully, and after 1 year is symptom-free. **Conclusion.** Long-lasting somatostatin analogue was used for the first time in treatment of gastroesophageal variceal hemorrhage in the patient with prehepatic portal hypertension. It was effective as temporary therapeutic option allowing the improvement of the patients general condition and adequate planning of elective surgical procedure. Futher reports are needed in order to compare efficacy in treatment of patients with variceal bleeding, where poor outcome is expected.

Key words:
esophageal and gastric varices; hemorrhage; venous thrombosis; diagnosis; octreotide; digestive system surgical procedures; treatment outcome.

Apstrakt

Uvod. Krvarenje iz variksa jednjaka predstavlja ozbiljno medicinsko stanje s obzirom na veliki rizik i od recidiva i smrtnog ishoda (17–54%). Variksi jednjaka i želuca razvijaju se kod 50% bolesnika sa cirozom jetre i portnom hipertenzijom, mada i druga pre- i posthepatična oboljenja mogu dovesti do njihovog stvaranja. **Prikaz bolesnika.** Prikazali smo bolesnicu staru 48 godina, sa portnom hipertenzijom prouzrokovanom mezenterijalnom venskom trombozom usled kongenitalne trombofilije. Bolesnica je više puta bila hospitalizivana zbog recidivantnog masivnog varikoznog krvarenja. Tromboza portne, lijenalne i mezenterijalne vene dijagnostikovan je uz pomoć multislajsne kompjuterizovane tomografije (MSCT) angiografije. Endoskopska terapija nije bila izvodljiva s obzirom na veličinu i lokalizaciju variksa. Krvarenje je svakog puta sanirano balon tamponadom i parenteralno sandostatinom. Beta blokatori bili su bez efekta. Hirurška terapija bila je veoma rizična zbog lošeg opšteg stanja i moguće postoperativne tromboze. Stoga je u terapiju uključen dugodelujući analog somatostatina – Sandostatin LAR u dozi od 30 mg im/mesečno. Bolesnica je adekvatno reagovala na ovu terapiju i bila bez epizoda krvarenja tri meseca. Nakon tri meseca ponovo su prokrvarili gastroezofagealni varikoziteti, ali s obzirom na značajno bolje opšte stanje odlučeno je da se bolesnica podvrgne hirurškom lečenju. Urađena je toatalna gastrektomija, nakon čega se bolesnica oporavila i godinu dana kasnije bila bez tegoba.

Correspondence to: Tamara Alempijević, Clinic for Gastroenterology, Clinical Center of Serbia, Dr Koste Todorovica 2, 11 000 Belgrade, Serbia. Phone: +381 11 3663 727, Fax: +381 11 3615 575. E-mail: tamara.alempijevic@med.bg.ac.rs

Zaključak. Lek dugotrajnog dejstva, analog somatostatina, prvi put smo primenili za lečenje krvarenja iz gastroezofagusnog variksa kod bolesnice sa prehepatičnom portalnom hipertenzijom. Pokazao se delotvornim kao privremena opcija lečenja koja je popravila opšte stanje bolesnice i omogućila adekvatno planiranje elektivne hiruške procedure. Neophodna su dalja saopštenja da bi se uporedila efikasnost lečenja bolesnika sa krvarenjem iz variksa, posebno tamo gde se ne očekuje povoljan ishod.

Ključne reči:
jednjak i želudac, variksi; krvarenje; tromboza, venska; dijagnoza; oktreotid; hirurgija digestivnog sistema, procedure; lečenje, ishod.

Introduction

The most important cause of portal hypertension is liver cirrhosis, nevertheless portal vein thrombosis can also occur as a consequence of procoagulant mutations. Factor II G20210A is a mutation found in 4 out of 10 patients with idiopathic portal vein thrombosis (PVT) [1].

Variceal hemorrhage is the most serious complication of portal hypertension and accounting for 17–57% of all deaths in cirrhotic patients [2, 3]. The prognosis associated with variceal bleeding is overall much better in patients without significant liver impairment, *ie* those with non-cirrhotic portal vein thrombosis. The main therapeutic goals in patients with gastroesophageal varrices is prevention of initial bleeding episode, control of acute varriceal bleeding and prevention of recurrent variceal bleeding. Endoscopic sclerotherapy, endoscopic variceal ligation and pharmacological treatment today have the overall success rate of 90%.

We presented a patient with recurrent variceal bleeding due to mesenterial vein thrombosis caused by congenital thrombophilia in whom long-acting somatostatin analogue was successfully used in control of repeated variceal bleeding for 3 months allowing patient stabilization and careful elective surgery planning.

Case report

A 48-year-old female was admitted with massive hematemesis and melena to the Clinic. Emergency esophagogastroduodenoscopy (EGD) revealed bleeding and esophageal and gastric varices (Figure 1.). Bleeding was controlled using blood volume restitution, vasoactive drugs and balloon tamponade. The personal history of the patient revealed 8 spontaneous miscarriages, one complicated with ileus. At the age of 28 variceal bleeding occurred, was treated with beta blockers and for 20 years no rebleeding occured. At the age of 44, routine ultrasound examination revealed enlarged spleen and PVT and congenital thrombophilia (mutation of protrombin gene G20210A) was diagnosed.

Thrombosis of portal, lienal and mesenteric veins was diagnosed using MSCT angiography. After repeated EGD it was concluded that sclerotherapy and/or variceal ligation would not be adequate solution due to variceal size and distribution. Beta blockers were introduced, but recurrent variceal bleeding occurred leading to multiple hospital admissions and blood transfusions. Balloon tamponade was used in massive bleeding episodes as a temporary therapy until definitive treatment. Octreotid was used for five days, but every time the therapy was discontinued, rebleeding occurred. There was no response to

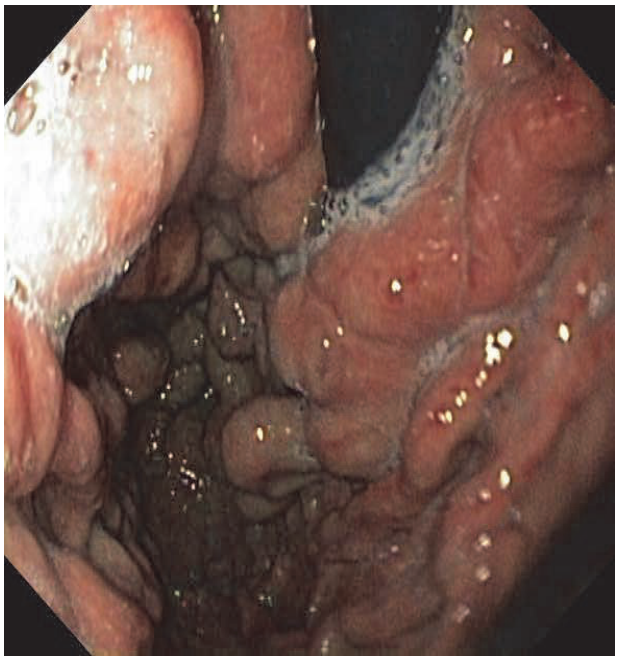

Fig. 1 – Macroscopic appearance of gastric varices and hypertensive gastropathy>

oral and parenteral treatment with beta blockers and the patient became dependent on continuous infusion of somatostatin analogs. At the same time acute deep venous thrombosis of the left leg developed and low-dose anticoagulant was introduced (nadroparin 2,500 ij *sc*/day). Carvedilol therapy was introduced according to Baveno V consensus but rebleeding occured. At that point surgical treatment was considered to be high risk procedure due to massive thrombosis of mesenterial veins, patient`s general condition and high risk of postoperative thrombotic events. Thus, long-acting somatostatin analogue – Sandostatin® LAR was initiated at a dose of 30mg i.m./month. The patient responded to therapy well and variceal bleeding did not occur for the following 3 months.

After 3 months another episode of gastric variceal hemorrhage occurred and surgical treatment was reconsidered. Total gastrectomy was performed in order to prevent repeated bleeding from large gastric varices and the patient recovered successfully (Figures 2 and 3).

Discussion

In non-cirrhotic portal hypertension endoscopic therapy is effective for control of acute variceal bleeding, while for secondary prophylaxis preliminary evidence suggests beta blockers to be as effective as endoscopic ligation. Decompressive surgery or interventional radiological procedures are considered in patients with failure of endoscopic therapy [3].

The Baveno V criteria do not provide consensus on the indications for anticoagulant treatment of extra hepatic portal vein thrombosis [3], but in our patient it was initiated for acute deep venous thrombosis of the left leg.

Somatostatin analogue octreotide is used for decades in treatment of acute variceal haemorrhage, with the well-known mechanisms of lowering portal pressure. It increases efficacy of endoscopic therapy and decreases rebleeding risk [4–7].

In the presented patient after all the other therapeutic options failed we introduced Sandostatin® LAR as an attempt of

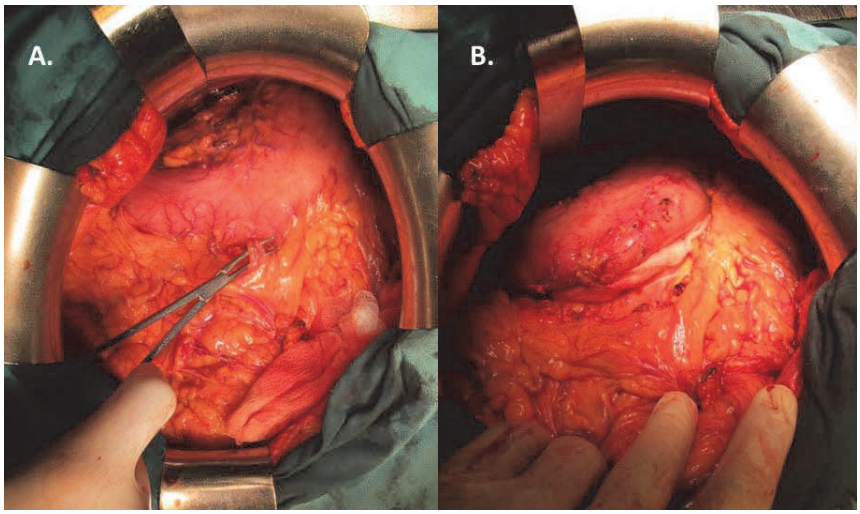

Fig. 2 – Perigastric devascularization – A) Surgical instrument inserted between greater curvature of the stomach, and the epiploic arcade along greater curvature; B) Great curvature of the stomach detached from the epiploic arcade.

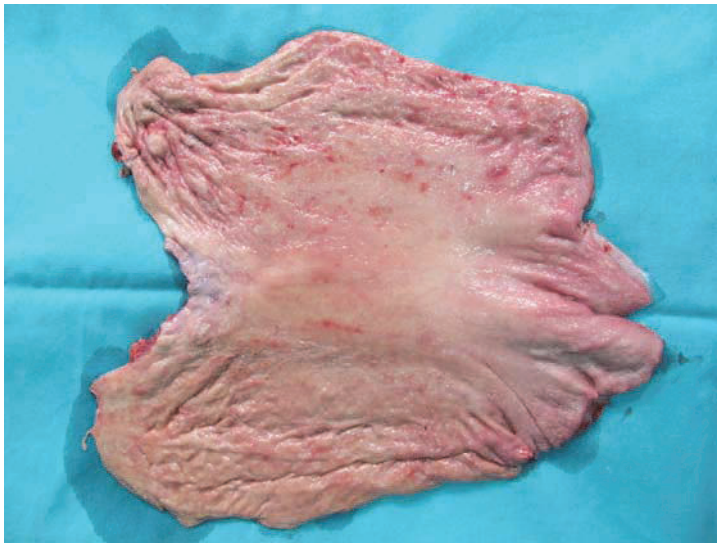

Fig. 3 – Specimen after total gastrectomy – the stomach open along the greater curvature. Macroscopic appearance of hypertensive gastropathy.

rescue therapy. Namely, data published by Spahr et al. [8] suggested that prolonged administration of a long-acting formulation of octreotide improves significantly portal hypertension in carefully selected cirrhosis patients. Sustained decrease in splanchnic hyperemia was proposed as underlying pharmacological mechanism of octreotid [8].

Conclusion

Long-lasting somatostatin analogue was used for the first time in treatment of gastroesophageal variceal hemorrhage in the patient with prehepatic portal hypertension. It was effective as temporary therapeutic option allowing the improvement of the patient's general condition and adequate planning of elective surgical procedure. Futher reports are needed in order to compare efficacy in treatment of patients with variceal bleeding, where poor outcome is expected.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. III41004)

R E F E R E N C E S

1. *Chamouard P, Pencreach E, Maloisel F, Grunebaum L, Ardizzone JF, Meyer A,* et al. Frequent factor II G20210A mutation in idiopathic portal vein thrombosis. Gastroenterology 1999; 116(1): 144−8.
2. *Jensen DM.* Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. Gastroenterology 2002; 122(6): 1620−30.
3. *de Franchis R.* Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol 2010; 53(4): 762−8.
4. *Chatila R, Ferayorni L, Gupta T, Groszmann RJ.* Local arterial vasoconstriction induced by octreotide in patients with cirrhosis. Hepatology 2000; 31(3): 572−6.
5. *Wiest R, Tsai MH, Groszmann RJ.* Octreotide potentiates PKC-dependent vasoconstrictors in portal-hypertensive and control rats. Gastroenterology 2001; 120(4): 975−83.
6. *Huang H, Lee F, Chan C, Chang F, Wang S, Lin H,* et al. Effects of somatostatin and octreotide on portal-systemic collaterals in portal hypertensive rats. J Hepatol 2002; 36(2): 163−8.
7. *Wahren J, Efendić S, Luft R, Hagenfeldt L, Björkman O, Felig P.* Influence of somatostatin on splanchnic glucose metabolism in postabsorptive and 60-hour fasted humans. J Clin Invest 1977; 59(2): 299−307.
8. *Spahr L, Giostra E, Frossard J, Morard I, Mentha G, Hadengue A.* A 3-month course of long-acting repeatable octreotide (sandostatin LAR) improves portal hypertension in patients with cirrhosis: a randomized controlled study. Am J Gastroenterol 2007; 102(7): 1397−405.

Received on September 16, 2013.
Accepted on February 13, 2014.

CASE REPORT

UDC: 617.7-089-06
DOI: 10.2298/VSP1503287V

Secondary surgical management of suprachoroidal hemorrhage during *pars plana* vitrectomy

Sekundarno hirurško rešavanje suprahoroidalne hemoragije nastale u toku *pars plana* vitrektomije

Dragan Vuković*†, Sanja Petrović†, Predrag Paović†

***Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Clinic of Eye Diseases, Clinical Center of Serbia, Belgrade, Serbia**

Abstract

Introduction. Suprachoroidal hemorrhage (SCH) is one of the most feared and devastating complications of intraocular surgery. Intraoperative SCH is defined as sudden hemorrhagic swelling of the choroid which develops at time of intraocular surgery, and is associated with expulsion of some or all of the intraocular contents. **Case report.** A 56-year-old man was admitted to our Clinic with bullose retinal detachment in the left eye. Intraoperatively, during the substitution of perfluorocarbone liquid (PFCL) with silicone oil, which is very rare situation, a sudden loss of red reflex happened and SCH was recognized as the cause. No attempt was made to drain the suprachoroidal blood. After 3 weeks the patient was scheduled for *pars plana* vitrectomy. Initial drainage of liqufied blood was made through a sclerotomy port during *pars plana* inferotemporally. Massive epiretinal proliferation with funnel shaped retinal detachment was solved during vitrectomy and internal tamponade with silicone oil was done. Postoperative visual aquity was 2/60 on the third postoperative day. **Conclusion.** Although suprachoroidal hemorrhage is one of most feared and devastating complications of intraocular surgery, it might have relatively good prognosis with proper preoperative, intraoperative and postoperative management.

Key words:
vitrectomy; eye hemorrhage; intraoperative complications; risk factors.

Apstrakt

Uvod. Suprahoroidalna hemoragija (SH) je komplikacija koje se najviše plašimo i koja je najrazornija u intraokularnoj hirurgiji. Intraoperativna SH definiše se kao iznenadni hemoragični otok horoidee koji se razvija tokom intraokularne operacije i koji je udružen sa delimičnim ili potpunim prolapsom intraokularnog sadržaja. **Prikaz bolesnika.** Muškarac, star 56 godina primljen je na našu kliniku sa buloznom ablacijom retine na levom oku. Intraoperativno, u toku izmene perfluorokarbona za silikonsko ulje, što se vrlo retko viđa, došlo je do iznenadnog gubitka crvenog refleksa, što je bila pojava SH. Nismo pokušali drenažu SH. Odlučili smo da bolesniku zakažemo vitrektomiju za tri nedelje. Početnu drenažu likveficirane SH uradili smo kroz preegzistirajuću standardnu *pars plana* sklerotomiju inferotemporalno. Masivna epiretinalna proliferacija sa levkastom ablacijom retine rešena je u toku vitrektomije i urađena je endotamponada sa silikonskim uljem. Postoperativni vizus bio je 2/60 trećeg postoperativnog dana. **Zaključak.** Iako je suprahoroidalna hemoragija komplikacija koje se najviše plašimo i koja je najrazornija u intraokularnoj hirurgiji, sa adekvatnim preoperativnim, intraoperativnim i postoperativnim tretmanom može, ipak, imati relativno dobru prognozu.

Ključne reči:
vitrektomija; oko, krvarenje; inraoperativne komplikacije; faktori rizika.

Introduction

Suprachoroidal hemorrhage (SCH) is a rare but dangerous complication of intraocular surgery [1]. SCH is defined as sudden hemorrhagic swelling of the choroid which develops at the time of intraocular surgery, which if associated with expulsion of some or all of the intraocular contents. SCH itself may be bad prognostic sign for postoperative outcome.

In myopic patients, SCH occurs in situations when the fragile vasculature is put under additional stress such as intraocular surgery [2]. We reported intraoperative and secondary surgical management of SCH during *pars plana* vitrectomy in the eye with rhegmatogenous retinal detachment.

Correspondence to: Dragan Vuković, Clinic of Eye Diseases, Clinical Center of Serbia, Pasterova 2, 11 000 Belgrade, Serbia.
Phone: +381 11 3430 804; Fax: +381 11 2688 164, E-mail: draganv@beotel.net

Case report

A 56-year-old man was admitted to our Clinic complaining of a 3-day history of decreased visual acuity in the left eye, followed by an episode of complete loss of vision in the inferior part of the visual field. He had no history of hypertension or diabetes, no previous operations or allergies, and no history of eye disease or trauma.

His axial lengths were 25.36 mm right and 25.37 mm left. His visual acuity in the right eye was 1.0 through a pinhole. The left eye's visual acuity was 1/60. The intraocular pressures were 19 mmHg in the right and 20 mmHg in the left. On fundoscopy he had extensive peripapillary atrophy and a myopic looking disc as well as several retinal tears located at 4, 5 and 8 o'clock accompanied with multiple areas of chorioretinal atrophy. Fundoscopy of his left eye revealed bullose retinal detachment with multiple retinal tears and chorioretinal degeneration. We decided to perform *pars plana* vitrectomy and tamponade with silicon oil.

We placed scleral buckle under the all rectus muscles, and then we made sclerotomies at 11, 2 and 4 o'clock and placed infusion cannula. Then *pars plana* vitrectomy was made, installation of perfluorocarbon liquid (PFCL) and endolaserphotocoagulation.

Intraoperatively, during the substitution of PFCL with silicone oil, shallowing of the anterior chamber was noticed, silicon oil mixed with PFCL started prolapsing through sclerotomies, there was a sudden loss of red glow and SCH was recognized as the cause. We used vacuum needle to drain as much of the remained PFCL and silicone oil as we could. Sclerothomies were closed, the infusion cannula was removed. No attempt was made to drain the suprachoroidal blood. The patient was started on topical corticosteroids along with anti-glaucoma medication. Ultrasound examination confirmed the diagnosis of hemorrhagic choroidal detachment with the prominency of 13.26 mm and vitreous hemorrhage mixed with the silicone oil. Vitreoretinal surgery was planned when liquefaction of blood in the suprachoroidal space happened, and not before two weeks after the SCH occurred.

After 3 weeks the patient was scheduled for *pars plana* vitrectomy. When admitted he had visual acuity in the operated eye of light perception with intraocular pressure (IOP) at 1 mmHg and advanced cataract. There was no fundal reflex. An ultrasound examination confirmed the decrease of hemorrhagic choroidal detachment to 5.36 mm accompanied with total retinal detachment.

Then a standard *pars plana* vitrectomy port was made at the *pars plana* inferotemporally. Dark red, liqufied blood gushed out. Once drainage from this site was completed, two other ports were made superonasally and superotemporally, at 10 and 2 o'clock.

The infusion cannula was placed through the standard *pars plana* site. Due to advanced cataract we had to perform lensectomy. Further limited vitrectomy and membranectomy was done to remove vitreous hemorrhage, membranes and proliferations, taking care to avoid the anteriorly displaced retina and choroid. When the visibility improved, we noticed incarceration of the retina in the wound so we had to perform retinectomy in order to release incarcerated retina.

When the media became clear, residual choroidal swellings were observed. PFC2 was instilled over the posterior pole, leading to further drainage of blood from the port sites. Photocoagulation with endolaser was performed. PFCL was then completely removed and substituted with silicon oil.

Postoperatively the retina was attached and patient had the best corrected visual acuity of 2/60 at the time of discharge 3 days later (Figure 1). With correction the vision after 4 weeks visual acuity was 4/60.

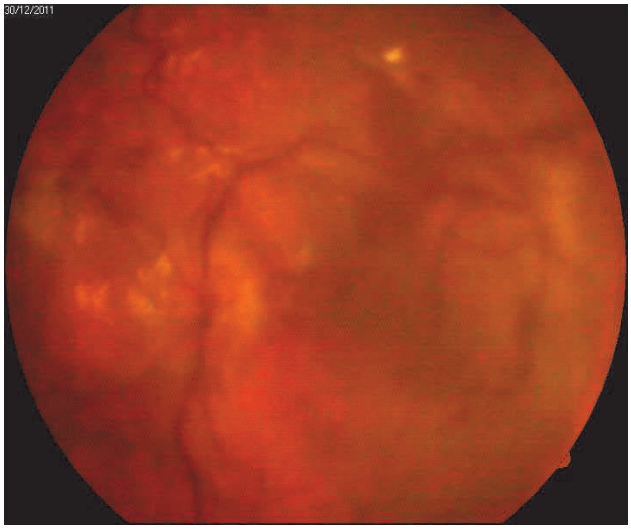

Fig. 1 – Fundus of the left eye 3 days after the surgery for suprachoroidal hemorrhage.

Discussion

Intraoperative SCH is defined as a sudden hemorrhagic swelling of the choroid which develops at the time of intraocular surgery, which if associated with expulsion of some or all of the intraocular contents [3]. Various studies have focused on identifying patients at risk and reduction of risk factors help to reduce the incidence [4]. Myopia is a risk factor for suprachoroidal hemorrhage because the longer axial length causes increased choroidal vascular fragility [5]. This case illustrates how myopia associated with choroidal vasculature fragility could cause intraoperative hemorrhage.

Some investigators have come to a conclusion that SCH itself is not prognostic sign of bad postoperative outcome and that some eyes may recover with useful visual acuity if proper intraoperative and secondary surgical management is conducted [6, 7].

Special attention should be paid in cases with risk factors for expulsive SCH. Risk factors for the development of intraoperative SCH during *paps plana* vitrectomy are high myopia, previous retinal detachment (RD) surgery, rhegmatogenous RD, cryotherapy, scleral buckling, external drainage of subretinal fluid, and intraoperative systemic hypertension [8]. Intraoperative, early recognition and immediate surgical

response are crucial for postoperative outcome. Intraoperatively early recognization and immediate rapid closure of the wound is important. Prolapsed intraocular contents should be reposited as quickly as possible; if this is not possible the eye can be softened by performing posterior sclerotomies [9]. If SCH happens during PFCL-silicon oil exchange (due to low IOP caused by inadvertent tubing occlusion) we think that evacuation of as much as possible silicon oil is crucial. Evacuation should be done through open sclerotomies.

Is it a good idea to make sclerotomies during an acute event? We believe that making sclerotomies during the acute event may be detrimental to eyes. Lakhanpal [10] showed in his study that the tamponading effect of raised IOP could be unsettled due to ooze through the sclerotomies and could cause re-bleed. Once acute MSCH is recognized intraoperatively, surgical decompression at that time should be avoided as MSCH itself may tamponade the choroidal bleed.

In the rabbit model of SCH, sclerotomy resulted in the marked extension of SCH. Intraoperative sclerotomy cannot therefore be recommended. Immediate closure of the open globe must remain the priority in intraoperative management of SCH [10].

Immediate sclerotomy during the acute formation of massive suprachoroidal hemorrhage resulted in the further increase in suprachoroidal hemorrhage, with marked extension of hemorrhage into the retina and vitreous humor. Therefore, we think that immediate sclerotomy during massive suprachoroidal hemorrhage is detrimental to the eye. Our clinical data show that eyes with massive suprachoroidal hemorrhage can be treated successfully by secondary surgery, and the majority of the eyes can be salvaged with good visual results [10].

The role of sclerotomies at the time of acute event is controversial. Blood clots rapidly extend in the suprachoroidal space and so it may not drain through emergency sclerotomies [9].

In most cases, intraoperative drainage of suprachoroidal hemorrhage is not associated with better outcomes. The prognosis is more favorable if suprachoroidal hemorrhage is localized and does not extend in to the posterior pole [11].

If there is retinal detachment, incarceration of the vitreous or retina into the wound, secondary surgical management should be planned. Ideal time for vitrectomy is suggested to be 7–14 days after the SCH because that is the period when blood in suprachoroidal space liquefies [10]. Some authors underwent *pars plana* vitrectomy after an interval of 19 (14–54) days [12].

The natural course of the disease suggests that there is a very little change in the size of the choroidal detachment in the first 7 days. Maximum liquefaction of the suprachoroidal hemorrhage clot was seen to occur between 7 and 14 days. However, increased retinal and ciliary body atrophy was also noted 14 days. Therefore, the optimum time to drain massive suprachoroidal hemorrhage appears to be between 7 and 14 days. Immediate sclerotomy during the acute formation of massive suprachoroidal hemorrhage resulted in further increase in suprachoroidal hemorrhage, with marked extension of the hemorrhage into the retina and vitreous. Therefore, we consider immediate sclerotomy during massive suprachoroidal hemorrhage detrimental to the eye. Our clinical data show that eyes with massive suprachoroidal hemorrhage can be treated successfully by secondary surgery, and the majority of the eyes can be salvaged with good visual results [10].

The role of sclerotomies at the time of acute event is controversial. Blood clots extend rapidly in the suprachoroidal space and so it may not drain through the emergency sclerotomies [9].

Postoperative B-scan ultrasonography can be used to monitor liquefaction of blood. In our case ultrasonography was not capable to give precise and detail status of blood in suprachoridal space due to the presence of silicon oil in the vitreal cavum.

It is recommended to drain blood through standard vitrectomy *pars plana* ports. If this is not possible drainage of hemorrhage should be done through posterior sclerotomies before *pars plana* ports are made in order to avoid iatrogenic damage to anterior retina unless the drainage can be accomplished through the standard ports for *pars plana* vitrectomy as it did in our case. We also used PFCL to facilitate drainage of SCH. To provide better attachment of the retina we performed tamponade with silicone oil. Tamponade with silicone oil was used for the presented patient because of uncertainty about the presence of unidentified retinal tears. Endolaser photocoagulation was done. Within one-month follow up, vision in the operated eye was 4/60.

Conclusion

Although suprachoroidal hemorrhage is one of the most feared and devastating complications of intraocular surgery, it might have relatively good prognosis with proper preoperative, intraoperative and postoperative management. We reported this case as an example of successful management of suprachoroidal hemorrhage during *pars plana* vitrectomy of rhegmatogenous retinal detachment in spite of the fact that the eye looked lost.

R E F E R E N C E S

1. *Mei H, Xing Y, Yang A, Wang J, Xu Y, Heiligenhaus A*. Suprachoroidal hemorrhage during pars plana vitrectomy in traumatized eyes. Retina 2009; 29(4): 473−6.
2. *Beatty S, Lotery A, Kent D, O'Driscoll A, Kilmartin DJ, Wallace D*, et al. Acute intraoperative suprachoroidal haemorrhage in ocular surgery. Eye (Lond) 1998; 12(Pt 5): 815−20.
3. *Chu TG, Green RL*. Suprachoroidal hemorrhage. Surv Ophthalmol 1999; 43(6): 471−86.
4. *Spaeth GL*. Suprchoroidal hemorrhage: no longer a disaster. Ophthalmic Surg 1987; 18(5): 329−33.
5. *Speaker MG, Guerriero PN, Met JA, Coad CT, Berger A, Marmor M*. A case-control study of risk factors for intraoperative suprachoroidal expulsive hemorrhage. Ophthalmology 1991; 98(2): 202−9.
6. *Spaeth GL, Baez KA*. Long term prognosis of eyes having had operative suprachoroidal expulsive hemorhage. Ger J Ophthalmol. 1994; 3(3): 159−63.
7. *Lambrou FH, Meredith TA, Kaplan HJ*. Secondary surgical management of expulsive choroidal hemorrhage. Arch Ophthalmol 1987; 105(9): 1195−8.
8. *Tabandeh H, Sullivan PM, Smahliuk P, Flynn HW, Schiffman J*. Suprachoroidal hemorrhage during pars plana vitrectomy. Risk factors and outcomes. Ophthalmology 1999; 106(2): 236−42.
9. *Sharma YR, Gaur A, Azad RV*. Suprachoroidal haemorrhage. Secondary management. Indian J Ophthalmol 2001; 49(3): 191−2.
10. *Lakhanpal V*. Experimental and clinical observation on massive suprachoroidal hemorrhage. Trans Am Ophthalmol Soc 1993; 91: 545−652.
11. *Tabandeh H, Flynn JH*. Suprachoroidal hemorrhage during pars plana vitrectomy. Curr Opin Ophthalmol 2001; 12(3): 179−85.
12. *Ling R, Cole M, James C, Kamalarajah S, Foot B, Shaw S*. Suprachoroidal haemorrhage complicating cataract surgery in the UK: epidemiology, clinical features, management, and outcomes. Br J Ophthalmol 2004; 88(4): 478−80.

Received on November 22, 2013.
Revised on January 7, 2014.
Accepted on February 3, 2014.

CASE REPORT

UDC: 616.12-006-07/-08
DOI: 10.2298/VSP140212031R

Massive right atrial myxoma with dyspnea at rest in an elderly patient: A case report

Veliki miksom desne pretkomore sa dispnejom u miru kod bolesnice u starijem životnom dobu

Radoslav Romanović*†, Nenad Ratković*, Žaklina Davičević†‡, Radoje Ilić†§

***Clinic for Urgent Internal Medicine, ‡Clinic for Cardiology, §Clinic for Cardiac and Thoracic Surgery, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Primary heart tumors are extremely rare and myxoma is the most common type of these tumors. Although intra-atrial presentation is a predilection place, right atrial localization is atypical. The symptom triad is characteristic in the clinical presentation of the tumor: embolic complication, intracardiac blood flow obstruction and systemic manifestations like elevated erythrocyte sedimentation rate, fever, anemia, body weight loss. **Case report.** We presented an elderly female patient with massive myxoma in the right atrium, 77 × 44 mm in diameter, which filled the entire right atrium and spread into the right ventricle, causing the tricuspid valve obstruction and dyspnea. It was visualized by transthoracic echocardiography and small and insignificant pericardial effusion was also seen. After surgical removal of the tumor, the patient remained without any symptoms and pericardial effusion. **Conclusion.** Tumors of the right heart have to be considered in the differential diagnosis of unexplained dyspnea in elderly patients. Transthoracic echocardiography is certainly necessary and mostly available diagnostic tool that can be of great help in diagnosing heart tumor as well as planning cardiac surgery, as it provides in most cases excellent visualization of the tumor and its relationship with other parts of the heart.

Key words:
myxoma; heart atria; dyspnea; aged; diagnosis; cardiac surgical procedures; treatment outcome.

Apstrakt

Uvod. Primarni tumori srca su izuzetno retki. Najčešće su to miksomi. Lokalizacija u desnoj pretkomori je atipična. Za kliničku sliku karakterističan je trijas simptoma: embolijske komplikacije, opstruktivne tegobe i sistemske manifestacije bolesti kao što su: povišena sedimentacija eritrocita, povišena telesna temperatura, anemija, gubitak telesne težine. **Prikaz bolesnice.** U radu je prikazana starija bolesnica sa velikim miksomom desne pretkomore, dimenzija 77 × 44 mm, koji je potpuno ispinjavao desnu pretkomoru i skoro potpuno desnu komoru, uzrokujući opstrukciju trikuspidnog protoka i dispneju. Miksom je dijagnostikovan transtorakalnom ehokardiografijom. Pored toga, ehokardiografski verifikovan je manji hemodinamski nesignifikantni perikardni izliv bez značaja. Nakon hirurškog odstranjenja tumora bolesnica je bila bez simptoma i bez perikardnog izliva. **Zaključak.** Tumore desnog srca trebalo bi imati u vidu u diferencijalnoj dijagnostici prilikom pojave neočekivane dispneje kod starijih bolesnika. Transtorakalna ehokardiografija neophodna je i dostupna dijagnostička procedura koja daje pouzdane rezultate u dijagnostici tumora srca, naročito kod bolesnika kod kojih se planira hirurška intervencija. Kod većine bolesnika ova metoda omogućava dobru vizualizaciju tumora i definiše njegove odnose sa ostalim strukturama srca.

Ključne reči:
miksom; srce, pretkomora; dispneja; stare osobe; dijagnoza; hirurgija, kardijalna, procedure; lečenje, ishod.

Introduction

Based on data from 22 large autopsy studies, the incidence of primary tumors of the heart at 731,309 autopsied was 157 or 0.02% [1]. In the Mayo Clinic series, that was conducted in a period 1954–1979, which including 23,673 patients, primary tumor of the heart was diagnosed in 0.17% of patients. Myxoma was present in 28 patients, 17 were localized in the left atrium and 4 in the right atrium [2]. In Military Medical Academy in Belgrade, overall 63 patients with

Correspondence to: Radoslav Romanović, Clinic for Urgent Internal Medicine, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 64 828 26 44 2. E- mail: rasaromanovic@yahoo.com

myxoma of the heart were diagnosed and treated from 1961 up to date. Among those patients, 12 had myxoma in the right atrium [3]. Myxoma can be asymptomatic, but clinical presentation may include nonspecific signs and symptoms: arthralgia, body weight loss, anemia, hypergammaglobulinemia, elevated erythrocyte sedimentation rate or dyspnea [4].

Pulmonary embolism as a right atrial myxoma complication is accompanied with dyspnea, hemoptysis, syncope, chest pain, right heart failure or sudden death [5]. Transthoracic echocardiography is a standard diagnostic procedure in revealing myxomas, and cardiac surgery procedure in most cases is the treatment of choice.

Case report

A 77-year-old female presented with 4 month lasting symptoms of progressive dyspnea on exertion with palpitations and gradual reduction in exercise tolerance and with symptoms of heart failure, NYHA class II/III. Chest pain and orthopnea were not present. The patient had a previous history of hypertension and diabetes mellitus type II.

The patient was dispnoic on the first clinical examination, and auscultation showed weakened respiratory sounds in the basal portions of the lungs. Cardiac auscultation was unremarkable, with normal blood pressure (BP) 110/70 mmHg. The patient had 5 cm hepatomegaly and bilateral pedal edema. Electrocardiogram showed a reduced amplitude of the R-wave in precordial leads (Figure 1).

Hematological and biochemical investigations demonstrated normal hemoglobin of 142 mg/L, a mildly elevated erythrocyte sedimentation rate (23 mm/h). Brain natriuretic peptide was not measured. Chest radiography showed a distorted right atrial border with small bilateral pleural effusion (Figure 2). Transthoracic echocardiography (TTE) showed a huge mobile mass, of 77 × 44 mm, occupying the entire right atrium. The mass was connected to the lower portion of the interatrial septum, and protruding with its floating part through the tricuspid valve into the normal-sized right ventricle (Figures 3 and 4). The inferior vena cava was dilated with minimal collapse on inspiration suggesting raised right atrial pressure. The left atrium, left ventricle, mitral valve, aortic valve, and pulmonary valve all appeared normal. Insignificant circular pericardial effusion of 10 mm, was also seen in TTE. Doppler ultrasonography of the major arteries

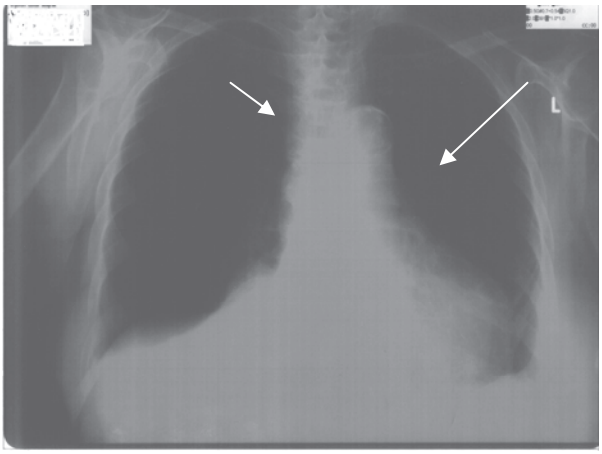

Fig. 2 – Chest radiography showing a distored right atrial border with small bilateral pleural effusion.

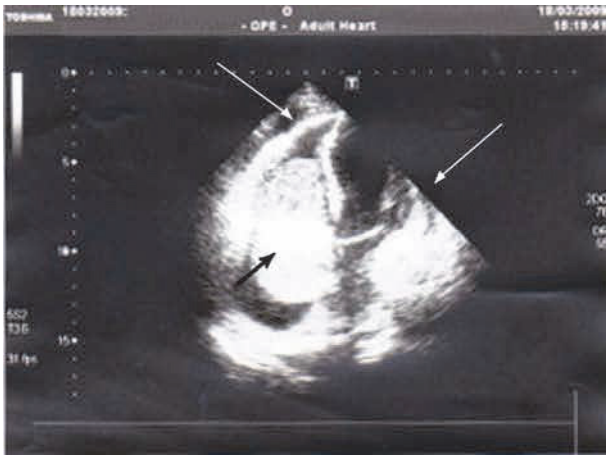

Fig. 3 – Transthoracic echocardiography (TTE) – 4 chamber view with a small pericardial effusion behind the posterior left ventricular wall and in front of the right ventricle (white arrows) and massive myxoma (black arrow).

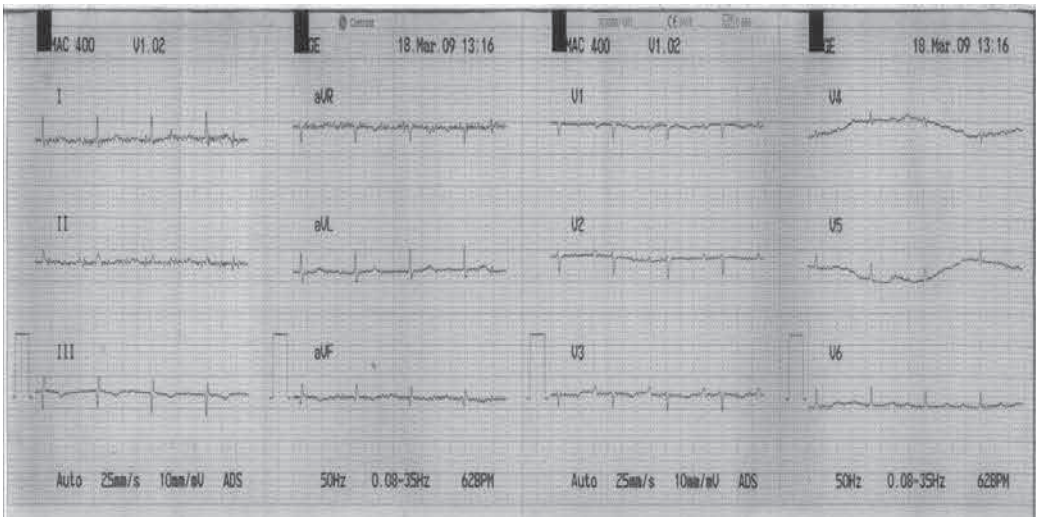

Fig. 1 – A reduced R-wave amplitude in precordial leads in electrocardiogram.

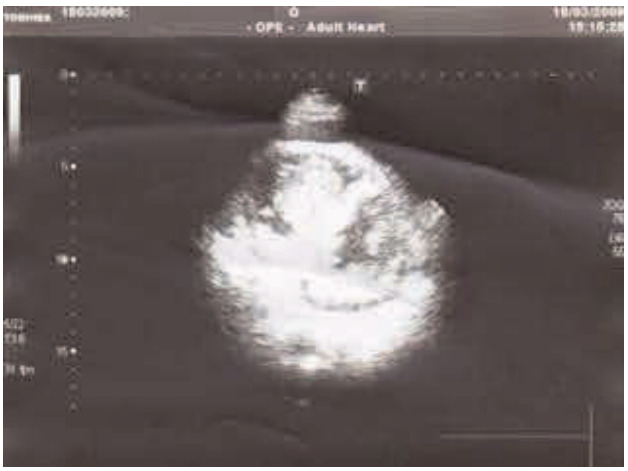

Fig. 4 – Transthoracic echocardiography (TTE) short axis parasternal view, with a small circular pericardial effusion behind the posterolateral wall and a bigger one in front of the right ventricle.

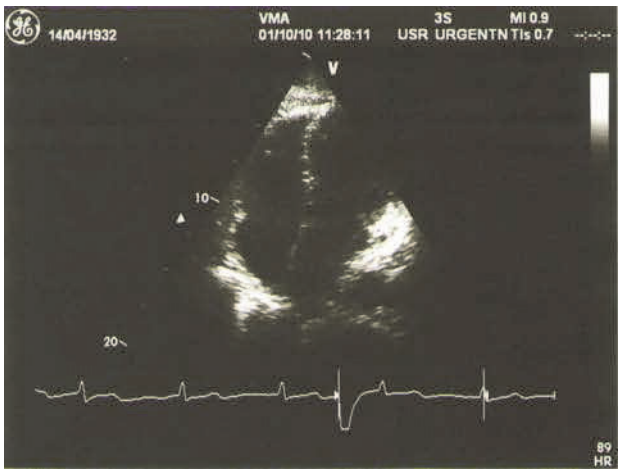

Fig. 6 – Transthoracic echocardiography (TTE) after a year: the normal right atrium and ventricle, with no tumor, nor pericardial effusion.

in the neck showed hemodynamically insignificant atherosclerotic changes, and on selective coronary angiography, only mild insignificant atherosclerosis was seen.

At surgery, during cardioplegia and with extracorporeal blood flow, right atrium was open and the tumor on a tin stalk connected to interatrial septum was identified. It filled the entire right atrium protruding with its floating part through tricuspid valve into the majority right ventricular space. The tumor was extirpated together with the connection part of endocardia.

Pathologically, the macroscopic specimen showed a nodular formation measuring 77 × 43 × 34 mm and containing hemorrhagic areas (Figure 5). Benign myxoma was confirmed histologically, but no residual tumor at the base of the stalk.

Discussion

Right atrial myxoma is extremely rare, and accompanied with pericardial effusion has not been published so far. Three-quarters of neoplasms of the heart are benign tumors, and among them the most common is myxoma, representing about 50% of all primary tumors of the heart. The largest series of patients have shown that females, with the average age of 50 years, are more commonly involved with this type of tumors [1].

The presented patient had myxoma in a less typical localization – the right atrium. The patient presented with the symptoms in her elderly age of 77 years. Although the oldest described patient with myxoma was 95 years old, these tumors are extremely rare after the age of 60 [2].

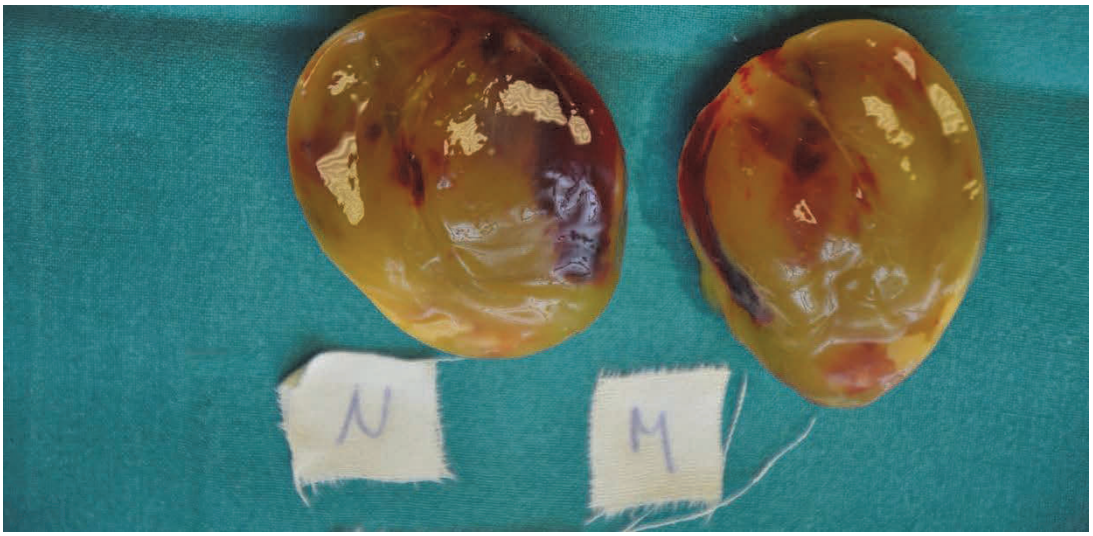

Fig. 5 – Macroscopic longitudinal tumor cut.

At 12-month follow-up, the patient's exertional capacity much improved, free of other preoperative signs and symptoms. TTE after 12 month showed normal echocardiogram without signs of tumor recurrence, tricuspid insufficiency or dilatation of the right heart cavities (Figure 6).

Our hospital has been treated a large series of patients for tumors of the heart since 1961. The presented patient was the oldest in the group of 63 patients within more than 50 years long history of the treatment of patients with heart tumors in the Military Medical Academy in Belgrade [3]. In 75%

of cases myxomas are located in the left atrium, while 23% in the right atrium. The remaining myxomas are of the ventricular origin [6].

The average tumor size at the time of diagnosis is about 50–60 mm [2]. It remains unclear whether the tumor size is directly related to the presence of symptoms, but it has been reported that signs and symptoms usually occur with the minimal tumor size of 50 mm [2, 7]. The presented patient's myxoma measured 77 mm in diameter.

The clinical presentation of patients with myxoma can be quite different. Symptoms of heart obstruction, embolic complications and systemic manifestations are the components of the classical triad, but rarely are present all. However, at least one of the triad symptoms is present.

In a study on 116 patients with left atrial myxoma 65% of patients had signs of intracardiac obstruction, 28% had signs of embolic complications and 33% presented with systemic events. All patients had at least one of the characteristic manifestations of myxoma [8]. In our group of 63 patients with myxoma in any side of the heart, 62% had symptoms and signs of intracardiac obstruction, 26% had signs of embolization, and 30% had systemic symptoms. All patients had at least one component of the classic triad [9, 10]. The presented patient had symptoms and signs of obstructive syndrome of the right heart cavities and dyspnea which is the most common manifestation of myxoma logged in the right atrium. In addition, the presented patient had a small, hemodynamically insignificant pericardial effusion, which disappeared immediately after surgical removal of the tumor. Pericardial and pleural effusion as a systemic manifestation was previously seen in only one 46-year-old woman with left atrial myxoma. These effusions disappeared soon after the surgical removal of the tumor [11]. The most common differential diagnostic problem after discovering a mass formation in the heart cavities is to differentiate myxoma from the thrombus. The morphology and motility of myxoma and thrombus may be similar and difficult to distinguish by echocardiography, and surgical excision may be necessary for certain diagnosis [4].

Conclusion

Right atrial myxoma is extremely rare, and accompanied with pericardial effusion has not been published so far. Tumors of the right heart have to be considered in the differential diagnosis of unexplained dyspnea in elderly patients. Transthoracic echocardiography is certainly necessary and most available diagnostic tool that can be of great help in diagnosing heart tumor, as well as planning cardiac surgery, as in most cases it provides excellent visualization of the tumor and its relationship with other parts of the heart.

REFERENCES

1. *Reynen K.* Cardiac myxomas. N Engl J Med 1995; 333(24): 1610−7.
2. *Wold LE, Lie JT.* Cardiac myxomas: a clinicopathologic profile. Am J Pathol 1980; 101(1): 219−40.
3. *Rafajlovski S.* Cardiac myxoma. In: *Rafajlovski* S, editor. Tumors of the heart. Belgrade: Military Medical Academy; 2010. p. 45−52. (Serbian)
4. *Yuce M, Dagdelen S, Ergelen M, Eren N, Caglar N.* A huge obstructive myxoma located in the right heart without causing any symptom. Int J Cardiol 2007; 114(3): 405−6.
5. *Alsafwah S, Lababidi Z.* Recurrent pulmonary embolism originating from right atrial myxoma. J Am Soc Echocardiogr 2001; 14(4): 305−7.
6. *Topol E.* Cardiac neoplasms. In: *Topol E*, editor. Textbook of cardiovascular medicine. 2nd ed. Philadelphia, PA: Lippincott, Williams and Wilkins; 2002. p. 921−3.
7. *Bilku RS, Loubani M, Been M, Patel RL.* Massive right atrial myxoma causing exertional dyspnoea. Eur J Echocardiogr 2008; 9(1): 130−2.
8. *Pinede L, Duhaut P, Loire R.* Clinical presentation of left atrial cardiac myxoma. A series of 112 consecutive cases. Medicine (Baltimore) 2001; 80(3): 159−72.
9. *Todorić M, Jablanov J, Albreht M, Ilić R, Tatić V, Stojnić B.* Immediate results of surgical treatment of intracardiac myxomas in 45 patients. Vojnosanit Pregl 1993; 50(4): 353−8. (Serbian)
10. *Sanna A, Porcu M, Basciu M, Floris E, Martelli V.* Left atrial myxoma simulating a systemic disease with pleural-pericardial effusion. Detection by two-dimensional echocardiography. Ann Ital Med Int 1989; 4(1): 44−7.
11. *Rafajlovski S, Ilić R, Gligić B, Kanjuh V, Tatić V, Ristić A,* et al. Impact of heart myxoma localization upon its clinical course and outcome. Vojnosanit Pregl 2012; 69(3): 270−6.

Received on February 12, 2014.
Accepted on February 18, 2014.
OnLine-First April, 2014.

CASE REPORT

UDC: 618.11-006
DOI: 10.2298/VSP1503295I

Hypercalcemic type of small cell carcinoma of the ovary

Hiperkalcemični tip sitnoćelijskog karcinoma jajnika

Milena B. Ilić*, Dalibor V. Jovanović*, Miloš Z. Milosavljević†, Vesna Stanković*†, Gordana Djordjević*, Zoran Protrka*‡, Jasmina Nedović§, Slobodanka Lj. Mitrović*†

***Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; †Department of Pathology, ‡Department of Gynaecology and Obstretics, §Department of Oncology, Clinical Center Kragujevac, Kragujevac, Serbia**

Abstract

Introduction. Extrapulmonary small cell carcinoma is a rare, prognostically bad tumor category. Primary, it can be localized in every organ, even in the ovary, where, due to its clinical specificities, it represents a challenge in diagnosis, as well as in therapy. Small cell ovarian carcinoma (SCOC) is biologically very aggressive malignant tumor of unknown histogenesis. We presented a rare case of SCOC with hypercalcemia of aggressive course and fatal outcome in a postmenopausal woman at International Federation of Gynecology and Obstetrics (FIGO) Ia stage. **Case report.** A 60-year-old woman, Caucasian, came to the doctor because of discomfort in the lower abdomen and pain of greater intensity in last few days. Ultrasound examination and CT scan of the abdomen confirmed the presence of large adnexal masses of cystic-solid appearance with the largest diameter of 13 cm, regular structure of the other gynecological organs, without verifying the existence of metastatic deposits. All the results of laboratory analysis gave normal values, except for calcium, which was elevated. Explorative laparotomy with complete hysterectomy, bilateral salpingo-oophorectomy, dissection of lymph nodes and omentectomy were conducted. Based on pathohistological analysis of the operative material, SCOC at FIGO Ia stage was diagnosed. No complications were observed in a postsurgery period and after 10 days the patient was discharged in a good condition and with normal calcemia. The treatment was continued with concurrent radiotherapy and chemotherapy. However, in spite of overall treatment, the disease progressed, and the patient died of disseminated metastatic disease, 26 months after the diagnosis. **Conclusion.** Small cell carcinoma localized in the ovary is generally a tumor category with bad prognosis depending on the stage of the disease.

Key words:
carcinoma, small cell; ovarian neoplasms; diagnosis; immunohistochemistry; neoplasm metastasis; treatment outcome.

Apstrakt

Uvod. Ekstrapulmonalni sitnoćelijski karcinom je retka, prognostički loša kategorija tumora. Primarno, može da se lokalizuje u skoro svim organima, pa i u jajniku, gde zbog svojih kliničkih specifičnosti, često predstavlja pravi dijagnostički i terapijski izazov. Sitnoćelijski karcinom jajnika (*small cell ovarian carcinoma* – SCOC) biološki je vrlo agresivan maligni tumor nepoznate histogeneze. U radu je prikazana bolesnica u post-menopauzi sa sitnoćelijskim karcinomom jajnika i hiperkalcemijom, agresivnog toka i fatalnog ishoda, u *International Federation of Gynecology and Obstetrics* – FIGO Ia stadijumu. **Prikaz bolesnika.** Žena stara 60 godina, javila se zbog tegoba u donjem abdomenu praćenih bolom jakog intenziteta. Ultrazvučnim pregledom i kompjuterizovanom tomografijom abdomena ustanovljeno je prisustvo velike adneksalne mase cističnog izgleda sa najvećim promerom od 13 cm, sa urednom strukturom drugih ginekoloških organa i bez prisustva metastatskih depozita. Svi rezultati laboratorijske analize bili su u granicama normalnih vrednosti izuzev povišenih nivoa kalcijuma u serumu. Izvršena je eksplorativna laparotomija sa kompletnom histerektomijom, bileteralnom salpingooforektomijom, disekcijom limfnih čvorova i omentektomijom. Na osnovu patohistološke analize operatvnog materijala postavljena je dijagnoza SCOC stadijuma Ia. U postoperativnom periodu nije bilo komplikacija i bolesnica je otpuštena deset dana posle operacije u dobrom stanju i normalizovanom kalcemijom. Lečenje je nastavljeno istovremenom radio- i hemioterapijom. Međutim, bez obzira na preduzete mere lečenja, bolest je progradirala i bolesnica je umrla zbog diseminovane metastatske bolesti 26 meseci posle postavljanja dijagnoze. **Zaključak.** Sitnoćelijski karcinom lokalizovan u jajniku najčešće je kategorija tumora sa lošom prognozom u zavisnosti od faze bolesti.

Ključne reči:
karcinom malih ćelija; jajnik, neoplazme; dijagnoza; imunohistohemija; neoplazme, metastaze; lečenje, ishod.

Correspondence to: Miloš Z. Milosavljević, Department of Pathology, Clinical Center Kragujevac, Zmaj Jovina 30, 34 000 Kragujevac, Serbia. Phone: +381 64 950 10 39. E-mail: drmilosavljevic@sbb.rs

Introduction

Extrapulmonary small cell carcinoma is rare, prognostically bad tumor category [1–3]. Primary, it can be present in almost every organ and very often it represents a real challenge in diagnosis and therapy. Small cell carcinoma localized in the ovary (SCOC) is very rare tumor of aggressive course with relatively fast and fatal outcome.

The first description of SCOC dates back to 1975, but seven years later, while describing some other cases Cannon et al. [4], and Dickersin et al. [5] defined SCOC as separate tumor category. World Health Organization (WHO) classification from 2003, puts SCOC into the group of different ovary tumors [6]. Works of Young et al. [7] and multicentric study of Harrison et al. [8] (with the precise description of diagnosis, treatment and course of the disease in 17 patients from Australia, Canada and Europe for the period of time between 1989 to 2004) give us the greatest amount of information about SCOC.

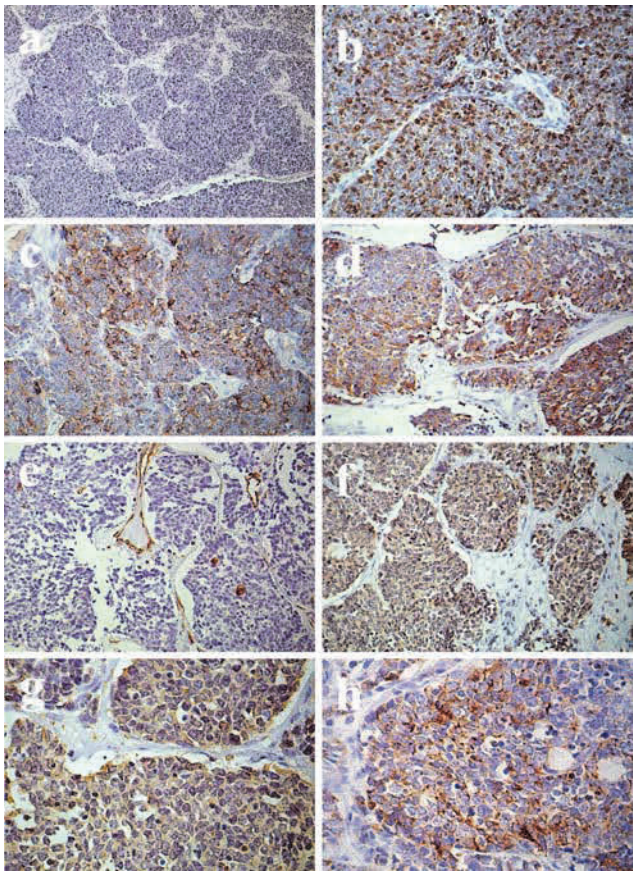

Fig. 1 – Histological features of ovarian small cell carcinoma: a) tumor islands formed by small cells with scant cytoplasm and hyperchromatic round to oval nuclei (HE, ×100); b) proliferative activity is very high; about 90% of nuclei show immunoreactivity for Ki-67 (×200); tumor cells are positive to: c) low molecular weight cytokeratin (CK LMW) (×200); d) Epithelial membrane antigen (EMA) (×200); e) vimentin positive staining in endothelial cells, while tumor cells are negative (×200); and positive for: f) sinaptophysin (Syn) (×200); g) neuron specific enolose (NSE) (×400) and h) chromogramin A (ChroA) (×400).

It most commonly arises in younger women between the age of 13 and 55. Single cases of SCOC in girls and postmenopausal women are described [9, 10]. Clinical presentation is deficient. Symptomatology and manifestation usually appear in the sense of discomfort or pain in the abdomen when the tumor reaches greater size, and with 2/3 of the patients paraneoplastic hypercalcemia also appears [7, 11].

SCOC prognosis is generally bad even tough aggressive regime of treatment is applied. The overall survival of patients at Ic *International Federation of Gynecology and Obstetrics* (FIGO) stage is a bit higher than 10% and for those in higher stages is only 6.5% [7].

Case report

A 60-year-old woman, Caucasian, gravida 4, para 2, menarche at 17 and menopause at 48, came to the doctor because of discomfort in the lower abdomen and pain of greater intensity in last few days. Apart from the echinococcal liver cyst operation five years ago, the patient did not mention any other significant gynecological or other health problems. The report of physical examination showed a soft abdomen with sensitivity to pain and fullness in the right lower quadrant, and bimanual gynecological examination showed the presence of large adnexal masses on the right. Ultrasound examination and CT scan of the abdomen confirmed the presence of large adnexal masses of cystic-solid appearance with the largest diameter of 13 cm, regular structure of the other gynecological organs, without verifying the existence of metastatic deposits. All the results of laboratory analysis gave normal values, except for calcium, which was elevated (2.69 mmol/L) (normal range 2.10–2.55 mmol/L). Medical council of gynecologists recommended operative treatment. Explorative laparotomy with complete hysterectomy, bilateral salpingo-oophorectomy, dissection of lymph nodes and omentectomy was conducted.

The operative material was submitted for pathohistological analysis. The omentum, lymph nodes, uterus with cervix, both uterine tubes and the left ovary were without significant micromorphological changes. The sample of the right ovary was of irregular spherical shape with the smooth, pale gray surface, with diameter of 13 × 11 cm, and the weight of 330 g. The cross section showed a whitish tumor mass, mostly solid with a small cystic part, with extensive areas of bleeding and necrosis. Microscopically, the tumor was formed of relatively uniform small cells which made diffuse field or forming solid nests, thick ribbons and follicle–like structures. Tumor cells were spherical or with slight spindle elongation, with scant of cytoplasm, oval nuclei with granular chromatin pattern and sporadically prominent nucleoli (Figure 1a). Mitoses were numerous; more than 20 mitotic figures could be seen in 10 fields of high power magnification (Figure 1b). Fields of extensive necrosis were present, as well as bleeding focuses, capsular infiltration without its bursting, with evident invasion of lymphatic vessels. Immunohistochemically (Figure 1c-h), tumor cells were vimentin negative, and positive to epithelial membrane antigen (EMA), low molecular weight cytokeratin (LMW CK) and to neuroendocrine differentiation markers:

neuron specific enolase (NSE), chromogranin A (Chro A) and synaptophysin (Syn). Thyroid transcripton factor-1 (TTF-1) negativity with external positive control (Figure 2a and 2b), excluded small cell carcinoma of the lung. Proliferative activity was very high; about 90% of nuclei showed immunoreactivity for Ki-67 (Figure 1b). Based on this pathohistological analysis, SCOC at FIGO Ia stage was diagnosed.

No complications were observed in a postsurgery period and after 10 days the patient was discharged in a good condition and with normal calcemia. The treatment was continued with concurrent radiotherapy and chemotherapy. However, in spite of overall treatment, the disease progressed, and the patient died of disseminated metastatic disease, 26 months after the diagnosis

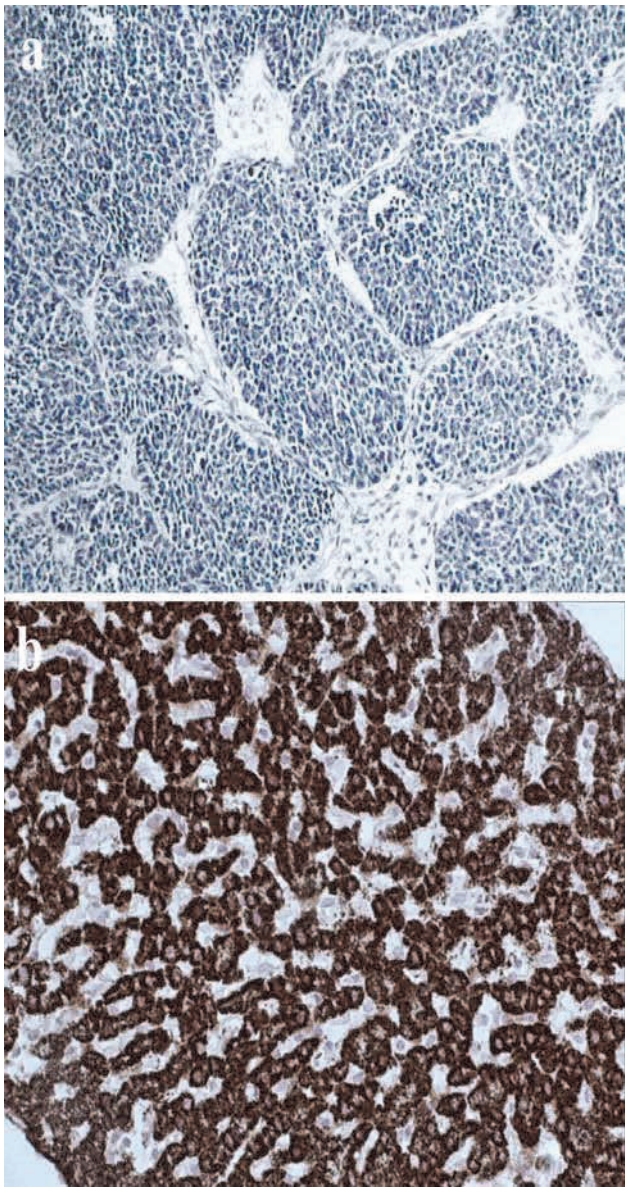

Fig. 2 – Immunoexpression of Thyroid Transcription Factor-1 (TTF-1): a) tumor cells are negative for TTF-1 (×200); b) positive control for TTF-1 (cytoplasmatic expression in the liver tissue, ×200).

Discussion

Primary extrapulmonary small cell carcinomas are very rare, making 2.5–4% of all small cell carcinomas [1–3]. They are of aggressive course with bad prognosis and usually recidivate shortly after the therapy [2]. They can be localized in various organs including the pleura, thymus, kidney, prostate, ovary, cervix, larynx, trachea, thyroid gland, lymph node, CNS, liver, skin, sinuses, salivary glands, peritoneum, stomach, esophagus, pancreas, gall bladder, colon, skin etc [2, 3].

In the series by Kim et al. [12, 13], the most common extrapulmonary localization was cervix while the another authors indicated that those are esophagus and thymus.

SCOC commonly arises between the age of 13 and 55, more often with younger women [7]. However, Pantier et al. [10] presented 11 cases of menopausal and postmenopausal women with histologically and immunohistochemically confirmed SCOC. It is considered that about two thirds of the patients have hypercalcemia at the moment of diagnosis, although symptomatology of hypercalcemia is rarely present [14]. Our presentation also referred to these rare cases of SCOC arising in postmenopausal women, with elevated level of calcium in the serum, and without clinical manifestation of hypercalcemia.

SCOC is unilateral, while bilaterality is usually present in patients with wide-spread metastatic disease and in this case tumor deposits on the other ovary are actually the result of metastasis [7]. There are also descriptions of bilateral SCOC which appear within a family [7, 15]. In the series by Young et al. [7] there was 1 pair of first-degree relatives (mother and daughter) and 1 pair of second-degree relatives (cousins).

The histogenesis of SCOC is unknown. Malignant cells do not originate from the surface epithelium of ovary, sex cords and stroma, as well as germinative and neuroendocrine cells [16]. Animal models used in the research of SCOC origin did not show the connection of SCOC with epithelial and germinative ovary tumors either [17].

Some authors believe that for the diagnosis the classical histological picture of small cell carcinoma on tissue sections stained with the standard Hematoxylin and Eosin (H&E) method is sufficient. However, since differential diagnostical dilemma is likely to appear, it is necessary to detect the immunophenotype and to prove a neuroendocrine differentiation in a special way. A certain immunophenotype helps in differentiation of SCOC from the other tumors of epithelial origin, lymphoma, melanoma, primary neuroectodermal tumors, tumors of germinative origin and the sex cords and stromal origine [18, 19]. In the presented case, neuroendocrine differentiation was imunohistohemically proved in combination with classical hystomorphological picture; tumor cells are Chro A, Syn and NSE positive. Immunohistochemically, small cell carcinomas generally show the sporadic positivity for LMW CK, and 30–75% is immunoreactive to EMA [20]. Although the positivity for vimentin is often present, this staining is considered to be nonspecific. In the original description of this tumor, 5 out of 7 cases showed positive staining to a parathyroid hormone–related protein (PTHrP) [21]. Differential diagnosis

excludes metastasis of the small cell lung carcinoma with negativity for TTF1 [22].

SCOC treatment is not clearly defined. The multiple model which includes surgery, chemo- and radiotherapy is recommended [2, 8, 10]. In unilateral cases the usual procedure is hycterectomy with bilateral salpingo-oophorectomy, while in younger women unilateral oophorectomy is an option. Chemotherapy and radiotherapy are applied concurrently or sequentially, and depending on the stage of disease they are applied preoperatively or adjuvantly. In the cases of complete therapeutic response prophylactic cranial irradiation is applied. The base of chemotherapy regime consists of cisplatin, etoposide, cyclophosphamide and doxorubicin in a variety of combinations. The overall response rate in the extensive disease, using cisplatin-based or cyclophosphamide/doxorubicin with vincristine or etoposide chemotherapy, is 70–90% [2, 3, 12]. However, despite the aggressive complex model of treatment more than 50% of women at Ia stage of the disease die within 2 years, while 33% of women have a 6-year interval without the disease [7]. Women with smaller tumors at Ia stage have better prognosis than those with tumor masses larger than 10 cm [7]. The presented patient belonged to this prognostically worse category. Although the disease was at Ia FIGO stage, with optimal treatment regime, the patient died 26 months after the SCOC diagnosis.

Conclusion

Small cell carcinoma localized in the ovary is generally a tumor category with bad prognosis depending on the stage of the disease.

REFERENCES

1. *van der Heijden HF, Heijdra YF*. Extrapulmonary small cell carcinoma. South Med J 2005; 98(3): 345−9.
2. *Woopen H, Sehouli J, Pietzner K, Darb-Esfahani S, Braicu EI, Fotopoulou C*. Clinical experience of young patients with small cell ovarian carcinoma of the hypercalcemic type (OSCCHT). Eur J Obstet Gynecol Reprod Biol 2012; 165(2): 313−7.
3. *Haider K, Shahid RK, Finch D, Sami A, Ahmad I, Yadav S*, et al. Extrapulmonary small cell cancer: a Canadian province's experience. Cancer 2006; 107(9): 2262−9.
4. *Cannon PM, Smart CR, Wilson ML, Edwards CB*. Hypercalcemia with ovarian granulosa cell carcinoma. Rocky Mt Med J 1975; 72(2): 72−4.
5. *Dickersin GR, Kline IW, Scully RE*. Small cell carcinoma of the ovary with hypercalcemia: a report of eleven cases. Cancer 1982; 49(1): 188−97.
6. *Tavassoli FA, Devilee P*. Worald Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Breast and Female Genital Organs. Lyon: IARC Press; 2003.
7. *Young RH, Oliva E, Scully RE*. Small cell carcinoma of the ovary, hypercalcemic type. A clinicopathological analysis of 150 cases. Am J Surg Pathol 1994; 18(11): 1102−16.
8. *Harrison ML, Hoskins P, du Bois A, Quinn M, Rustin GJ, Ledermann JA*, et al. Small cell of the ovary, hypercalcemic type -- analysis of combined experience and recommendation for management. A GCIG study. Gynecol Oncol 2006; 100(2): 233−8.
9. *Kopp LM, Desoky S, Pugh J, Herzog CE*. Small cell carcinoma of the ovary of the hypercalcemic type presenting in a 5-year-old girl. J Pediatr Hematol Oncol 2013; 35(5): e217−8.
10. *Pautier P, Ribrag V, Duvillard P, Rey A, Elghissassi I, Sillet-Bach I*, et al. Results of a prospective dose-intensive regimen in 27 patients with small cell carcinoma of the ovary of the hypercalcemic type. Ann Oncol 2007; 18(12): 1985−9.
11. *Hamilton S, Beattie GJ, Williams AR*. Small cell carcinoma of the ovary: a case report of 3 cases and review of the literature. J Obstet Gynaecol 2004; 24(2): 169−72.
12. *Kim JH, Lee SH, Park J, Kim HY, Lee SI, Nam EM*, et al. Extrapulmonary small-cell carcinoma: a single-institution experience. Jpn J Clin Oncol 2004; 34(5): 250−4.
13. *Kim K, Lee H, Chun S, Shin S, Kim M, Lee K*, et al. Clinical overview of extrapulmonary small cell carcinoma. J Korean Med Sci 2006; 21(5): 833−7.
14. *Estel R, Hackethal A, Kalder M, Münstedt K*. Small cell carcinoma of the ovary of the hypercalcaemic type: an analysis of clinical and prognostic aspects of a rare disease on the basis of cases published in the literature. Arch Gynecol Obstet 2011; 284(5): 1277−82.
15. *Martinez-Borges AR, Petty JK, Hurt G, Stribling JT, Press JZ, Castellino SM*. Familial small cell carcinoma of the ovary. Pediatr Blood Cancer 2009; 53(7): 1334−6.
16. *Dickersin GR*. The role of electron microscopy in gynecological pathology. Int J Gynecol Pathol 2000; 19(1): 56−66.
17. *Walt H, Hornung R, Fink D, Dobler-Girdziunaite D, Stallmach T, Spycher MA*, et al. Hypercalcemic-type of small cell carcinoma of the ovary: characterization of a new tumor line. Anticancer Res 2001; 21(5): 3253−9.
18. *McCluggage GW*. Ovarian neoplasms composed of small round cells: a review. Adv Anat Pathol 2004; 11(6): 288−96.
19. *McCluggage WG, Oliva E, Connolly LE, McBride HA, Young RH*. An immunohistochemical analysis of ovarian small cell carcinoma of hypercalcemic type. Int J Gynecol Pathol 2004; 23(4): 330−6.
20. *Dabbs D*. Diagnostic immunohistochemistry. 3rd ed. Philadelphia: Saunders & Elsevier; 2010.
21. *Chen L, Dinh TA, Haque A*. Small cell carcinoma of the ovary with hypercalcemia and ectopic parathyroid hormone production. Arch Pathol Lab Med 2005; 129(4): 531−3.
22. *Matoso A, Singh K, Jacob R, Greaves WO, Tavares R, Noble L*, et al. Comparison of thyroid transcription factor-1 expression by 2 monoclonal antibodies in pulmonary and nonpulmonary primary tumors. Appl Immunohistochem Mol Morphol 2010; 18(2): 142−9.

Received on January 9, 2013.
Revised on January 22, 2014.
Accepted on January 30, 2014.

C O R R I G E N D A

In the article by Vladimir Djordjević, Bojana Bukurov, Nenad Arsović, Snežana Ješić, Jovica Milovanović, Vladimir Nešić "Long term complications of ventilation tube insertion in children with otitis media with effusion". Vojnosanit Pregl 2015; 72(1): 40–43 (DOI:10.2298/VSP131210041D), on the page 43 after Conclusion it should be added:

Acknowledgements

The study was supported within the project from the Ministry of Education, Science and Technological Development of the Republic of Serbia "Influence of cochlear implantation on education of deaf and hard-of-hearing" (No. 179055).

INSTRUCTIONS TO THE AUTHORS

Vojnosanitetski pregled (VSP) publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that makes them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The VSP reserves all copyrights for the published papers. Accepted are only papers in English.

On January 1, 2012 the *Vojnosanitetski pregled* turned to the electronic editing system e-Ur: Electronic Journal Editing.

All the users of the system: authors, editors and reviewers have to be registered at:

http://aseestant.ceon.rs/index.php

The VSP publishes: **editorials, original articles**, **short communications**, **reviews/meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mm Hg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: *, †, ‡, §, ‖, ¶, **, ††, ‡‡ .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

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

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

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

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

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

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

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

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and symbols

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

Detailed Instructions are available at the web site:
www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

Vojnosanitetski pregled (VSP) objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“ neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu autora mora potpisati i od svakog autora rada, treba skenirati i poslata uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Za objavljene radove VSP zadržava autorsko pravo. **Primaju se radovi napisani samo na engleskom jeziku.**

Od 1. januara 2012. godine Vojnosanitetski pregled prešao je na e-Ur: Elektronsko uređivanje časopisa.

Svi korisnici sistema: autori, recezenti i urednici moraju biti registrovani jednoznačnom e-mail adresom. Registraciju je moguće izvršiti na:

http://aseestant.ceon.rs/index.php

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

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).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

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

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

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.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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

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

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

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

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

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.

Skraćenice i simboli

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

Detaljno uputstvo može se dobiti u redakciji ili na sajtu:www.vma.mod.gov.rs/vsp

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

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2015. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

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

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

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

Datum _____	Potpis _____

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

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2015. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

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

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

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

Datum _____	Potpis _____