YU ISSN 0042-8450

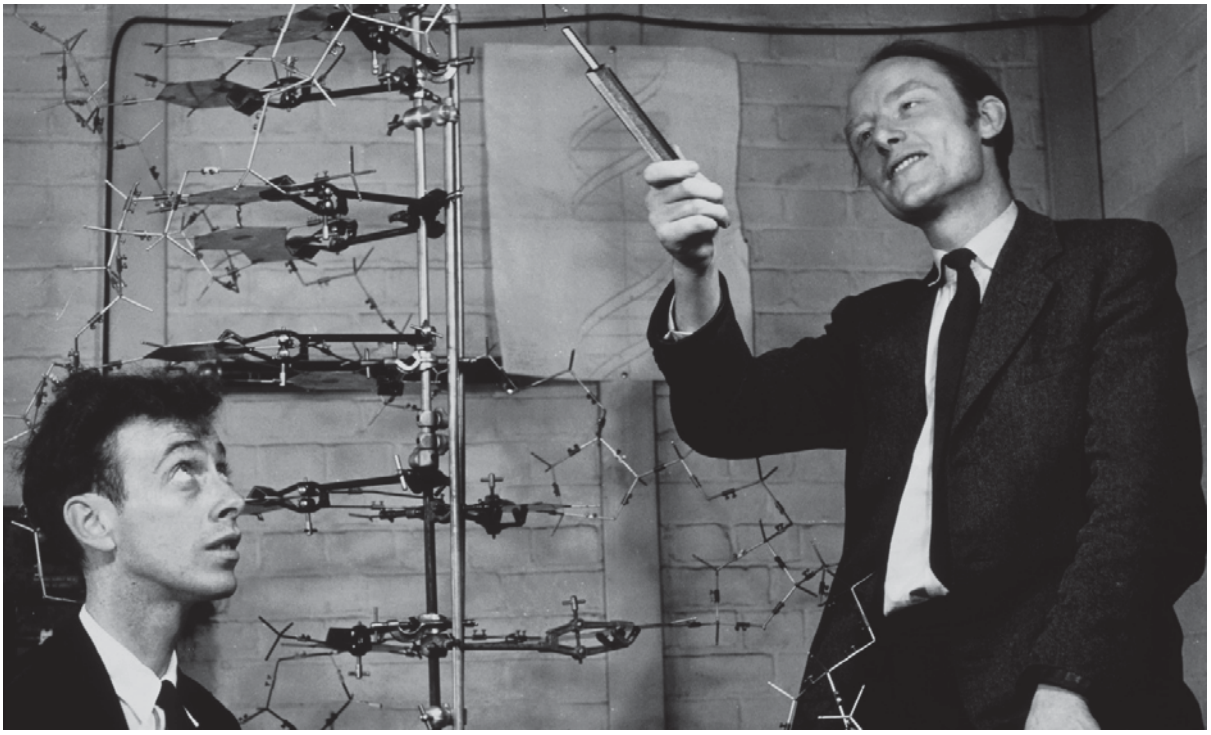
ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2013; December Vol. 70 (No. 12): p. 1075-1180.



YU ISSN 0042-8450 vol. 70, br. 12, 2013.

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. 70 No. 12, 2013

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.

Poštovani autori, urednici, recenzenti i saradnici Vojnosanitetskog pregleda,

Opraštajući se od stare godine, zahvaljujem vam na izuzetnoj saradnji i podršci uz želje da nam nastupajuća 2014. godina donese još više uspeha i radosti!

SREĆNA NOVA GODINA I BOŽIĆNI PRAZNICI!

Srdačno,
prof. dr Silva Dobrić,
glavni i odgovorni urednik

Dear Authors, Editors, Reviewers, and Collaborators of the *Vojnosanitetski Pregled*,

Saying farewell to 2013, I express my deep gratitute to your extraordinary cooperation and support along with my best whishes that the coming New Year 2014 bring us more success and happiness!

MARRY CHRISTMAS AND A HAPPY NEW YEAR!

Cordially,
Prof. Dr. Silva Dobrić
Editor-in-Chief

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

Poziv za reklamiranje u 2014. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je dostupan *on line* od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

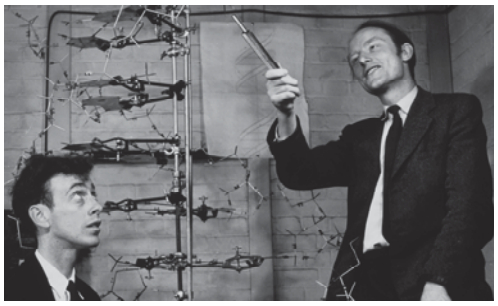
Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2014. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
2.	Oglas u c/b tehnici A4 formata za celu godinu (11-12 brojeva)	200 000,00 dinara
3.	Oglas u boji A4 formata za jedan broj	35 000,00 dinara
4.	Oglas u boji A4 formata za celu godinu (11-12 brojeva)	330 000,00 dinara
5.	Oglas u boji na koricama K3 za jedan broj	50 000,00 dinara
6.	Oglas u boji na koricama K3 za celu godinu (11-12 brojeva)	455 000,00 dinara
7.	Oglas u boji na koricama K2 i K4 za jedan broj	55 000,00 dinara
8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

Za sva obaveštenja, uputstva i ponude obratiti se redakciji časopisa „Vojnosanitetski pregled“. Sredstva se uplaćuju na žiro račun kod Uprave javnih plaćanja u Beogradu broj: 840-941621-02 **VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om) na adresu: Vojnosanitetski pregled, Crnotravska 17, 11000 Beograd; tel/faks: 011 2669 689, e-mail: vsp@vma.mod.gov.rs ili vmavsp@hotmail.com

CONTENTS / SADRŽAJ

ORIGINAL ARTICLES / ORIGINALNI ČLANCI



Watson & Crick`s deoxyribonucleic acid (DNA) model in 1953
The discovery in 1953 of the double helix, the twisted-ladder structure of DNA, by James Watson and Francis Crick marked a milestone in the history of science and gave rise in modern molecular biology, wich is largely concerned with understanding how genes control biochemical processes within cells. This year 60th anniversary of this discovery has been marked around the world (see pages 1165–70).

Watson-ov i Crick-ov model dezoksiribonukleinske kiseline (DNK) iz 1953. godine
Otkriće Watson-a i Crick-a iz 1953. godine da DNK ima strukturu dvostruke spirale u obliku uvijenih stepenica, označilo je prekretnicu u istoriji nauke i dovelo do brzog razvoja moderne molekularne biologije i razumevanja načina na koji geni kontrolišu biohemijske procese unutar ćelije. Ove godine, širom sveta obeležena je 60. godišnjica tog otkrića (vidi str. 1165–70).

ORIGINAL ARTICLES

UDC: 616.9-053.2-02:578.822]:616.511
DOI: 10.2298/VSP110607026P

Epidemiological and clinical features of erythema infectiosum in children in Novi Sad from 2000 to 2009

Epidemiološke i kliničke karakteristike infektivnog eritema kod dece u Novom Sadu u periodu 2000–2009.

Sonja Prćić*, Zorica Gajinov†, Bogdan Zrnić‡, Anica Radulović*, Milan Matić†, Verica Djuran†

***Department of Dermatovenerology, Institute for Child and Youth Health Care of Vojvodina, Novi Sad, Serbia; †Clinic of Dermatovenerology Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia; ‡Faculty of Medicine, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Erythema infectiosum (EI) is a common childhood illness, caused by human parvovirus B19. It occurs sporadically or in epidemics and is characterized by mild constitutional symptoms and a blotchy or maculopapular lacy rash on the cheeks (slapped-cheek) spreading primarily to the extremities and trunk. The aim of our study was to analyse the epidemiological and clinical characteristics of erythema infectiosum in children. **Methods.** This study included 88 children observed in the Department of Dermatology of the Institute for Child and Youth Health Care of Vojvodina, in Novi Sad, during the period January 2000–December 2009. We compared the data about the clinical characteristics during and after the outbreak of EI observed from December 2001 to September 2002. The data were retrieved from the hospital database. **Results.** During the study period, EI was detected in 88 children (44 females and 44 males), 0.213% of the total number of 41,345 children observed in the Department of Dermatology. An outbreak of erythema infectiosum was observed from December 2001 to September 2002, with the peak frequency in April and May 2002 and 39 diagnosed cases, and stable number of cases from 2005 to 2009 (a total of 49 diagnosed cases). The average age of infected children was 7.59 ± 3.339. Eleven (12.5%) children were referred from primary care pediatricians with the diagnosis of urticaria or rash of allergic origin. The most constant clinical sign was reticular exanthema on the limbs, present in 100% of the cases, followed by 89.77% of cheek erythema. Pruritus was present in 9.09% of the children, mild constitutional symptoms in 5.68% and palpable lymph glands in 3.41% of the children. In all the cases the course of the disease was without complications. **Conclusion.** The results of this study confirm the presence of EI (the fifth disease) in our area with a mild course in the majority of patients. Since the diagnosis of EI is usually based on clinical findings, continuing medical education of primary health care pediatricians is essential for reducing the number of misdiagnosed cases.

Key words:
erythema infectiosum; child; serbia; disease outbreaks; epidemiology; drug therapy; prognosis.

Apstrakt

Uvod/Cilj. Infektivni eritem je dečja osipna bolest, koja se karakteriše homogenim, veoma izraženim eritemom obraza i mrežastim osipom, lokalizovanim na ekstremitetima i trupu, bez značajnijeg poremećaja opšteg stanja. Izazivač je parvovirus B19. Javlja se sporadično ili periodično, u vidu manjih epidemija. Cilj našeg rada bio je da se utvrde epidemiološke i kliničke karakteristike infektivnog eritema. **Metode.** Studijom je bilo obuhvaćeno 88 obolele dece 44 devojčice i 44 dečaka koja su se javila na pregled u Odseku za dermatologiju, Instituta za zdravstvenu zaštitu dece i omladine u Novom Sadu, u periodu od januara 2000. do decembra 2009. godine. Podaci o kliničkim karakteristikama bolesnika dijagnostikovanih tokom epidemije 2001/2002. godine (39 obolelih) poređeni su sa periodom od 2005 do 2009. godine kada je broj dijagnostikovanih slučajeva bio ujednačen (ukupno 49). **Rezultati.** Infektivni eritem dijagnostikovan je kod 0,213% dece od ukupno 41 345 pregledane dece u Odseku za dermatologiju. Od decembra 2001. do septembra 2002. godine došlo je do naglog porasta broja obolelih od infektivnog eritema, dok je najveći broj obolelih bio u maju i aprilu 2002. godine. Prosečan uzrast obolele dece iznosio je 7,59 ± 3,339 godine. Ukupno 12,5% bolesnika upućeno je pod sumnjom na urtikariju ili osip alergijskog porekla. Mrežast osip na ekstremitetima bio je prisutan kod

Correspondence to: Sonja Prćić, Department Of Dermatovenereology, Institute for Child and Youth Health Care of Vojvodina, Hajduk Veljkova 10, Novi Sad, Serbia. Phone: +381 21 488 0444. Fax: +381 21 520 436. E-mail: buki97@eunet.rs

svih obolelih, eritem obraza kod 89,77%, svrab je bio prisutan kod 9,09% dece, blagi opšti simptomi infekcije kod 5,68%, a limfadenopatija kod 3,41% dece. Nisu zabeležene komplikacije oboljenja. **Zaključak.** Sva obolela deca imala su karakterističnu kliničku sliku i dobroćudan tok bolesti. Pošto se dijagnoza infektivnog eritema u dečjem uzrastu postavlja pretežno na osnovu kliničke slike, naglašavamo važnost kontinuiranog obrazovanja pedijatara koji pružaju primarnu zdravstvenu zaštitu sa kliničkim tokom i diferencijalnom dijagnozom ovog oboljenja.

Ključne reči:
eritem, infektivni; deca; srbija; epidemije; epidemiologija; lečenje lekovima; prognoza.

Introduction

Erythema infectiosum (EI) is an acute childhood illness, characterized by blotchy or maculopapular rash starting on cheeks, spreading to extremities and the trunk, giving a typical slapped cheek appearance and lacy configuration on limbs. Constitutional symptoms are mild [1, 2]. The disease is caused by human parvovirus B19 (Pv B19), the route of natural transmission is presumably the respiratory route, and parenteral and transplacental transmission have been proved, as well. A receptor molecule for B19 is glycolipid antigen on the erythrocyte surface, and hosts with the decreased production or increased destruction of red blood cells (such as hemolytic anemia or pure red cell aplasia due to various causes) are prone to aplastic crises and protracted severe anemia upon infection with human Pv B19 [2–4]. In immunocompromised hosts the course of Pv B19 infection can be severe, even caused by hemophagocytic syndrome [4]. Acute infection during pregnancy can result in hydrops fetalis (risk estimated in 10% of cases) [5]. The incubation period is 4–14 days, during which patients are infectious, only prior to the onset of the rash [6, 7].

EI usually develops suddenly, prodromal symptoms are mild or may be absent. The course of the disease is threephasic: facial "slapped-cheeks" rash, followed by lacy or reticular rash of the upper extremities and an evanescence/recrudescence stage. Eruption is pruritic in about 15% of children. EI usually lasts for 2 weeks, but can recur with mechanical, physical or emotional triggers. The diagnosis of EI is usually made on the basis of the characteristic clinical features. The differential diagnosis includes the other viral rashes (rubella, measles, enteroviral infection), scarlet fever, cheek erysipelas, hypersensitivity reaction (urticaria, drug reaction or allergic exanthemata) and collagen vascular diseases (systemic lupus erythematosus). Due to the mild course only symptomatic treatment is necessary (antipyretics, antihistamines) [1, 7, 8].

Methods

This retrospective study was conducted in the Department of Dermatology, Institute for Child and Youth Health Care of Vojvodina in Novi Sad, as tertiary referral center for the South Bačka region in the Province Vojvodina. Age, sex distribution, the presence of constitutional symptoms and clinical characteristics of the disease were retrieved from the medical records of all EI patients diagnosed between January 2000 and December 2009. The data from the period 2005–2009, and those previously published for the period 2000–2004 were compared [9].

All the patients were diagnosed by one of the dermatologists (authors), based on the clinical findings in the majority of cases. A total of 12 patients (2 during the period of 2005–2009) serum samples were additionally analyzed using SERION ELISA (enzyme-linked immunosorbent assay) classic parvovirus B19 IgG/IgM quantitative and qualitative tests for identification of specific antibodies against human parvovirus B19. Complement-fixing reactions (CFR) to viruses (Rubella, Adenovirus, Coxsackie virus) were performed in 5 children, with constitutional symptoms and palpable lymph nodes.

Results

During the study period (from the beginning of 2000 to the end of 2009), at the Department of Dermatology, the total number of first visits was 41,345 and erythema infectiosum was diagnosed in 88 children (0.213%). Figure 1 shows the number of patients during a 10-year period. Patients with EI were not observed within 2000, and sporadic cases emerged by the end of 2001. A sudden outbreak was noted from December 2001 to September 2002 with the highest number of cases recorded in April and May, 2002 [9]. In a subsequent 5 year period (2005–2009) EI was diagnosed in 49 children, 22 girls and 27 boys, average age being 7.98 ± 3.554 years. During a total period of 10 years, the average age of EI patients was 7.59 ± 3.339 years, with the majority of patients in the 5–10 years group, and equal sex ratio (44 girls and 44 boys affected).

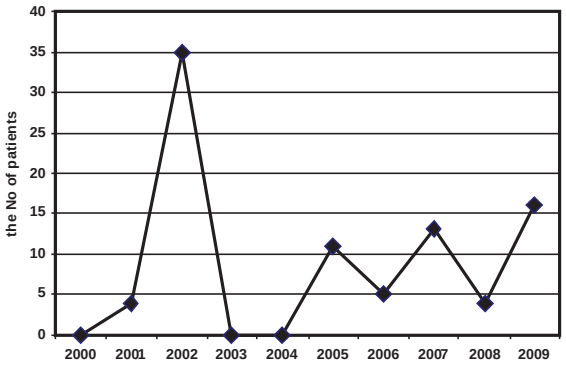


Fig. 1 – The number of patients with erythema infectiosum in a 10-year research period.

During the whole study period 11 children (12.5%) were referred from primary care pediatricians with the diagnosis of urticaria or rash of allergic origin, 7 during the pe-

riod of outbreak and 4 patients (8.16%) during the second period. These children were on diet and recevied oral antihistamines, while 5 of them (5.68%) had been prescribed parenteral corticosteroid treatment, 2 (4.08%) in the second period after the outbreak. Mild pruritus was present in 8 (9.09%) of the children and mild constitutional symptoms were present in only 5 (5.68%) of the children. All the patients had typical clinical picture, and atypical forms of the disease were not recorded in the studied pediatric population (*ie*, papular-purpuric gloves and socks syndrome or acropetechial syndrome) (Figure 2). The most constant clinical sign

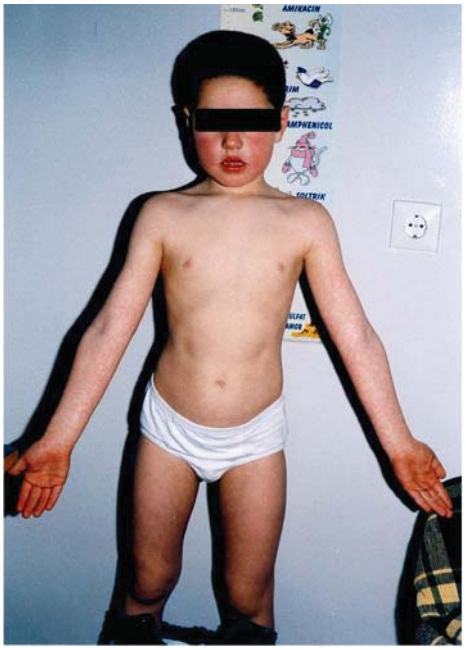


Fig. 2 – Erythema infectiosum in a 6-year-old boy.

was reticular exanthema on upper extremities, present in 100% of the cases, followed by intensive erythema of the cheeks (slapped-cheek appearance) in 79 (89.77%). Exanthema on trunk and extremities was present in 12 (13.63%) children. One (1.14%) patient had palmar and plantar erythema. Occipital lymphadenopathy was present in 3 (3.41%) children**.** Clinical findings of EI patients from the study period 2005–2009 are presented in Table 1. All the 12 tested

Table 1

Clinical findings of erythema infectiosum (EI) from 2005 to 2009

Clinical findings of EI	Children	
	n	%
Pruritus	4	8.16
Constitutional symptoms	3	6.12
Exanthema on upper extremities	49	100.00
Exanthema on extremities and trunk	9	18.37
Erythema of the cheeks	45	91.84
Palmar-plantar erythema	0	0
Occipital lymphadenopathy	1	2.04
Total	49	100.00

children were positive for IgM antibodies against human parvovirus B19, confirmative of the acute Pv B19 infection. CFR assays for Rubella, Adenovirus and Coxsackie were negative in all the five children tested. On control examination after 14 days rash was resolved in all the patients, while physical examination showed normal results.

Discussion

Infection with PvB19 is ubiquitous and occurs worldwide. EI is common, mildly contagious, and occurs sporadically or in epidemics [1]. The rise in the number of EI patients noted during the period December 2000 to May 2002, with the peak in April and May 2001, was not repeated in the later study period when an average number of patients was relatively stable [9]. That is in concordance with data form the literature on EI epidemics occurring in cyclical fashion, every 4–7 years, with more frequent outbreaks in winter and spring [3, 6, 8, 9]. Localized outbreaks among schoolchildren are common [6]. The first outbreak of EI in Serbia was reported from October 1987 to May 1988 that occurred among school children in part of Belgrade [10]. The majority of our EI patients were 5–10 years old, that is in concordance with literature data [1]. No gender difference in susceptibility to EI was noted in the literature, similar to our case series [5]. Lacy exanthema on proximal extremities was the most prominent symptom noted in all our patients, that disagrees with the study by Revilla et al. [11] where erythema of the cheeks (slapped-cheek appearance) was the most frequent one. Lacy exanthema of the extremities was the most frequent in the study by Bukumirović et al. [10], and in our previous report, also [9]. Palmar and plantar erythema occurs only rarely in EI [6]. In our study it was present only in one boy during the 2001–2002 outbreak, and in was not observed a later period [9]. None of the children had any further complications. In a similar study by Rewilla et al. [11] the illness had a mild course. The only difference in clinical presentation of EI patients between the two study periods is that exanthema affecting both trunk and limbs occurred more frequently during the study period 2005–2009 than in the previous 5 years (18.37% and 7.69% correspondingly) [9].

In our study lymph nodes enlargement was present in 3.41% of the patients (occipital lymph nodes), and in those children serological analyses for Rubella, Adenovirus and Coxsackie virus were performed, proved negative in all of them. In all 12 tested serum samples Pv B19 infection was serologically detected. According to the literature, laboratory proof of Pv B19 infection is not necessary in cases with characteristic clinical picture and uncomplicated course in previously healthy children [1, 7, 8]. Therefore, EI should not be a diagnostic problem because of its characteristic presentation and the course of the illness. EI description as “geographical map with lakes” as the most picturesque of all the rashes, together with phasic course with slapped cheeks or sun-burnt facial aspect preceding rash, are pathognomonic, and seen only in this disease [1, 7, 8]. However, the number of patients had been referred to the Institute because of suspected allergic rash, and a relatively large proportion of children (12.15%) had been previously prescribed antihistamine and corticosteroid treatment and

elimination diet. Even though EI is not a rare disease, it appears periodically every 4–7 years in the form of sporadic epidemics lasting for several months, that could be the reason not to be easily recognized among primary health care physicians. Albeit, during 2001/2002 outbreak when 18% of children were referred from primary care as suspect allergic reaction, in the period 2005–2009 proportion of unrecognized cases decreased to 8%. That is an encouraging result pointing that previous epidemics increased awareness and enhanced recognition of EI among non-dermatologists [9].

Conclusion

The results of this study confirm the presence of EI (the fifth disease) in our area with a mild course in the majority of patients. Since the diagnosis of EI is usually based on clinical findings, the continuing medical education of primary health care pediatricians is essential for reducing the number of misdiagnosed cases. Further studies within longer observation periods and larger groups of patients are necessary to determine the epidemiological and clinical characteristics of erythema infectiosum in Serbia.

R E F E R E N C E S

1. *Vafaie J, Schwartz RA*. Erythema infectiosum. J Cutan Med Surg 2005; 9(4): 159−61.
2. *Drew WL*. Parvoviruses. In: *Wilson WR*, editor. Current diagnosis and treatment in infectiosus diseases. New York: Mc Graw-Hill Co; 2001. p. 451−3.
3. *American Academy of Pediatrics Committee on Infectious Diseases*. Parvovirus B19. In: *Pickering LK, Baker CJ, Kimberlin DW, Long SS*, editorss. 2009 Red Book: Report of the Committee on Infectious Diseases. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009. p. 491−3.
4. *Heegaard ED, Brown KE*. Human parvovirus B19. Clin Microbiol Rev 2002; 15(3): 485–505.
5. *Anderson LJ, Hurwitz ES*. Human parvovirus B19 and pregnancy. Clin Perinatol 1988; 15(2): 273−86.
6. *Mancinci A*. Exanthems in childhood: an update. Pediatr Ann 1998; 27(3):163−70.
7. *Wiss K*. Erythema infectiosum and Parvovirus B19 infection. In: *Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF*, editors. Dermatology in general medicine. 5th ed. New York: Mc Graw-Hill; 2003. p. 2409−13.
8. *Zellman G*. Erythema Infectiosum (Fifth Disease). Emedicine Dermatology (journal series online). 2002. Available at: http://author.emedicine.com/derm/topic 136.htm.
9. *Prcic S, Jakovljević A, Djuran V, Gajinov Z*. Erythema infectiosum in children: a clinical study. Med Pregl 2006; 59(1−2): 5−10. (Serbian, English)
10. *Bukumirović K, Radosavljević M, Milkić V, Antonijević B, Polak D, Dodić M,* et al. Epidemiologic characteristics of human parvovirus B19. Report of an epidemic of erythema infectiosum in the area of Belgrade. Srp Arh Celok Lek 1992; 120(5−6): 171−4. (Serbian)
11. *Revilla G, Carro G, Sanchez DM, Galan C, Nebreda M*. Outbreak of infectious erythema at a urban health center. Aten Primaria 2000; 26(3): 172−5. (Spanish)

Received on Jun 7, 2011.
Revised on Avgust 18, 2011.
Accepted on September 5, 2011.
OnLine-First May, 2013.

ORIGINAL ARTICLE

UDC: 616-036.22::616.61-076
DOI: 10.2298/VSP110614027S

Histomorphological and clinical study of primary and secondary glomerulopathies in Southeast Serbia (20-year period of analysis)

Histomorfološko i kliničko ispitivanje primarnih i sekundarnih glomerulopatija u jugoistočnoj Srbiji (analiza u periodu od 20 godina)

Nikola Stanković*, Predrag Vlahović†, Vojin Savić‡

***Mother and Child Health Care Institute, Belgrade, Serbia; †Center for Medical Bichemistry, ‡Institute of Nephrology and Hemodialysis, Clinical Center Niš, Niš, Serbia**

Abstract

Background/Aim. Epidemiological studies of renal biopsies have been performed to follow up the incidence of glomerular diseases on a specified territory and to compare the obtained results with results from other regions. The aim of this study was to analyze the frequency of certain histopathophysiological types of glomerular diseases on the territory of Southeast Serbia. **Methods.** In a 20-year period (1986–2006), 316 kidney biopsies were performed in patients with clinicall signs of impaired renal function, in Southeast Serbia. On average 1.6 biopsies were made per year per 100 000 inhabitants. **Results.** Biopsies of adult patients represented 88% of all biopsies, biopsies in children (aged under 18 years) represented 8%, while biopsies of elderly patients (more than 60 years) represented 4% of all biopsies. The predominance of male patients was described with male/female ratio of 1.4. The most frequent clinical manifestation in patients at the time of biopsy were nephrotic syndrome (42.5%), and asymptomatic proteinuria and/or hematuria (31.3%) and nephritic syndrome (14.9%). The most common glomerular disease was IgA nephropathy with an incidence of 21.5% of total biopsy diagnosed glomerulopathies, followed by: membranous glomerulonephritis (12.6%), focal segmental proliferative and sclerosing glomerulonephritis (10.7%), lupus nephritis (8.4%), nephroangiosclerosis (7.0%), mesangio-proliferative glomerulonephritis (6.1%), minimal change disease (2.8%), mesangiocapillary glomerulonephritis (2.3%). **Conclusion.** The frequency of certain histopathologic findings significantly correlated with data from studies that we used for comparison, with the exception of minimal change disease whose incidence in our study was smaller.

Key words:
kidney diseases; glomerulonephritis; biopsy; histocytochemistry; epidemiology; serbia.

Apstrakt

Uvod/Cilj. Epidemiološke analize biopsija bubrega imaju za cilj praćenje učestalosti pojedinih glomerulskih bolesti na određenom području i upoređivanje dobijenih rezultata sa rezultatima iz drugih regiona. Cilj ovog istraživanja bio je da se analizira učestalost pojedinih histopatofizioloških tipova glomerulskih bolesti na teritoriji jugoistočne Srbije. **Metode.** U periodu od 20 godina (1986–2006), na teritoriji jugoistočne Srbije, urađena je biopsija bubrega kod 316 bolesnika kod kojih su klinički bili prisutni znaci poremećene bubrežne funkcije. Prosečno je urađeno 1,6 biopsija godišnje na 100 000 stanovnika. **Rezultati.** Od urađenih biopsija 88% su bile biopsije kod odraslih osoba, 8% kod dece, dok su biopsije kod starijih osoba činile 4%. Predominacija bolesnika muškog pola, iskazana muško/ženskim odnosom biopsiranih bolesnika iznosila je 1,4. Najzastupljenije kliničke prezentacije kod bolesnika u vreme biopsije bile su nefrotski sindrom (42,5%), zatim asimptomatska proteinurija i/ili hematurija (31,3%) i nefritski sindrom (14,9%). Najučestalija glomerulska bolest bila je IgA nefropatija sa učestalošću od 21,5% od ukupnog broja biopsijom dijagnostifikovanih glomerulopatija, a sledili su: membranski glomerulonefritis (12,6%), fokalnosegmentalni proliferativni i sklerozirajući glomerulonefritis (10,7%), lupus nefritis (8,4%), nefroangioskleroza (7,0%), mezangioproliferativni glomerulonefritis (6,1%), bolest minimalnih promena (2,8%), mezangiokapilarni glomerulonefritis (2,3%). **Zaključak.** Učestalost pojedinih patohistoloških nalaza u značajnoj meri koreliše sa podacima iz studija koje su nam služile za komparaciju, sa izuzetkom bolesti minimalnih promena čija je incidencija niža u našoj studiji.

Ključne reči:
bubreg, bolesti; glomerulonefritis; biopsija; histocitohemija; epidemiologija; srbija.

Correspondence to: Nikola Stanković, Mother and Child Health Care Institute, Cetinjska 32/7, 11 000 Belgrade, Serbia.
Phone: +381 60 013 57 13. E-mail: kokanasta@gmail.com

Introduction

Glomerulonephritis (GN) is a bilateral, non-bacterial, non-suppurative inflammation of kidney which primarily affects glomerulus and then the other kidney structures.

Histological features are the most important criterion for the nomenclature and classification of glomerular disease. In addition to localization, the histological characteristics define the nature of glomerular lesion as well as cell proliferation, deposit of immune complexes or extracellular components lesions.

Immune processes underlie most cases of primary GN, but they are also present in many secondary events in the glomerulus. Therefore, the division of etiopathologic mechanisms of glomerulopathy is reduced to immunological and non-immunological [1].

Glomerular lesion is clinically expressed with a relatively small number of signs and symptoms. There are five main clinical syndromes which may be manifested in glomerular disease: acute GN syndrome (acute nephritic syndrome), syndrome of rapidly progressive GN, nephrotic syndrome, asymptomatic urinary abnormalities (asymptomatic proteinuria and hematuria) and the syndrome of chronic GN.

The diagnosis of glomerular disease involves a complete understanding of the clinical condition of the patient, laboratory diagnostics, immunological and morphological studies. Diagnosis based only on the clinical manifestations may be unsuccessful because different glomerular diseases are characterized by similar or the same clinical presentation. On the other hand, the same glomerular disease may be presented differently in different patients [2].

Therapeutic approach, response to therapy and prognosis, depend on the type of GN. The frequency of certain forms of GN is different in different geographical areas and associated with genetic and environmental factors.

This retrospective study was conducted with the aim to determine the frequency of certain types of glomerular disease in Southeast Serbia on the basis of which may be possible to predict the response to therapy and prognosis.

Methods

During a 20-year period (1986–2006), 316 kidney biopsies were performed in patients with clinically and laboratory shown signs of some primary or secondary glomerulopathy (nephritic or nephrotic syndrome, syndrome of rapide progressive GN and asymptomatic hematuria or proteinuria). All biopsies were done at the Institute of Nephrology and Hemodialysis in Clinical Center Niš and the whole tissue biopsy material was processed in the histopathological laboratory of this institute. The histopathologist has always taken biopsy material from a patient and prepared it for light microscopic analysis, immunofluorescence and if necessary for electron microscopy.

The same method for taking biopsy material was always used – blind percutaneous renal biopsy with previous intravenous pyelography or ultrasound examination. In parallel to light microscopy, immunofluorescence with IgG, IgA, IgM, C1q, C3, fibrinogen, albumin, kappa and lambda chains was always done. Electron microscopy was performed in 3–4% of cases when it was possible (during the difficult economic crisis) and when indications were present (other morphological examination aroused suspicion of a change in the GBM structure; existence of deposit which could not been precisely localize in immunofluorescence findings).

Of the total number of samples, 214 biopsies had positive histopathological findings in the glomeruli, while 102 findings were not included in this study. Only those cases who clearly indicated the existence of glomerulopathy were included. The patients whose clinical presentation and laboratory findings did not fit into diagnosis of glomerulopathy or tubulointerstitial changes were clinically and histopathologicaly confirmed, were not included in this study. Among samples which were not included in the study, 26 had negative glomerular findings, 50 of them did not have enough material for analysis, and in 26 of them findings in tubulointerstitial system were dominant (chronic pyelonephritis, acute tubular necrosis, balkan endemic nephropathy, etc).

Immunological tests included measurements of serum immunoglobulins concentration, complement fractions in serum, immune complexes while the presence of certain autoantibodies were detected by direct immunofluorescence. Immunoglobulins and complement fractions were measured by the nephelometric method (II BEN nefelometar – Dade-Behring, Magdeburg, Germany). Immune complexes were measured by the deposition of immunoglobulins with polyethylene glycol (PEG-6000). The presence of autoantibodies in biopsy specimens was performed by direct immunofluorescence with autoantibodies labeled with fluorescein (FITC).

Other analysis have included detailed medical history, routine biochemical tests as well as other relevant testing. The following findings have been specifically analyzed: duration of clinical manifestations of impaired kidney function, previous diseases, edema, the appearance of micro- or macrohematuria, blood pressure, finding of proteinuria, hypoproteinemia and hipoalbuminemia findings, and elevated serum cholesterol and triglycerides.

The data were analyzed by descriptive statistics using the program Sigma Stat.

Results

Of 214 patients with biopsy confirmed diagnosis of a glomerulopathy, 125 (58.4%) were males and 89 (41.6%) females.

Age and gender distributions are shown in Table 1. Age data were available for 204 patients. Most of the patients were aged 31–50 years, a total of 98 patients (61 males and 37 females), that was 45.8% of the total number of biopsies with positive findings. Within this age group, most were male patients with primary proliferative GN (33% or 33.7%).

Clinical presentation of the patients with GN is shown in Table 2. Of the 7 described syndromes, the most common was nephrotic syndrome in 91 (42.5%) patients.

Table 1

Age and gender distribution of the biopsied patients in the groups of glomerular disease

Type of glomerulopathy	≤ 18 years		19–30 years		31–50 years		51–60 years		> 61 years		All ages	
	m	f	m	f	m	f	m	f	m	f	m	f
Primary nonproliferative GN	2	–	8	–	12	3	6	6	3	1	31	10
Primary proliferative GN	6	2	13	14	33	12	8	–	3	–	68	31
Secondary GN	–	2	–	4	3	11	–	2	–	–	3	19
Non-immunological and other glomerulopathies	2	1	5	8	13	10	2	6	1	3	23	29
Σ	10	5	26	26	61	35	16	14	7	4	125	89

GN – glomerulonephritis; m – male; f – female

Of a total number, 140 biopsied patients had pathological findings of the primary forms of GN, which was 65.4%, of which the proliferative forms were much more frequent (Table 2). Secondary forms of GN had 22 (10.3%) patients, non-immunological glomerular lesions (diabetes, amyloidosis, and hypertensive changes) were recorded at 22 (10.3%) patients, while in the other group, terminally damaged renal parenchyma (end stage renal diseases-ESRD) occurred in 13 (6.1%) of the cases.

Primary nonproliferative forms of GN were found in 41 of 214 patients (29.3% of all the patients with primary GN). The most frequent nonproliferative GN was membranous GN, followed by focal segmental sclerosis and minimal change disease (Table 3).

Membranous GN was present in 27 patients (7 females and 20 males) of which hypertension was registered in 13 (severe stages in 4), edema in 23, hematuria in 22, while nephrotic proteinuria was present in 15 of the patients. The

Table 2

Clinical syndrome at the time of biopsy in the patients with biopsy findings of glomerular lesion

Clinical syndrome	Patients	
	n	%
Nephrotic	91	42.5
Nephritic	32	14.9
Asymptomatic proteinuria and hematuria	67	31.3
Asymptomatic proteinuria	7	3.3
Asymptomatic hematuria	2	0.9
Rapidly progressive glomerulonephritis	2	0.9
Chronic renal failure	13	6.2
Type of glomerular lesion		
Primary nonproliferative GN	41	19.1
Primary proliferative GN	99	46.3
Secondary GN	22	10.3
Non-immunological glomerulopathy	22	10.3
Other	30	14.0

GN – glomerulonephritis

Table 3

Glomerular diseases in the biopsied patients

Type of glomerulopathy	Glomerular diseases	Patients	
		n	%
Primary nonproliferative GN	Minimal-change disease	6	2.8
	Focal segmental sclerosis	8	3.7
	Membranous GN	27	12.6
Primary proliferative GN	Focal segmental proliferative and sclerosing GN	23	10.7
	Endoteliomesangial GN	10	4.7
	Mesangioproliferative GN	13	6.1
	IgA nephropathy	46	21.5
	Mesangiocapillary GN	5	2.3
	Rapidly progressive GN	2	0.9
Secondary GN	Lupus GN	18	8.4
	Henock-Schoenlein purpura	4	1.9
Non-immunological glomerulopathy	Nephroangiosclerosis	15	7.0
	Diabetic nephropathy	4	1.9
	Amyloidosis	3	1.4
Other	End-stage glomerulonephritis	13	6.1
	Sy. Alport	2	0.9
	Postpartal thrombotic microangiopathy	1	0.5
	Status after kidney transplantation	1	0.5
	Unknown entity	13	6.1

GN – glomerulonephritis

findings of immunofluorescence of IgG were positive in 25 of the patients.

Detection of primary proliferative GN was present in 99 patients of 214 which is 70.7% of the biopsied patients with findings of primary glomerulonephritis. The most common form of primary proliferative GN was IgA nephropathy, then focal segmantal praliferative and sclerosing GN, mesangioproliferative GN, endoteliomezangial GN, mesangiocapillary GN and rapidly progressive GN (Table 3).

IgA nephropathy was diagnosed in 46 patients (14 females and 32 males). The youngest patients belonged to the age group of 11–20 years while the oldest were between 51 and 60 years. Hypertension was present in 21, edema in 10, the finding of hematuria was present in all the patients, while proteinuria greater than 3.5 g/L was present in 8 patients with this finding. The largest number of light microscopy findings belonged to the group with focal segmental-proliferative and sclerosing GN. Second most frequent findings matched to focal segmental proliferative GN with mesangial area lesions. Diffuse changes in the glomeruli, such as diffuse proliferative and diffuse proliferative and sclerosing GN were registrated in a much lower number of cases. Extracapillary proliferation with less than 50% of affected glomeruli was observed in only one biopsy. Considering immunofluorescence findings all the patients of this group were positive for IgA.

Secondary forms of GN were registered in 22 out of 214 biopsied patients, which is 10.3%. The definitive diagnosis in these cases was confirmed by other immunological tests (primarily by determining the presence of serum autoantibodies). Out of 22 patients 18 had systemic lupus erythematosus, and 4 showed light microscopic signs of focal proliferative and sclerosing GN with changes in the skin and positive immunofluorescence finding, which is definitely pointing on Henoch-Schonlein purpura (Table 3).

Lupus nephritis was present in 18 cases. All the patients were females, aged 17 to 48 years. Hypertension was registrated in 7 of the patients, edema in 13, proteinuria and hematuria in all the patients. Serum levels of C3 and C4 were reduced in most patients, while immune complexes and antinuclear antibodies were present in 9 of the patients to whom these measurements were made. Non-immunological and other glomerulopathies were diagnosed in 52 patients, which is 24.3%. Nephroangiosclerosis was diagnosed in 15 of the patients, glomerular lesion in diabetic nephropathy in 4 patients and glomerular lesions in amyloidosis in 3 patients (Table 3). Other biopsy material presented 14% of the biopsied patients with positive findings and spoke in support of end-stage GN (13 patients), Alport syndrome (2 patients), postpartal thrombotic microangiopathy (1 patient), status after kidney transplantation (1 patient) or it was not possible to come out of any known entity.

Discussion

Epidemiology of glomerular disease often shows some peculiarities related to the geographic area, requiring a regional monitoring and mutual comparison of morbidity in different territories. This study presents the frequency of some glomerular diseases diagnosed by biopsy of renal tissue in the period of 20 years in Southeast Serbia (population over 1 million).

A large number of biopsy samples with insufficient material is probably due to technical reasons of biopsy procedure (blind percutaneous renal biopsy) and in some degree disparage the preciseness of results. According to our results, 1.6 biopsies were performed per 100 000 inhabitants per year. This number is several times lower than the rates present in Western European countries like Spain [3], Italy [4], France [5], and the Czech Republic [6], or Australia [7], but is similar to the rate of biopsies in Romania [8], whose registry is one of the few renal biopsy registers of Eastern Europe, and to the rate of biopsies obtained in another single centre study conducted in Serbia, for the same period in a much larger sample [9].

Biopsies in adult patients represented 88% of all biopsies, biopsies in children (aged under 18 years) represented 8%, while the biopsies in elderly patients (more than 60 years) represented 4% of all biopsies (Table 1). This relationship between the number of biopsies of children and the elderly is quite different from the relationship presented in the Spanish registry of renal biopsies, where renal biopsy in children represented 7% and 22% in the elderly [3]. These results show predominance of adults in the biopsied patients and that a large number of elderly patients and children were treated on the basis of clinical manifestations.

The predominance of male patients is reported with male/female ratio of 1.4, which is consistent with data from other international studies [3,7,10]. From this, we can exclude secondary forms of glomerulonephritis, in which the predominance of females was present, what can be explained by a much higher incidence of systemic lupus in women (Table 1). However, male/female ratio is significantly different from the results obtained in another single centre study conducted in Serbia, for the same period in a much larger sample [9].

The most frequent clinical presentations in patients at the time of biopsy were nephrotic syndrome (42.5%), asymptomatic proteinuria and/or hematuria (31.3%) and nephritic syndrome (14.9%). Other syndromes were significantly less frequent (Table 2). These data are consistent with the results from the Spanish [3] and Czech register [6], as well as with data from a Japanese study with 1850 biopsied patients. Also, data from another single centre study conducted in Serbia were similar, except that the incidence of nephrotic syndrome showed more pronounced dominance [9]. However, the obtained data differ the results from the Italian registry where asymptomatic urinary abnormalities were more frequent than nephrotic syndrome [4] and the results from the Romanian registry where asymptomatic urinary abnormalities were less frequent than all other syndromes [8] (Table 4).

According to the literature and larger registers [4, 11–13], a difference between glomerular pathology in children, adult and elderly population is clearly visible. With the increase of the average number of years of biopsied patients reduces the incidence of primary GN and increases the incidence of secondary forms of glomerular disease [3]. The results of this

Table 4

Most common clinical syndromes in the biopsied patients in 4 regions

Clinical syndrome	Southeast Serbia (%)	Romania (%)[6]	Italia (%)[2]	Another single centre in Serbia (%)[9]
Nephrotic syndrome	42.5	52.3	27.1	63.8
Asymptomatic proteinuria and/or hematuria	31.3	3.3	39.5	15.2
Nephritic syndrome	14.9	21.9	5.4	7.9
Other syndroms	11.3	22.5	28.0	13.1

study confirm the distribution of certain glomerular patologic entities by the age of the group, although with less certainty, possibly due to the relatively small number of biopsied patients (Table 2).

Similar to other registers, primary GN was proven in about two thirds of biopsied patients [5, 6], while the secondary form and non-immunological GN were equally presented with one tenth of biopsied patients.

The most common was IgA nephropathy with an incidence of 21.5% of total diagnosed glomerulopathies. This frequency is most similar to the frequency of this entity in the Netherlands [14] and Spain [3], and significantly lower than reported in other European studies [4, 6, 8, 12]. The most frequent clinical presentation among this patients was asymptomatic proteinuria and/or hematuria. All the patients with this histopathological diagnosis in our study were younger than 50 years and only 4 of them were younger than 19 years.

Second most frequent glomerular disease, with an incidence of 12.6% was membranous glomerulopathy. The patients in this group had a higher incidence of developed nephrotic syndrome than all other histopathological groups. In this group, histopathological finding of focal segmental proliferative and sclerosing GN with an incidence of 10.7%, was three times as frequent as isolated sclerosing form of this finding.

Despite the fact that a small number of biopsies was performed in children, the incidence of minimal change disease was significantly lower (2.8%) than in other European and world studies [3, 4, 7, 15]. Conservative treatment until symptoms withdrawal and lack of technical conditions for electron microscopy during the bombing of Serbia could be just some of the reasons for these results.

In the group of secondary GN, lupus nephritis as a dominant finding with the frequency of 8.4% is consistent with all known registries. At the time of biopsy these patients presented predominantlly nephrotic syndrome and asymptomatic proteinuria and/or hematuria. The most frequent histological form of lupus nephritis was diffuse proliferative lupus nephritis (class IV) registered in 12 out of 18 patients, followed by focal segmental lupus nephritis (class III) in 4 patients, mesangial lupus nephritis (Class II) in 1 patient, lupus nephritis without histological changes (Class I) in 1 patient, while patients with membranousand (Class V) and end-stage lupus nephritis (Class VI) was not registrated. Although in a small sample and with lower frequency of mesangial lupus nephritis, these those are similar to results obtained by Dimitrijevic et al. [16] on a sample of 311 patients with lupus nephritis. In all the patients immunofluorescence findings were positive primarily for IgG and C3 and in the lower percentage on IgA, IgM and C4. All of 18 biopsy specimens with this findings belong to women aged between 19 and 50 years, except in two cases of 17 and 14 years of age (Table 3).

Conclusion

The obtained results lead to a conclusion that in the region of Southeast Serbia the rate of biopsied patients in order to obtain accurate histological diagnosis of clinically manifested glomerular disorders is significantly lower than in European countries. Also, the age distribution of the biopsied patients is significantly different compared to western European countries due to a small number of renal biopsies in children and in elderly. Primary proliferative forms of glomerular disease is a dominant finding in the study group. The frequency of respective histopathologic findings significantly correlated with data from studies that we used for comparison, with the exception of minimal change disease whose incidence in our study is lower. These data are a significant contribution to the epidemiological analisys of glomerular disease in Serbia and represent a database for comparison and improvement of renal diseases treatment.

REFERENCES

1. *Churg J, Bernstein J, Glassock JR.* Renal disease, classification and atlas of glomerular disease. New York: Igaku-Shoin; 1995.
2. *Marcussen N, Olsen S, Larsen S, Starklint H, Thomsen OF.* Reproducability of the WHO classification of glomerulonephritis. Clin Nephrol 1995; 44(4): 220−4.
3. *Rivera F, Lopez-Gomez JM, Perez-Garcıa R.* Frequency of renal pathology in Spain 1994–1999. Nephrol Dial Transplant 2002; 17(9): 1594–602.
4. *Schena FP.* Survey of the Italian Registry of Renal Biopsies.Frequency of the enal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. Nephrol Dial Transplant 1997; 12(3): 418–26.
5. *Simon P, Ramée MP, Autuly V, Laruelle E, Charasse C, Cam G, Ang KS.* Epidemiology of primary glomerular diseases in a French region. Variations according to period and age. Kidney Int 1994; 46(4): 1192−8.
6. *Rychlík I, Jancová E, Tesar V, Kolsky A, Lácha J, Stejskal J,* et al. The Czech registry of renal biopsies. Occurrence of renal diseases in the years 1994–2000. Nephrol Dial Transplant 2004; 19(12): 3040–9.
7. *Briganti EM, Dowling J, Finlay M, Hill PA, Jones CL, Kincaid-Smith PS,* et al. The incidence of biopsy-proven glomerulonephritis in Australia. Nephrol Dial Transplant 2001; 16(7): 1364–7.

8. *Covic A*, *Schiller A*, *Volovat C*, *Gluhovschi G*, *Gusbeth-Tatomir P*, *Petrica L*, et al. Epidemiology of renal disease in Romania: a 10 year review of two regional renal biopsy databases. Nephrol Dial Transplant 2006; 21(2): 419−24.
9. *Naumovic R, Pavlovic S, Stojkovic D, Basta-Jovanovic G, Nesic V*. Renal biopsy registry from a single centre in Serbia: 20 years of experience. Nephrol Dial Transplant 2009; 24(3): 877–85.
10. *Malafronte P, Mastroianni-Kirsztajn G, Betônico GN, Romão JE Jr, Alves MA, Carvalho MF, et al.* Paulista registry of glomerulonephritis: 5-year data report. Nephrol Dial Transplant 2006; 21(11): 3098–105.
11. *Research Group on Progressive Chronic Renal Disease*. Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1,850 biopsied cases. Nephron 1999; 82(3): 205–13.
12. *Heaf J, Okkegaard H, Larsen S*. The epidemiology and prognosis of glomerulonephritis in Denmark 1985–1997. Nephrol Dial Transplant 1999; 14(8): 1889–97.
13. *Werner T, Brodersen, HP, Janssen U*. Analysis of the spectrum of nephropathies over 24 years in a West German center based on native kidney biopsies. Med Klin (Munich) 2009; 104(10): 753−9.
14. *van Paassen P, van Breda Vriesman PJ, van Rie H,Tervaert JW*. Signs and symptoms of thin basement membrane nephropathy: A prospective regional study on primary glomerular disease – The Limburg Renal Registry. Kidney Int 2004; 66(3): 909–13.
15. *Choi IJ, Jeong HJ, Han DS, Lee JS, Choi KH, Kang SW, et al.* An analysis of 4,514 cases of renal biopsy in Korea. Yonsei Med J 2001; 42(2): 247–54.
16. *Dimitrijević J, Dukanović L, Kovacević Z, Bogdanović R, Maksić D, Hrvacević R,* et al. Lupus nephritis: histopathologic features, classification and histologic scoring in renal biopsy. Vojnosanit Pregl 2002; 59(6 Suppl): 21−31.

Received on June 14, 2011.
Revised on October 11, 2011.
Accepted on January 25, 2012.
OnLine-First May, 2013.

ORIGINAL ARTICLE

UDC: 616.65-08
DOI: 10.2298/VSP110620029A

Does the addition of *Serenoa repens* to tamsulosin improve its therapeutical efficacy in benign prostatic hyperplasia?

Da li dodavanje *Serenoa repens* tamsulosinu poboljšava njegovu terapeutsku efikasnost kod benigne hiperplazije prostate?

Aleksandar Argirović*, Djordje Argirović†

***Department of Urology, Clinical Hospital Center Zemun, Belgrade, Serbia;**
†Outpatient Urology Clinic "Argirović", Belgrade, Serbia

Abstract

Background/Aim. It has been observed that a large number of patients with low urinary tract symptoms due to benign prostatic hyperplasia (LUTS/BPH)) has been treated with a combination of tamsulosin (TAM) + *Serenoa repens* (SR) (TAM + SR). The aim of this study was to compare a combination TAM + SR with TAM and SR alone, to see if there was any difference in efficacy and tolerance of each in patients with LUTS/BPH. **Methods.** In this prospective study patients had to have prostate volume (PV) < 50 mL, International Prostate Symptom Score (IPSS) of 7–18, Quality of Life score (QoLs) > 3, a maximal flow rate (Qmax) of 5–15 mL/s, with post voiding residual volume (PVR) < 150 mL and serum prostatic antigen (PSA) < 4 ng/mL. TAM (0.4 mg) was administered once a day, SR (320 mg) daily or SR (320 mg) + TAM (0.4 mg) daily for a median period of 6 months. **Results.** A total of 297 patients were recruited, whereas 265 patients were fully available: 87 into the group TAM, 97 into the group SR and 81 into the group TAM + SR. There was no statistically significant difference between the treatment groups in the sense of demographic and other baseline parameters. No difference was found among the 3 treatment groups, neither in the major endpoint of the study in the sense of a change between baseline and final evaluation in total IPSS, obstructive and irritative subscores, improvement of QoLs, increase in Qmax, nor for the second endpoint including diminution of PV, PSA and PVR. During the treatment period 20 (23%) of the patients managed with TAM and 17 (21%) with TAM + SR had drug- treated with related adverse reactions. No adverse effect was detected in the group SR. **Conclusion.** Treatment of BPH by both SR and TAM seems to be efficacious alone. None of them had superiority over another and, additionally, a combined therapy (TAM + SR) does not provide extra benefits. Furthermore, SR is a well-tolerated agent that can be used alternatively in the treatment of LUTS/BPH.

Key words:
prosthatic hyperplasia; adrenergic alpha-antagonists; phytotherapy; treatment outcome.

Apstrakt

Uvod/Cilj. Uočeno je da veliki broj bolesnika sa simptomima od strane donjih partija urotrakta izazvanih benignom hiperplazijom prostate (SDPU/BHP) ima terapiju sa kombinacijom tamsulosina (TAM) + *Serenoa repens* (SR) (TAM + SR). Ova studija imala je za cilj da uporedi kombinaciju TAM + SR sa samo TAM i SR, da bi se videlo da li postoji razlika između njih u pogledu efikasnosti i podnošljivosti kod bolesnika sa SDPU/BHP. **Metode.** U ovoj prospektivnoj studiji bolesnici su imali volumen prostate (VP) < 50 mL, internacionalni prostata simptom skor (IPSS) 7–18, ocenu kvaliteta života (KŽ) > 3, maksimalni protok urina (MPU) 5–15 mL/s, sa volumenom rezidualnog urina (RU) < 150 mL i prostata specifičnim antigenom (PSA) < 4 ng/mL. TAM (0,4 mg) je bio primenjivan jedan put dnevno, SR u dozi od 320 mg dnevno, a kombinacija SR (320 mg) + TAM (0,4 mg) dnevno za prosečni period od 6 meseci. **Rezultati.** Ukupno 297 bolesnika bilo je uključeno u studiju, s tim da je 265 bolesnika bilo dostupno potpunoj proceni: 87 u TAM grupi, 97 u SR grupi i 81 u TAM + SR grupi. Nije bilo statistički značajne razlike između grupa u pogledu demografskih i drugih parametara pri početnoj proceni. Takođe, nije bilo razlike između tri grupe, kako u pogledu primarnog cilja studije, promene između početne i završne ocene ukupnog IPSS, opstruktivnom i nadražujućem supskoru, poboljšanju KŽ, povećanju MPU, kao ni u pogledu sekundarnog cilja studije uključujući smanjenje VP, PSA i RU. Tokom lečenja, 20 (23%) bolesnika lečenih TAM i 17 (21%) bolesnika na kombinaicji TAM + SR imali su neželjene reakcije. Nije bilo neželjenih sporednih efekata u grupi SR. **Zaključak.** Lečenje BPH sa SR i TAM je podjednako efikasno. Ni jedan od tretmana nema superiornost u odnosu na drugi i dodatno, kombinovana terapija (TAM + SR) ne doprinosi dodatnom poboljšanju efikasnosti. Štaviše, izgleda da je SR dobro podnošljiv fitopreparat koji se može alternativno primeniti u lečenju SDPU/BHP.

Ključne reči:
prostata, hipertrofija; alfa blokatori; fitoterapija; lečenje, ishod.

Correspondence to: Aleksandar Argirović, Department of Urology, Clinical Hospital Center Zemun, Vukova 9, 11 080 Belgrade, Serbia. Phone: + 38162 23 66 59. E-mail: sasa_argirovic@yahoo.com

Introduction

Low urinary tract symptoms (LUTS) are frequently associated with benign prostatic hyperplasia (BPH) caused by cellular hyperplasia of both glandular and stromal elements. With an aging population the number of men affected by BPH is likely to increase [1, 2]. Symptoms severity appears to be dependent, at least in part, on smooth muscle tone in the prostate and bladder neck [3]. Since medical inhibitors, including alpha-blockers (ABs) [4], alpha-reductase inhibitors (5-ARIs) and phytotherapeutic agents offer an attractive alternative to surgery, the number of transurethral resections of the prostate has declined in recent years [5, 6]. However, the tolerability of these agents varies. Some ABs are associated with cardiovascular adverse events (AEs) (postural hypotension, dizziness, and headache) and 5-ARIs can lead to sexual dysfunction [7]. Conversely, a drug with high affinity for alpha 1A-adrenoreceptors (tamsulosin) (TAM) may be more prostate specific and may maintain the therapeutic response in the treatment of symptomatic BPH with less effect on blood pressure and fewer cardiovascular AEs [8]. In selected patients, a combination of AB and 5-ARI is the most effective form of BPH medical therapy to reduce the risk of clinical progression, i.e. acute urinary retention (AUR) and BPH-related surgery [9]. On the other hand, increasing attention has been focused on the use of phytotherapeutic agents to alleviate the symptoms of BPH. The most described and studied phytotherapeutic agent for the medical treatment of BPH is etahnolic extract of *Serenoa repens* (SR) (*Sabal serrulata*) derived from the berry of the American dwarf palm tree [10–12]. The antiandrogenic, antiproliferative and anti-inflammatory complementary activities of SR extracts could constitute an advantage over ABs to treated symptomatic BPH where both "obstruction" and "irritation" are involved.

The aim of this prospective pilot study was to test the hypothesis that the efficacy of combination TAM + SR is superior to TAM and SR alone for the relief of LUTS/BPH. The main endpoints of the study were changes in the total International Prostate Symptom Score (IPSS), Quality of a life score (QoLs), maximal flow rate (Qmax) and post voiding residual volume (PVR) from baseline to the last observation carried forward. This was applied only in naive patients suffering from LUTS/BPH without previous treatment with ABs, 5-ARIs or phytotherapy.

Methods

Between June 2008 and September 2010, 297 men aged 50–87 years, with symptomatic BPH were included in the study containing 3 regimens: TAM (Tamsol®) 0.4 mg daily (n = 98), SR (Prostamol uno®) and TAM 0.4 mg + SR 320 mg daily (n = 92), to compare the efficacy of each of these treatment regimens. All the patients signed informed consent form before any treatment. Pre-treatment procedures consisted of collection of the medical history (including urologic history), check of concomitant medications, physical examination [including digital rectal examination (DRE)], routine laboratory tests [urine analysis, urine culture, creatinine, prostate specific antigen (PSA)], total IPSS, irritative and obstructive subscores, QoLs, prostate volumen (PV), Qmax and PVR. The study was specially designed for medical treatment of patients suffering from low risk of AUR and BPH-related surgery. Inclusion criteria were men > 50 years of age, a total IPSS of 7–18, QoLs >3, Qmax of 5–15 mL/s, with PVR < 150 mL, PV < 50 mL, measured by transrectal ultrasound (TRUS) and serum PSA 1.5–4 ng/mL. TRUS-guided biopsies of the prostate were performed in patients with PSA > 4 ng/mL, abnormal DRE, and/or suspicious echogenicity on TRUS. The subjects with a significant bladder outlet obstruction (BOO) were excluded *a priori* from the study (PVR > 200 mL, Qmax < 5 mL/s). Patients were excluded from the study if they had the history of bladder disease likely to affect micturition, urethral stenosis, prostate and/or bladder cancer, bladder stone, previous pelvic radiotherapy, recurrent urinary retention, neurogenic lower urinary tract dysfunction, repeated infection of the urinary tract, chronic bacterial prostatitis, or any other disease that can cause urinary problems. Assessment visits were performed head to head and were scheduled at randomization (day 0), and latter at months 3 and 6. PV and PSA were measured at selection and at endpoint, whereas the total IPSS, obstructive and irritative subscore, Qols, Qmax and PVR were evaluated at baseline and later every 3 months. Responders were defined on the basis of IPSS and Qmax by decrease of > 25% and increase of > 30% from baseline, respectively. The patients without subjective and objective improvement were rejected from the study within 3 months from the initiation of the treatment, after both patients and physicians had agreed about that.

The Kruskal-Wallis test was used for comparison of the groups and the Wilcoxon signed-rank test for analysis of the baseline and a 6-month treatment parameters. A p value < 0.05 was considered statistically significant. Data processing was done by SPSS package for Windows version 11.0.

Results

A total of 87, 97 and 81 patients were fully available regarding the treatment regimen, according to TAM, SR and TAM + SR, respectively. The main reason for study discontinuation was voluntary withdrawal (1.3%), protocol violation (2.4%), lack of efficacy (3.03%) and other reasons (2.7%). Four (1.3%) patients were lost to follow-up (Table 1).

The treatment groups had comparable distribution in terms of age, body mass index, a total IPSS, irritative and obstructive subscores, QoLs, Qmax, PVR, PV and PSA (Table 2). The mean age was 64.9 ± 7.6 years.

After 6 months of the treatment, the mean decrease in IPSS was -4.6, -6.1 and -4.9 in the TAM, SR and TAM + SR groups respectively. The difference between IPSS values at baseline and 6 months later were significant in each group (p < 0.05). The patients in the group SR had a greater reduction in symptoms than the other group. However, statistical analysis did not reveal this expected difference between the treatment regimens (p = 0.1). This difference between the

Table 1

Reasons for premature discontinuation per the treatment group

Variable	TAM (n = 98) n (%)	SR (n = 107) n (%)	TAM + SR (n = 92) n (%)
Completed study treatments	87 (88.8)	97 (80.7)	81 (88.0)
Discontinued study treatment	11 (11.2)	10 (9.3)	11 (12)
Lost to follow-up	1 (1.02)	1 (0.9)	2 (2.2)
Discontinued due to:			
protocol violation	3 (3.06)	2 (1.8)	2 (2.2)
patient's decision	2 (2.04)	1 (0.9)	1 (1.1)
other reasons	2 (2.04)	2 (1.8)	4 (4.4)
lack of efficacy	3 (3.06)	4 (3.6)	2 (2.2)

TAM – tamsulosin; SR – *Serenoa repens*; n – number of patients.

Table 2

Demographic and other baseline parameters ($\bar{x} \pm SD$)

Parameters	TAM (n = 87)	SR (n = 97)	TAM + SR (n = 81)	*p*
Age (years)	56.8 ± 7.7	59.2 ± 7.8	65.9 ± 7.4	0.27
Body mass index (kg/m^2)	28.0 ± 3.4	26.7 ± 2.5	27.8 ± 2.3	0.40
IPSS total score	16.2 ± 4.7	18.0 ± 4.9	15.6 ± 3.2	0.21
IPSS obstructive subscore	9.0 ± 3.5	10.1 ± 4.2	8.6 ± 3.2	0.85
IPSS irritative subscore	6.4 ± 2.8	6.7 ± 3.1	6.6 ± 2.5	0.91
QoL score	3.5 ± 1.1	4.2 ± 1.2	3.5 ± 1.1	0.08
Qmax (mL/s)	10.5 ± 2.8	9.4 ± 2.9	9.9 ± 2.4	0.49
PVR (mL)	65.5 ± 33.3	67.4 ± 27.7	63.7 ± 23.7	0.76
PV (mL)	38.6 ± 11.6	35.2 ± 10.3	31.2 ± 4.2	0.07
PSA (ng/mL)	2.1 ± 0.9	1.9 ± 0.9	1.7 ± 0.7	0.41

SD – standard deviation, TAM – tamsulosin; SR – *Serenoa repens;* IPPS – International Prostate Symptom Score; QoL – Quality of life; Qmax – maximal flow rate; PVR – post-voiding residual volume; PV – prostate volume; PSA – prostate specific antigen.

groups in the mean total IPSS decrease was not observed in the irritative part -1.7, -1.8 and -1.9 ($p = 0.6$) and the obstructive part -1.5, -1.4 and -1.3 ($p = 0.5$), for TAM, SR and TAM + SR, respectively. For the QoLs, the group TAM had an initial mean score of 3.5, which decreased for 2.1; the group SR 4.2 which decreased to 2.6; the group TAM + SR had the initial mean score of 3.5 which decreased for 2.2 after 6 months. The 3 groups had lower mean score after the treatment but the difference was not significant ($p = 0.1$). Six months following the treatment, the mean increase in Qmax was similar in both TAM and SR group (3.7 mL/s for TAM, 3.2 mL/s for SR), but was slightly greater in the group TAM + SR (4.2 mL/s). The patients in each group improved flow rates and the difference between the Qmax values at baseline and 6 months later was statistical significance in each group ($p < 0.005$), although the difference was not statistically significant among groups with regard to increase in Qmax values ($p = 0.3$). The improvement of PVR volume was not statistically different among the groups which decreased by 29.6, 28.1 and 25.4 mL, respectively ($p = 0.4$). Six months following the treatment the mean PV had decreased by 1.0, 0.7 and 0.8 mL, respectively. The difference was not significant ($p = 0.6$). The decrease in PSA was more pronounced in the groups SR, but the difference was not statistically significant ($p = 0.25$) (Table 3).

Table 3

Mean changes and ameliration rate in efficacy parameters from baseline to endpoint of the study

Parameters	TAM (n = 87)	SR (n = 97)	TAM + SR (n = 81)	*p*
IPSS total score, $\bar{x} \pm SD$ [amelioration rate (%)]	- 4.6 ± 3.3, (28.4)	- 6.1 ± 2.7, (33.9)	- 4.9 ± 2.3, (31.4)	0.1
IPSS obstructive subscore, $\bar{x} \pm SD$ [amelioration rate (%)]	-1.5 ± 2.4, (16.7)	-1.4 ± 3.1, (13.8)	-1.3 ± 2.8, (15.1)	0.5
IPSS irritative subscore, $\bar{x} \pm SD$ [amelioration rate (%)]	-1.7 ± 2.8, (26.6)	-1.8 ± 0.9, (26.9)	-1.9 ± 9.4, (28.8)	0.6
QoL score, $\bar{x} \pm SD$ [amelioration rate (%)]	- 2.1 ± 0.8, (60)	-2.6 ± 0.9, (61.9)	- 2.2 ± 1.0, (62.9)	0.1
Qmax (mL/s), $\bar{x} \pm SD$ [amelioration rate (%)]	+3.7 ± 2.6, (35.2)	+3.2 ± 2.2, (34.1)	+4.2 ± 2.5, (42.4)	0.3
PVR (mL), $\bar{x} \pm SD$ [amelioration rate (%)]	- 23.6 ±20.2, (36.0)	-28.1 ± 22.6, (41.7)	-25.4 ± 14.8, (39.9)	0.4
PV (mL), $\bar{x} \pm SD$ [amelioration rate (%)]	- 1.0 ± 0.6, (2.6)	-0.7 ± 0.1, (2.0)	- 0.8 ± 0.3, (2.6)	0.6
PSA (ng/mL), $\bar{x} \pm SD$ [amelioration rate (%)]	- 0.1 ± 0.2, (4.8)	-0.3 ± 1.4, (15)	- 0.25 ± 0.2, (14.7)	0.25

SD – standard deviation, TAM – tamsulosin; SR – *Serenoa repens;* IPPS – International Prostate Symptom Score; QoL – Quality of life; Qmax – maximal flow rate; PVR – post-voiding residual volume; PV – prostate volume; PSA – prostate specific antigen.

At endpoint, the percentage of patients negatively affected by urinary symptoms (feeling mostly dissatisfied, unhappy, and terrible) was reduced by > 50% in the groups TAM, SR and TAM + SR (from 66.7% to 34.5%, from 63.1% to 34.7% and from 64.2% to 22.7%, respectively) ($p < 0.001$) (Table 4).

During a 6-month treatment period, 20 (23%) of the patients managed with TAM and 17 (21%) patients with TAM + SR, had some degree of drug related AEs. For most of these patients (79%) the AEs were mild. The most frequently reported AEs were reduced or absent ejaculations during orgasm and a headache. The mean improvement of total IPSS was greater in the men experiencing ejaculatory disorders (10.7%) than in those who did not (-7.3 ± 3.3 *vs* -6.1 ± 2.3) ($p = 0.04$) but not regarding Qmax (-4.0 ± 2.3 *vs* -3.4 ± 2.5) ($p = 0.07$). A headache was reported by a slightly higher percentage of subjects in the group TAM + SR (6.1%) compared to the group TAM (5.9%), but without statistically significant difference. However, these AEs did not result in withdrawal from the study. No AE, were detected in the group SR (Table 5).

oica), the 5-ARI (finasteride), or AB (TAM). The mean follow-up of these trials varied between 4 and 60 weeks. The Cochrane report concluded that SR was not superior to placebo, finasteride, or TAM with regard to IPSS improvement, increase in Qmax or prostate size reduction. For nocturia SR was significantly better than placebo (mean weight difference -0.78) [14].

Direct comparative randomized controlled trials have shown the superior efficacy of ABs over placebo, whereas the combination of 5-ARI and an AB was more effective than the AB alone [15]. Although the combination of an AB and SR was frequently used in some European countries, including Serbia, at the time of preparing this study, its superior efficacy over AB and SR alone had not been fully investigated. Therefore, this question was addressed to direct comparative trial.

The results of our study demonstrate that TAM and SR are equivalent to a combination TAM + SR in the management of these patients. After 6 months, all the treatment groups induced practically the same mean reduction in total IPSS (-4.6 *vs* -6.1 *vs* -4.9 points) with 2/3 of men responding

Table 4

Summary of quality of life scores related to urinary simptoms

Variable	TAM (n = 87) n (%)	SR (n = 97) n (%)	TAM + SR (n = 81) n (%)
Baseline			
Delighted, pleased or mostly satisfied	7 (8.0)	9 (8.1)	7 (8.6)
Mixed: about equally satisfied and unsatisfied	22 (25.3)	28 (28.6)	22 (27.2)
Mostly dissatisfied, unhappy or terrible	58 (66.7)	60 (63.1)	52 (64.2)
Endpoint			
Delighted, pleased or mostly satisfied	38 (43.7)	42 (44.2)	36 (44.4)
Mixed: about equally satisfied and unsatisfied	19 (21.8)	22 (23.2)	21 (25.9)
Mostly dissatisfied, unhappy or terrible	30 (34.5)	33 (34.7)	24 (22.7)

n – number of patients; TAM – tamsulosin.

Table 5

Adverse events summary

Parameters	TAM (n = 87)	SR (n = 97)	TAM + SR (n = 81)
Any, n (%)	67 (77.0)	97 (100)	64 (79.0)
Rhinitis, n	1	–	–
Fatigue, n	1	–	–
Dizziness, n	1	–	1
Postural hypotension, n	1	–	1
Dry mouth, n	1	–	1
Libido decrease, n	1	–	1
Ejaculation disorders, n	10	–	8
Headache, n	4	–	5
Total adverse events, n (%)	20 (23.0)	–	17 (21.0)

TAM – tamsulosin; SR – *Serenoa repens*; n – number of patients.

Discussion

The use of phytotherapy in treating LUTS/BPH has been popular in Europe for many years and has recently spread in the USA. In some studies the efficacy of SR was found to be equivalent to 5-ARI and Abs [12, 13]. However, recently updated Cochrane report summarized the clinical results of 30 randomized trials comprising 5.222 men. SR was compared as mono or combination preparations either with placebo, other plant extracts (*Pygeum africanum*, *Ustica di-*

to the treatment by a decrease of 3 points or more. For all the treatment groups the mean percentage change from baseline and after 6- months was similar (28.4% for TAM, 33.9% for SR and 31.4% for TAM + SR). This data correlates with the results reported in other series [14, 16]. However, the difference in total IPSS in TAM *vs* the group TAM + SR was slightly higher in the study of Glemain et al. [17] (-5.2 *vs* -6.0). The greatest improvement in total IPSS was observed in those patients with greatest severity of disease [18]. No differences were observed among the treatment groups from baseline to

endpoint of the study in terms of irritative and obstructive symptoms, corresponding with data providing from other studies [16, 17].

We reported the improvement in QoLs of -2.1, -2.6 and -2.2 in each group. However, the improvement of QoLs was lower for TAM *vs* TAM + SR, -1.0 *vs* -1.3, in the study reported by Glemain et al. [17].

In the present study, the mean increase in Qmax (3.7 mL/s, 3.2mL/s and 4.2 mL/s) strongly correlates with data providing from Hizli and Uygur [16], whereas mean changes of 1.3mL/s (TAM) and 1.2mL/s (TAM+SR) are reported in other study [17].

Limited studies have evaluated PVR in measuring the response to treatment [16]. We measured PVR to assess the efficacy of treatment regimens and found a mean decrease of 23.6 mL, 28.1 mL and 25.4 mL, respectively.

We found that the addition of SR has no significant effect on PSA levels, consistent with earlier results [19]. In fact, decreasing PSA would not be a desire result of a BPH medication, because it may mask or delay the detection of prostatic carcinoma. This is in contrast with 5-ARI [15].

PV was found to be decreased by SR in 3 uncontrolled stdies [20–22], but this was not confirmed in controlled studies [10, 11].We found the mean decrease in PV of -1.0 mL, -0.7 mL and 0.8 mL for the groups TAM, SR and TAM + SR, respectively, but they were not statistically significant. TAM efficacy does not depend on prostate size and is similar across age group. However, TAM does not reduce prostate size [23].

The occurrence of AEs was similar in the groups TAM and TAM + SR (23% *vs* 21%). Retrograde ejaculation was the most common TAM related AE (10.7%). The mean improvement of IPSS was greater in the men experiencing this AE than in the men who did not. For the older BPH patients who experienced retrograde ejaculation, it might be a small trade for the rapid and significant relief of urinary symptoms that treatment with TAM offered. Retrograde ejaculation is a characteristic AE of ABs with the occurrence in 4%–11% of patients and that has been shown to be reversible after administration of the drug has been stopped [24]. In short, our results confirm that AEs are commonly associated with TAM, whereas SR is a well-tolerated agent used for LUTS/BPH.

The best of our knowledge shows that only one study has compared TAM and SR alone with combination of TAM +S R in treatment of LUTS/BPH [16, 17]. The number of 60 patients included in this study (20 in each group) with follow-up of 6 months, is too small to be absolutely confident about these results. The OCOS trial included 329 patients managed with TAM (n = 161) and TAM + SR (n = 168) with the mean follow-up of 52 weeks. No statistically significant difference was found between these groups, neither for the change in IPSS between the baseline and final evaluation, nor for the improvement of the irritative and obstructive subscore, QoLs and Qmax. However, the group SR was not included in this study [17].

Although the efficacy of the 3 treatment groups can only be reliably determined with placebo-controlled studies, clinically relevant information can still be gained from comparative trials, and for this reason a placebo group was not included in the present study. The follow-up was relatively short (6 months) in comparison to 12 months in other trials [11, 14, 17]. There is also a financial implication with the use of SR because reimbursement of cost by health insurance in Europe, including Serbia, is not contemplated. However, trials exploring efficacy of new AB silodosin have a follow-up of only 3 months [25, 26]. We investigate currently the combination of AB with phytotherapeutical agent isoflavone extracted from red clover (*trifolium pretense*) during the treatment period of 3 months in patients with mild and moderate symptomatic BPH.

Overall, it appears that phytotherapy with SR is as valid pharmacotherapy as Abs in management of men with LUTS/BPH. Indeed, it may have less adverse effects and be better tolerated. What is certain is that urologist should be aware and informed about phytotherapy as it inevitably becomes part of the standard medical therapy for men with LUTS/BPH.

Conclusion

We find that treatment of BPH with both TAM and SR alone seems to be equally effective in reducing urinary obstruction, in proving symptomatology and QoLs, whereas a combined therapy (TAM + SR) does not provide extra benefits. Furthermore, SR is a well-tolerated agent that can be used alternatively in treatment of LUTS/BPH. The limitation of our study is a relatively short follow-up. Large prospective randomized studies with longer follow-up periods are needed to clarify more the efficacy of SR in treatment of BPH.

REFERENCES

1. *Wellch G, Weinger K, Barry MJ.* Quality- of- life impact of lower urinary tract symptom severity: results from Health Professionals Follow-up Study. Urology 2002; 59(2): 245−50.
2. *Fitzpatrick JM.* The natural history of benign prostatic hyperplasia. BJU Int 2006; 97(Suppl 2): 3−6.
3. *Anderson KE, Gratzke C.* Pharmacology of alpha1-adrenoreceptor antagonist in the lower urinary tract. Nat Clin Pract Urol 2007; 4(7): 368−78.
4. *Madersbacher S, Alivizatos G, Nordling J, Rioja Sans C, Emberton M, de la Rosette JJ.* EAU 2004 guidelines on assessment, therapy and follow-up of men with lower urinary tract symptoms suggestive of benign prostatic obstruction (BPH guidelines). Eur Urol 2004; 46(5): 547−54.
5. *Djavan B.* Lower urinary tract symptoms/ benign prostatic hyperplasia: fast control of the patient's quality of life. Urology 2003; 62 (3 Suppl 1): 6−14.
6. *Chacon A, Monga M.* Medical management of benign prostatic hyperplsia. Geriatr Nephrol Urol 1999; 9(1): 39−48.
7. *Clifford GM, Farmer RD.* Medical therapy for benign prostate hyperplasia. A review of the literature. Eur Urol 2000; 38(1): 2−19.
8. *Chapple CR.* Pharmacological therapy of benign prostatic hyperplasia/lower urinary tract symptoms: an overview for the practicing clinician. BJU Int 2004; 94(5): 738−44.

9. *Roehborn CG.* BPH progression: concept and key learning from MTOPS, ALTESS, COMBAT and ALF-ONE. BJU Int 2008; 101(Suppl 3): 17−21.
10. *Boyle P, Robertson C, Lowe F, Roehborn C.* Update meta-analysis of clinical trials of Serenoa repens extract in the treatment of asymptomatic benign prostatic hyperplasia. BJU Int 2004; 93(6): 751−6.
11. *Willets KE, Clements MS, Champion S, Eschman S, Eden JA.* Serenoa repens extracts for benign prostate hyperplasia: a randomized controlled trial. BJU Int 2003; 92(3): 267−70.
12. *Lowe FC.* Phytotherapy in the management of benign prostatic hyperplasia. Urology 2001; 58(6 Suppl 1): 71−6; discussion 76−7.
13. *Debruyne F, Koch G, Boyle P, Da Silva FG, Gillenwater JG, Hamdy FC,* et al. Comparison of a phytotherapeutic agent (Permixon) with an alpha blocker (Tamsulosin) in the treatment of benign prostatic hyperplasia. A 1-year randomized international study. Eur Urol 2002; 41(5): 497−506; discussion 506−7.
14. *Wilt T, Ishani A, Mac Donald R.* Serenoa repens for benign prostatic hyperplasia. Cochrane Database Syst Rev 2002; (3): CD001423.
15. *Roehrborn CG, Siami P, Barkin J, Damião R, Becher E, Miñana B,* et al. The influence of baseline parameters on changes in international prostate symptom score with dutasteride, tamsulosin, and combination therapy among men with symptomatic benign prostatic hyperplasia and an enlarged prostate: 2-year data from the CombAT study. Eur Urol 2009; 55(2): 461−71.
16. *Hizli F, Uygur CM.* A prospective study of the efficacy of Serenoa repens, tamsulosin and Serenoa repens plus tamsulosin treatment for patients with benign prostate hyperplasia. Int Urol Nephrol 2007; 39(3): 879−86.
17. *Glemain P, Coulange C, Billebaud T, Gattegno B, Muczynski R, Loeb G.* Tamsulosin with or without Serenoa repens in benign prostatic hyperplasia: the OCOS trial. Prog Urol 2002; 12(3): 395−403; discussion 404. (French)
18. *Debruyne F, Boyle P, Calais Da Silva F, Gillenwater JG, Hamdy FC, Perrin P,* et al. Evaluation of the clinical benefit of Permixon and Tamsulosin in severe BPH patients-PERMAL study subset analysis. Eur Urol 2004; 14(3): 326−31. (French)
19. *Gerber GS, Zgaja GP, Bales GT, Chodak GW, Contreras BA.* Saw palmetto (Serenoa repens) in men with lower urinary tract symptoms: effects on urodynamic parameters and voiding symptoms. Urology 1998; 51(6): 1003–7.
20. *Braeckman J.* The extract of serenoa repens in the treatment of benign prostatic hyperplasia: a multicenter study. Curr Ther Res 1994; 55: 776−85.
21. *Romicz I, Schmitz H, Frang D.* Experience in treating benign prostatic hypertrophy with Sabal serrulata for one year. Int Urol Nephrol 1993; 25(6): 565−9.
22. *Kondás J, Philipp V, Diószeghy G.*. Sabal serulata (Strogen forte) in the treatment of symptomatic benign prostatic hyperplasia. Int Urol Nephrol 1996; 28(6): 767−72.
23. *Wilt TJ, Mac Donald R, Rutks J.* Tamsulosin for benign prostatic hyperplasia. Cochrane Data Base Syst Rev 2003; (1) CD002081.
24. *van Dijk MM, de la Rosette JJ, Michel MC.* Effects of alpha1-adrenoeceptor antagonist in male sexual function. Drugs 2006; 66(3): 287−301.
25. *Chapple CR, Montorsi F, Tammella TL, Wirth M, Koldewiju E, Fernández Fernández E.* Silodosin therapy for lower urinary tract symptoms in men with suspected benign prostatic hyperplasia: Results of an international, randomized, double-blind, placebo- and active-controlled clinical trial performed in Europe. Eur Urol 2011; 59(3): 342−52.
26. *Marks LS, Gittelman MC, Hill LA, Volinn W, Hoel G.* Rapid efficacy of the highly selective alpha1A-adrenoceptor antagonist silodosin in men with signs and symptoms of benign prostatic hyperplasia: pooled results of 2 phase 3 studies. J Urol 2009; 181(6): 2634−40.

Received on June 20, 2011.
Revised on November 24, 2011.
Accepted on November 30, 2011.
OnLine-First June, 2013.

ORIGINAL ARTICLE

UDC: 613.99::618.11-006
DOI: 10.2298/VSP110629030G

Risk factors for epithelial ovarian cancer in the female population of Belgrade, Serbia: A case-control study

Faktori rizika od epitelijalnog karcinoma ovarijuma u ženskoj populaciji Beograda

Tatjana Gazibara*, Aleksandra Filipović†, Vesna Kesić‡, Darija Kisić-Tepavčević*, Tatjana Pekmezović*

***Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Public Health Center "Dr Simo Milošević", Belgrade, Serbia; ‡Department of Gynecology and Obstretics, Clinical Center of Serbia, Belgrade, Serbia**

Abstract

Background/Aim. Ovarian cancer (OC) comprises 3% of all cancers, but it is the fifth most common cause of cancer death in women. The aim of this case-control study was to determine the risk factors for OC in the female population of Belgrade, Serbia. **Methods.** A total of 80 consecutive patients were enrolled in the study between 2006 and 2008 in two national referral centers for OC in Serbia. The control subjects were recruited during the regular gynecological check-ups in the Public Health Center of the corresponding municipalities. All the study participants were interviewed during their visits to the above mentioned institutions by two physicians using the same questionnaire. In order to analyze the influence of specific exposure to the risk of the disease, we categorized variables according to the cut-off values. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated separately for each variable using univariate conditional logistic regression analysis. **Results.** There were no statistically significant differences in educational level, years of schooling, occupational and employment status between patients with OC and women in the control group. Oral contraceptives use and other contraceptive methods (condoms, mechanical contraceptive devices) were highly statistically significantly more frequent among women in the control group (OR = 0.2, 95% CI 0.1–0.7, $p = 0.005$; OR = 0.1, 95% CI 0.01–0.5, $p = 0.001$, respectively). The patients with OC practiced sports for 6.3 ± 2.1 years, and controls for 11.8 ± 9.9 years. Sport and recreation activities were statistically significantly protective (OR = 0.2, $p = 0.011$; OR = 0.4, $p = 0.019$). Tea consumption on daily basis had a highly statistically significat protective effect (OR = 0.3, $p = 0.001$). **Conclusions.** Oral contraceptives use and physical activity were independent protective factors for OC in this study.

Key words:
ovarian neoplasms; risk factors; motor activity; contraceptive agents.

Apstrakt

Uvod/Cilj. Karcinom ovarijuma čini 3% od svih karicnoma, i zauzima 5. mesto među najčešćim uzrocima smrti od karcinoma kod žena. Cilj ove studije bio je da se utvrde faktori rizika od nastanka epitelijalnog karcinoma ovarijuma u ženskoj populaciji Beograda. **Metode.** Studija je izvedena u periodu od 2006. do 2008. godine. Ukupno 80 bolesnica iz dva tercijarna centra u Beogradu sa dijagnozom epitelijalnog karcinoma ovarijuma uključeno je u studijsku grupu. Kontrolnu grupu činile su žene koje su dolazile na redovne godišnje ginekološke preglede u domove zdravlja opština u kojima su živele. Podatke su prikupljala dva lekara putem upitnika. U cilju procene uticaja specifične izloženosti riziku od pojave bolesti, varijable su kategorisane prema graničnim (*cut-off*) vrednostima. Unakrsni odnos (*odds ratio* – OR) i 95% interval poverenja (95% IP) izračunavani su za svaku varijablu pojedinačno korišćenjem univarijantne logističke regresije. **Rezultati.** Između grupe bolesnica i kontrole grupe nije postojala statistički značajna razlika u nivou obrazovanja, dužini školovanja, zanimanju i zaposlenosti. Oralni kontraceptivi i druge metode kontracepcije (prezervativi, mehanička kontraceptivna sredstva) bili su statistički visokoznačajno češće korišćeni među ženama u kontrolnoj grupi nego kod bolesnica sa ovarijalnim karcinomom (OR = 0,2; 95% IP 0,1–0,7; $p = 0,005$ za oralne kontraceptive; OR = 0,1, 95% IP 0,01–0,5 $p = 0,001$ za druge metode kontracepcije). Ispitanice sa ovarijalnim karcinomom bavile su se sportom u proseku 6,3 ± 2,1 godine, dok je za one iz kontrolne grupe taj period u proseku iznosio 11,8 ± 9,9 godina. Sport i rekreacija imali su statistički značajan zaštitni efekat na nastanak ovog tumora (OR = 0,2, $p = 0.011$; OR = 0,4, $p = 0,019$, redom). Statistički visokoznačajan zaštitni efekat imala je dnevna konzumacija čaja (OR = 0,3, $p = 0,001$). **Zaključak.** Upotreba oralnih kontraceptiva i fizička aktivnost bili su nezavisni zaštitni faktori od nastanka epitelijalnog karcinoma ovarijuma u našoj studiji.

Ključne reči:
jajnik, neoplazme; faktori rizika; motorna aktivnost; kontrola rađanja, sredstva.

Correspondence to: Tatjana Pekmezović, Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Visegradska 26A, Belgrade 11000, Serbia. Phone: +381 11 3607 062. E-mail: pekmezovic@sezampro.rs

Introduction

Ovarian cancer (OC) comprises 3% of all cancers, but it is the fifth most common cause of cancer death in women [1]. The 5-year relative survival rate ranges from 30% to 45%, without significant improvement in the past years even though the new methods in therapy have been used [2–4]. The highest incidence rates of OC have been registered in Scandinavia, Eastern Europe and Canada, where it varies between 10 and 15 per 100,000 women [5]. The lowest ones were found in Asia (excluding Japan) and Africa, with less than 5 per 100,000 women [6]. In Serbia, the standardized incidence rate in 1999 was 9.7 per 100.000, while in 2005 it rose up to 11.5 per 100.000 [7].

Risk factors for OC are still not well established. Age, family history of OC, infertility treatment and assisted fertilization, hormonal substitution in menopause, and obesity are potential factors in favor of developing the OC [8, 9]. Also, it has been noted that nulliparous women have an increased risk for OC [8, 10]. On the other hand, it has been well defined and quantified that the use of oral contraceptives decreases the risk [6] as well as multiple pregnancies. In addition, tubal ligation, hysterectomy and lactation are found to be protective factors, too [11].

Genetic factors also play an important role in the etiology of this tumor. The mutation of genes BRCA-1 and BRCA-2 in 17q are identified in two separate types of hereditary carcinomas. Another hereditary type of OC is found in cancer family syndrome Lynch type 2 [12]. As for environmental factors, it has been suspected that talc and asbestos may influence the onset of the disease, due to the fact that the highest incidence rates occur in highly industrialized countries. Some migrant studies have shown that when women from an undeveloped or developing country move to industrialized country develop OC [10], while certain case-control studies pointed out that high intake of animal fat, alcohol and smoking may increase the risk [13].

Regarding burden of OC in our country, official data revealed it as the 7th most frequent cause of cancer-related death as well as the 2nd most common cause in gynecological cancer deaths in the Serbian female population [7]. Furthermore, recent investigation showed statistically significant increase in OC mortality trend during the period 1976–2007 [14]. Thus, the aim of this study was to determine the risk factors for OC in the population of Belgrade (Serbia).

Methods

This case-control study included 80 consecutive patients treated and followed in the Department of Gynecology and Obstetrics of the Clinical Center of Serbia and the Clinic of Gynecology and Obstetrics "Narodni Front" in Belgrade, between 2006 and 2008. Both of these hospitals are the national referral centers for OC in Serbia. All cases resided on the territory of Belgrade and had histologically verified diagnose of epithelial OC. The control group consisted of 160 women, double matched according to age (± 2 years) and municipality of residence. For the study period, the control subjects were recruited during regular gynecological check-ups in the Public Health Center in corresponding municipalities. These women had no malignant tumors and/or hormone-dependent problems. All women, in both groups, signed the informed consent for the participation in the study. The research was approved by the Institutional Review Committee. Written informed consents were obtained from the study participants.

All the study participants were interviewed during their visits to the above mentioned institutions by two physicians using the same questionnaire to collect demographic information, as well as information regarding personal and family history, lifetime residence, particular lifestyle (smoking, alcohol and coffee intake), occupational exposures to radiation and chemicals, as well as reproductive history. Smokers were defined as persons who reported everyday smoking during a 60-day period prior to completing the questionnaire. To assess sport and recreation activities participants were asked if they do moderate activities for at least 10 minutes at a time, such as brisk walking, cycling, swimming, or any other activity that causes some increase in breathing or heart rate. In order to analyze the influence of specific exposure to the risk of the disease, we categorized variables according to the cut-off values. These values were determined based on the mean (± SD) level of variables investigated in the control group. All questions were referring to the 5-year period prior to the diagnosis or the corresponding period for the controls. This was supplemented and validated by an examination of the medical records.

Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated separately for each variable using univariate conditional logistic regression analysis. Variables that were related to OC at a significant level of $p < 0.05$, entered the final model of multivariate conditional logistic regression analysis to evaluate their independent contribution to the overall risk of OC.

Results

The average age of women in the study and the control groups was 56.1 ± 10.8 years and 56.7 ± 10.6 years, respectively.

Average income in patients was 245.52 ± 10.2 euros as opposed to the control group, where it was around 534 ± 146.5 euros. Lower family income (≤ 250 euros a month) was statistically significant risk factor for OC, with OR = 2.5 (95% CI 1.4–4.3), $p = 0.001$. Also, the living space in the study group it was in average 56.5 ± 23.0 m² while in the control group was 64.3 ± 67.3 m², thus the calculated OR was 2.0 (95% CI 1.1–3.6), $p = 0.018$, which was statistically significant.

The characteristics of OC cases and controls regarding education level and occupational history are presented in Table 1. There were no statistically significant differences in educational level, years of schooling, occupational and employment status between patients and the controls.

Considering the existing major chronic diseases in both study groups, occurrence of cardiovascular (OR = 2.0, 95%

Table 1

Characteristics of the ovarian cancer cases and the controls regarding education level and occupational history

Variable	Cases (n = 80) n	Controls (n = 160) n	OR (95% CI)	*p*
Education				
no/incomplete primary school	4	1	0.6	0.064
primary school	8	10	(0.3–1.1)	
manufacturer	4	9		
secondary school	32	95		
superior school	20	19		
university	12	26		
Schooling (years)				
≤ 12	48	109	0.7	0.161
≥ 13	32	51	(0.9–1.2)	
Occupation				
housewife	13	11	0.9	0.762
worker	24	65	(0.5–1.7)	
administrator	24	48		
professional	19	36		
Employment status				
employed	28	69	0.7	0.227
unemployed/retired	52	91	(0.4–1.2)	
Duration of employment (years)				
≤ 27	29	59	1.1	0.787
≥ 28	45	99	(0.6–1.9)	

OR – odds ratios; CI – confidence intervals.

CI 1.1–3.4, $p = 0.015$), gynecological (OR = 2.2, 95% CI 1.2–4.2, $p = 0.015$) and other chronic diseases (degenerative rheumatic diseases and chronic obstructive pulmonary disease) was found statistically significantly more frequent in women with OC (OR = 1.9, 95% CI 1.1–3.4, $p = 0.025$). The most common benign gynecological disorders were myomas and polyps of the uterus and condylomata.

Analysis of diseases in the family history showed that only cardiovascular diseases were more frequently registered in the study group compared to the controls (OR = 2.6, 95% CI 1.5–4.1, $p = 0.001$).

There were no statistically significant differences between the OC patients and their controls in terms of mean age at first menarche (OR = 1.5, 95% CI 0.7–3.4, $p = 0.303$), lenght of menstrual cycle (OR = 1.5, 95% CI 0.6–3.7, $p = 0.388$) and its duration (OR = 1.1, 95% CI 0.5–2.8, $p = 0.757$). Statistically significant differences were not registered regarding the number of pregnancies (OR = 1.0, 95% CI 0.6–1.8, $p = 0.926$) and the number of deliveries (OR = 0.7, 95% CI 0.3–1.6, $p = 0.372$). Less duration of breastfeeding (5.7 ± 7.2 months *vs* 7.2 ± 4.9 months) was frequently observed in the OC group than in the control group (OR = 1.7, 95% CI 0.9–2.9, $p = 0.082$).

Oral contraceptives use and other contraceptive methods (condoms, mechanical contraceptive devices) was highly statistically significantly more frequent among women in the control group (OR = 0.2, 95% CI 0.1–0.7, $p = 0.005$; OR = 0.1, 95% CI 0.01–0.5, $p = 0.001$, respectively) (Table 2). Hormone replacement therapy use was more frequent in the control group without statistical significance. Hormone therapy for any other reason including infertility treatment was more frequent among the OC patients but also without a statistical significance.

Table 2

Use of oral contraceptives and hormones

Variable	Cases (n = 80) n	Controls (n = 160) n	OR (95% CI)	*p*
Use of oral contracepties			0.2	
yes	2	23	(0.1–0.7)	0.005
no	77	137		
Use of other contraceptive methods			0.1	
yes	1	23	(0.01–0.5)	0.001
no	78	135		
Use of hormonal supstitution therapy			0.8	
yes	2	5	(0.1–4.8)	0.571
no	78	155		
Hormonal treatement for any reason			1.8	
yes	12	14	(0.8–4.2)	0.151
no	68	145		
Hormonal therapy for infertility			2.9	0.074
yes	7	5	(0.9–9.5)	
no	73	152		

OR – odds ratios; CI – confidence intervals.

Women in the case group smoked for on average, 23.5 ± 8.9 years while in the control group 18.4 ± 6.8 years. The mean number of cigarettes smoked per day was 23.1 ± 6.4 in the OC group and 18.5 ± 6.4 among controls. Longer duration of smoking as well as higher amount of cigarettes (per day) were statistically significantly more frequent in the study group in comparison to the controls (Table 3). Coffee

Table 3

Smoking and coffee consumption

Variable	Cases (n = 80) n	Controls (n = 160) n	OR (95% CI)	*p*
Smoking				
yes	40	67	1.4 (0.8–2.3)	0.266
no	40	91		
Years of smoking				
21+	31	29	5.6 (2.2–14.6)	0.001
≤ 20	7	37		
Number of cigarettes smoked per day				
≥ 21	20	13	4.1 (1.7–9.9)	0.001
≤ 20	20	54		
Years of smokers for former smokers				
≤ 16	11	11	2.2 (0.6–7.8)	0.236
≥ 17	6	13		
Coffee consumption				
yes	78	140	5.6 (1.3–24.5)	0.023
no	2	20		
No. of cups per day				
≤ 3	47	75	1.3 (0.7–2.3)	0.341
≥ 2	31	65		
Years of coffee consumption				
≥ 21	47	42	3.8 (2.1–6.8)	0.001
≤ 20	28	94		
Tea consumption				
every day	13	49	0.3 (0.1–0.6)	0.001
from time to time	66	79		

***Current and former smokers; OR – odds ratios; CI – confidence intervals.**

intake was 5.6 times higher in women with OC ($p = 0.023$). Longer period of coffee consumption was also statistically more frequent in this group (OR = 3.8, $p = 0.001$). Tea consumption on daily basis had a highly statistically significant protective effect (OR = 0.3, $p = 0.001$).

Body height and body mass index (BMI) showed that women in both groups did not differ in mass at the age of 18 (56.5 ± 5.3 kg *vs* 57.5 ± 7.7 kg, $p = 0.340$), nor within a 5-year prior to their illness (71.2 ± 7.4 kg *vs* 71.0 ± 11.5 kg, $p = 0.888$). However, the average body height at the age of 18 was statistically significantly higher among the OC patients (170.8 ± 4.7 cm) compared to controls (168.4 ± 6.7 cm) ($p = 0.007$).

Sport and recreation activities of the study participants are presented in Table 4. The patients with OC practiced sports for 6.3 ± 2.1 years, and the controls for 11.8 ± 9.9 years. According to the results of our study, sport and recreation activities were statistically significantly protective (OR = 0.2, $p = 0.011$; OR = 0.4, $p = 0.019$). The women in

Table 4

Sports and recreation

Variable	Cases (n = 80) n	Controls (n = 160) n	OR (95% CI)	*p*
Practising sports				
yes	3	26	0.2 (0.1–0.7)	0.011
no	76	134		
Years of sport practise				
≥ 7	2	18	0.8 (0.1–10.0)	0.847
≤ 6	1	7		
Hours per week in sport practise				
≥ 5	1	15	0.2 (0.1–2.6)	0.222
≤ 4	2	6		
Recreational activities				
yes	8	37	0.4 (0.1–0.8)	0.019
no	71	123		
Intensity of physical activity				
quite exhausting	2	9	0.5 (0.3–1.1)	0.080
energetic	4	17		
moderate	74	132		
Grading of professional activities regarding physical effort*				
quite hard	0	10	0.3 (0.1–1.2)	0.090
hard	3	7		
average	24	63		
standing	8	25		
mostly sitting	22	51		
Number of hours *per* day seated in leisure				
≥ 4	46	61	1.8 (1.0–3.1)	0.048
≤ 3	32	75		

***Quite hard, hard vs. average, standing, mostly sitting; OR – odds ratios; CI – confidence intervals.**

the control group were practicing sports for more years and for more hours weekly than the cases, without a statistical significance.

Additionally, women in the study group were seated for 4.1 ± 3.1 h per day during their working time while for the women in the control group it was 4.6 ± 2.6 h (p = 0.366). The average time in sitting position in their leisure was 3.4 ± 1.4 h in the study group as opposed to the control group, where it was 4.0 ± 2.3 h (p = 0.033). A greater number of hours in sedentary position was statistically significantly more frequent in the study group than in the control one (OR = 1.8, 95% CI 1.0–3.1, p = 0.048).

All variables related to OC at a significant level of $p <$ 0.05 using univariate logistic regression analysis were included in the model of multivariate logistic regression analysis. According to multivariate analysis the following factors were significantly negatively related to OC: oral contraceptive use (OR = 0.1, 95% CI 0.1–0.5, p = 0.009) and recreation activities (OR = 0.3, 95% CI 0.1–0.7, p = 0.007).

Discussion

In our case-control study conducted between 2006 and 2008 in Belgrade, Serbia, we included 80 OC patients and 160 controls. The results we found in this study indicate that the use of oral contraceptives, as well as sports and recreation activities statistically significantly decrease the risk of OC.

A strong evidence of negative association between oral contraceptives use and the occurrence of OC has been recorded in a number of studies [15–19]. Oral contraceptives have a long-term favorable effect on the OC risk [20]. Specifically, this phenomenon occurs due to the reduction in estrogen levels in the ovaries and prevention of the ovulation [15, 21]. Even a short-term oral contraceptive use has been reported to reduce the risk [22]. Our results confirm this well-documented and defined association.

Even though the relationship between physical activity and the risk of hormone-dependent tumors (such as breast and endometrium cancer) have been known [23–26], there is only a small number of studies regarding the effect of exercising on OC. The two studies in the US and China reported a decreasing risk of OC among women who exercise some kind of physical activity [27, 28]. However, a Swedish prospective cohort study found no such evidence [29]. Being a hormone-dependent tumor, OC largely depends on oscilations of estrogen. Physical activity affects adipose tissue by reducing it, thus mobilizing hormone depots. Also, certain theories suggest that very strenuous physical activity induces late menarche, ammenorrhea and anovulatory cycles [30–32]. In addition, some authors [19, 33] registered that older age at menarche has a protective effect upon OC, but our study did not show any link between these two variables.

In a pooled analysis of case-control studies Ness et al. [34] found no association between the use of fertility drugs and overall risk of OC which is in accordance with the results obtained in our study. On the other hand, systematic review and meta-analysis performed by Greise et al. [35] showed that both menopausal estrogene and progestin therapies are the risk factors for OC.

Many authors [15–19] have reported nulliparity to be strongly associated with the ccurrence of OC, but in our sample of patients no significant difference in parity between the cases and the controls was found. Our patients also brestfed for shorter period. Moorman et al. [15] reported breastfeeding to be protective factor for the occurence of OC. They had lower family income and were of less educational level which is in concordance with the findings of El-Khwsky et al. [16] in Egypt and Song et al. [18] in China, whereas Zhang et al. [36] from the USA reported opposite results.

The women with OC were taller at the age of 18, but as for BMI no relationship was established. Moorman et al. [15] registered body height and BMI of 35 and over to be the risk factors for this tumor. Greer et al. [22] reported that women who had greater both recent weight and weight at the age of 18 were at higher risk of OC. Beehler et al. [37] showed that obese, premenopausal women have 2 times more chance of developing OC. However, they have not recorded any association between BMI in postmenopausal women and OC, which corresponds to our findings, that BMI does not carry any risk of this disease.

Smoking is often associated with many types of cancers [33] and our research confirmed that the cases smoke more than their controls. However, controls drank more tea and coffee on daily basis. Data on this issue in literature is controversial. Some studies [18, 36] have shown protective effect of the green tea, as opposed to black tea. Our study did not divide teas into categories. And that neither caffeinated or decaiffenated coffees were associated with the risk of OC, which opposes to our findings [18].

One can criticize that the recall bias may have influenced the results in this case-control study and the size of our study groups. In addition, population controls may have been more appropriate and therefore our study is certainly subject to selection bias. In the present research we did not look for specific dietary patterns of our patients, even though certain articles suggest micronutrients such as calcium, vitamin E and beta-carotene have protective effect upon occurrence of OC [38, 39] and that meat and fat are associated with an increased risk of OC [40].

Conclusion

Based on data obtained in our study, oral contraceptives use and physical activity are independent protective factors for OC. Overall, this is the first epidemiological study on risk factors for this cancer in our country. In future, attention should be paid on finding a larger sample, which would select participants throughout the country, not only from one major center (Belgrade) and broaden the scope of the specific questionnaire.

REFERENCES

1. *Roett MA, Evans P.* Ovarian Cancer: An Overview. Am Fam Physician 2009; 80(6): 609−16.
2. *Gonzalez-Diego P, Lopez-Abante G, Pollan M, Ruiz M.* Time trends in ovarian cancer mortality in Europe (1955-1993): effect of age, birth cohort and period of death. Eur J Cancer 2000; 36(14): 1816−24.
3. *Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C,* et al. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. Lancet 2011; 377(9760):127−38.
4. *Hamidou Z, Causeret S, Debakuyo TS, Gentil J, Arnould L, Roignot P,* et al. Population-based study of ovarian cancer in Cote d'Or: prognostic factors and trends in relative survival rates over the past 20 years. BMC Cancer 2010; 10:(2) 622.
5. *Colombo N, Van Grop T, Parma G. Amant F, Gatta G, Sessa C,* et al. Ovarian cancer. Crit Rev Oncol Hematol 2006; 60: 159−79.
6. *Bray F, Sankila R, Ferlay J, Parkin DM.* Estimates of cancer incidence and mortality in Europe in 1995. Eur J Cancer 2002; 38(1): 99-166.
7. Institute of Public Health of Serbia "Dr Milan Jovanovic – Batut". Center for prevention and control of noncommunicable diseases. Cancer incidence and mortality 1999-2005. In: Cancer registry of Central Serbia. Belgrade: Institute of Public Health of Serbia " Dr Milan Jovanovi - Batut"; 2007. (Serbian)
8. *DeVita VT, Hellman S, Rosenberg SA.* Cancer: Principles and Practice of Oncology. 8th ed. Baltimore: Lippincott Williams and Willkins; 2008.
9. *Gates AM, Rosner BA, Hecht JL, Tworoger SS.* Risk factors for Epithelial Ovarian Cancer by Histologic Sybtype. Am J Epidemol 2009; 171(1): 45−53.
10. *Hinkula M, Pukkala E, Kyyrönen P, Kauppila A.* Incidence of ovarian cancer of grand multiparous women. A population-based study in Finland. Gynecol Oncol 2006; 103(1): 207−11.
11. *Hankinson SE, Danforth KN.* Ovarian cancer. In: *Schottenfeld D, Fraumeni JF,* editors. Cancer epidemiology and prevention. New York: Oxford University Press; 2006.
12. *Boyd J.* Specific keynote: hereditary ovarian cancer: What we know. Gynecol Oncol 2003; 88(Suppl 8−10): 11−3.
13. *Huncharek M, Kupelnick B.* Dietary fat intake and risk of epithelial ovarian cancer: a meta-analysis of 6689 subjects from 8 observational studies. Nutr Cancer 2001; 40(2): 87−91.
14. *Kisic Tepavcevic D, Matejic B, Gazibara T, Pekmezovic T.* Trends and petterns of ovarian cancer mortality in Belgrade, Serbia: A join-point regression analysis. Int J Gynecol Cancer 2011; 21(6): 1018−23.
15. *Moorman PG, Calingaert B, Palmieri RT, Iversen ES, Bentley RC, Halabi S,* et al. Hormonal risk factors for ovarian cancer in premenopausal and postmenopausal women. Am J Epidemiol 2008, 167(9): 1059−69.
16. *El-Khwsky FS, Maghraby HK, Rostom YA, Abd El-Rahman, AH.* Multivariate Analysis of Reproductive Risk factors for Ovarian Cancer in Alexandria, Egypt. J Egypt Natl Canc Inst 2006; 18(1): 30−4.
17. *Rossing MA, Cushing-Haugen KL, Wicklund KG, Doherty JA, Weiss NS.* Menopausal Hormone Therapy and Risk of Epithelial Ovaarian Cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(12): 2548−56.
18. *Song YJ, Kristal AR, Wicklund KG, Cushing-Haugen KL, Rossing MA.* Coffee, tea colas, and Risk of Epithelial Ovarian Cancer. Cancer Epidemiol Biomarkers Prev 2008; 17(13): 712−6.
19. *Riman T, Dickman PW, Nilsson S, Correia N, Nordlinder H, Magnusson CM* et al. Risk Factors for Invasive Epithelial Ovarian Cancer: Results from a Swedish Case-Control Study. Am J Epidemiol 2002; 156(4): 363−73.
20. *Bosetti C, Negri E, Trichopoulos D, Franceschi S, Beral V, Tzonou A* et al. Long-term effects of oral contraceptives on ovarian cancer risk. Int J Cancer 2002; 102(3): 262−5.
21. *Salehi F, Dunfield L, Phillips KP, Krewski D, Vanderhyden BC.* Risk factors for ovarian cancer: an overview with emphasis on hormonal factors J Toxicol Environ Health B Crit Rev 2008; 11(3−4): 301−21
22. *Greer JB, Modugno F, Allen GO, Ness RB.* Short-Term Oral Contraceptive Use and the Risk of Epithelial Ovarian Cancer. Am J Epidemiol 2005; 162(1): 66−72.
23. *Bernstein L, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK.* Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 1994; 86(18): 1403–8.
24. *Thune I, Brenn T, Lund E, Gaard M.* Physical activity and the risk of breast cancer. N Engl J Med 1997; 336(18): 1269–75.
25. *Shu XO, Hatch MC, Zheng W, Gao YT, Brinton LA.* Physical activity and risk of endometrial cancer. Epidemiology 1993; 4(4): 342–9.
26. *Levi F, La Vecchia C, Negi E, Franceschi S.* Selected physical activities and the risk of endometrial cancer. Br J Cancer 1993; 67(4): 846–51.
27. *Cottreau CM, Ness RB, Kriska AM.* Physical Activiry and Reduces Risk of Ovarian Cancer. Obstet Gynecol 2000; 96(4): 609−14.
28. *Zhang M, Lee AH, Binns CW.* Physical activity and epithelial ovarian cancer risk: a case-control study in China. Int J Cancer 2003; 105(6): 838−43.
29. *Weiderpass E, Margolis KL, Sandin S, Braaten T, Kumle M, Adami HO,* et al. Prospective study of physical activity in different periods of life and the risk of ovarian cancer. Int J Cancer 2006; 118(12): 3153−60.
30. *Merzenich H, Boeing H, Wahrendorf J.* Dietary fat and sports activity as determinants for age at menarche. Am J Epidemiol 1993; 138(4): 217–24.
31. *Frisch RE, Wyshak G, Vincent L.* Delayed menarche and amenorrhea in ballet dancers. N Engl J Med 1980; 303(1): 17–9.
32. *Bernstein L, Ross RK, Lobo RA, Hanisch R, Krailo MD, Henderson BE.* The effects of moderate physical activity on menstrual cycle patterns in adolescence: Implications for breast cancer prevention. Br J Cancer 1987; 55(6): 681–5.
33. *Fujita M, Tase T, Kakugawa Y, Hoshi S, Nishino Y, Nagase S* et al. Smoking, earlier menarche and low parity as independent risk factors for gynecologic cancers in Japanese: a case-control study. Tohoku J Exp Med 2008; 216(4): 297–307.
34. *Ness RB, Cramer DW, Goodman MT, Kruger Kjaer S, Mallin K, Mosgaard BJ* et al. Infertility, fertility drugs, and ovarian cancer: a pooled analysis of case-control studies**.** Am J Epidemiol 2002; 155(3): 217−23.
35. *Greise CM, Greiser EM, Doren M.* Menopausal hormone therapy and risk of ovarian cancer:systematic review and meta-analysis. Hum Reprod Update 2007; 13(5): 453−63.
36. *Zhang M, Binns CW, Lee AH.* Tea Consumption and Ovarian Cancer Risk: A Case-Control Study in China. Cancer Epidemiol Biomarkers Prev 2002; 11(8): 713−8.
37. *Beehler GP, Sekhon M, Baker JA, Teter BE, McCann SE, Rodabaugh KJ,* et al. Risk of Ovarian Cancer Associated with BMI Varies by Menopausal Status. J Nutr 2006; 136(11): 2881−6.
38. *Bidoli E, La Veccia C, Talamini R, Negri E, Papinel M, Conti E,* et al. Micronutrients and ovarian cancer: A case-control study in Italy. Ann Oncol 2001; 12(11): 1589−93.
39. *Gates MA, Vitonis AF, Tworoger SS, Rosner B, Titus-Ernstoff L, Hankinson SE,* et al. Flavonoid intake and ovarian cancer risk in a population-based case-control study. Int J Cancer 2009; 124(8): 1918−25.
40. *Kolahdooz F, Ibiele TI, van der Pols JC, Webb PM.* Dietary patterns and ovarian cancer risk. Am J Clin Nutr 2009; 89(1): 297−304.

Received on June 29, 2011.
Revised on October 5, 2011.
Accepted on December 25, 2011.
OnLine-First June, 2013.

ORIGINAL ARTICLE

UDC: 616.126.32-036-08:616.12-07
DOI: 10.2298/VSP110630031B

Association between aortic stenosis severity and contractile reserve measured by two-dimensional strain under low-dose dobutamine testing

Uticaj težine aortne stenoze na procenu kontraktilne rezerve procenjene pomoću dvodimenzionalnog naprezanja tokom niskodoznog dobutaminskog testa

Marko Banović*, Bosiljka Vujisić-Tešić*, Vuk Kujačić†, Slobodan Obradović‡§, Zdenko Crkvenac†, Miodrag Ostojić*

***Department of Cardiology, University Clinical Centre of Serbia, Belgrade, Serbia; †Sahlgrenska University Hospital/Östra, Gothenburg, Sweden; ‡Clinic of Urgent Internal Medicine, Military Medical Academy, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Early detection of left ventricle (LV) systolic dysfunction could be a clue for surgical treatment in patients with significant aortic stenosis (AS). Therefore, we evaluated LV peak of global longitudinal strain (PGLS) using speckle tracking imaging at rest and during low-dose dobutamine infusion in asymptomatic patients with moderate and severe AS and preserved LV ejection fraction (EF). **Methods.** All the patients underwent coronary angiography and had no obstructive coronary disease (defined as having no stenosis greater than 50% in diameter). The patients were divided into two groups: above and below median of 0.785 cm² aortic valve area (AVA). PGLS was measured from acquired apical 4-chamber and 2-chamber cine loops using a EchoPac PC-workstation at rest and during 5 μg/kg/min, 10 μg/kg/min, and 20 μg/kg/min dobutamine infusion, respectively. The global strain was the average of segment strains from the apical views. **Results**: A total of 62 patients with moderate and severe AS (AVA < = 1.5 cm²), the mean age 66.12 ± 9.91, (57.14% males), were enrolled in this prospective study. At rest, mean gradient was 43.57 ± 0.29 mmHg and mean EF was 72.24 ± 0.45%. When divided according to median AVA, both groups had decreased average PGLS at rest (-9.33 ± 4.46% *vs* -8.95 ± 3.08%; p = ns). During dobutamine both groups increased their average PGLS, but only the group with AVA > median reached the statistical significance (-8.71 ± 2.68% *vs* -11.93 ± 3.74%, p = 0.002). In addition, PGLS increase was also significant in 4-chamber view in the patients with AVA above median, but only when comparing baseline to peak 20 μg/kg/min (-10.72 ± 3.07% *vs* -13.14 ± 4.79%; p = 0.034). Conversely, in both groups the increase of PGLS in 2-chamber view did not reach significance. **Conclusion.** Two-dimensional strain speckle tracking analysis of myocardial deformation with measurement of peak systolic strain during dobutamine infusion is a feasible and accurate method to determine myocardial longitudinal systolic function and contractile reserve and may contribute to clinical decision making in patients with significant AS.

Key words:
ventricular function, left; myocardial contraction; aortic value stenosis; dobutamine; heart function tests; ultrasonography.

Apstrakt

Uvod/Cilj. Rano otkrivanje sistolne disfunkcije leve komore kod bolesnika sa znatnom aortnom stenozom (AS) je važno, jer nam može na vreme ukazati na potrebu da se bolesnik uputi na hirurško lečenje. Iz tog razloga, koristeći dvodimenzionalnu *speckle tracking* tehniku, ispitali smo kolika je vrednost maksimalnog globalnog longitudinalnog naprezanja (*maximal global longitudinal strain* – MGLS) u miru i kako se menja tokom niskodoznog dobutaminskog testa kod bolesnika sa umerenom i tesnom AS i očuvanom ejekcionom frakcijom (EF) u miru. **Metode.** Svim bolesnicima je urađen koronarni angiogram i nijedan bolesnik nije imao suženje veće od 50% prečnika epikardnog koronarnog krvnog suda. Bolesnici su na osnovu medijane površine aortnog ušća (PAŠ) koja je iznosila 0,785 cm² podeljeni u dve grupe: iznad i ispod medijane. MGLS je meren iz apikalnog četvoro i dvošupljinskog preseka, pomoću EchoPac PC-radne stanice u miru i tokom niskodoznog dobutaminskog testa koji je obuhvatao tri nivoa: 5, 10, i 20 μg/kg/min. Ukupno globalno naprezanje izračunato je kao srednja vrednost naprezanja izračunatog iz četiri i dve

Correspondence to: Marko Banović, Department of Cardiology, University Clinical Centre of Serbia, 8 Koste Todorovića, 11000 Belgrade, Serbia. Phone: +381 63 80 50 258; E-mail: markobanovic71@gmail.com, bamark@yubc.net

šupljine. **Rezultati.** Ukupno 62 bolesnika sa umerenom i tesnom AS (PAŠ ≤ 1,5 cm²), prosečne starosti 66.12 ± 9.91 godine, (57.14% muškarci), bile su uključena u ovu prospektivnu studiju. U miru, srednji gradijent preko aortnog ušća iznosio je 43.57 ± 0.29 mmHg, a srednja vrednost EF bila je 72.24 ± 0.45%. Obe grupe bolesnika imale su sniženu prosečnu vrednost MGLS u miru (-9.33 ± 4.46% *vs* -8.95 ± 3.08%, *p* = ns). Tokom dobutaminskog testa obe grupe bolesnika povećale su prosečnu vrednost MGLS, ali je samo u grupi bolesnika čija je PAŠ bila iznad medijane taj porast bio statistički značajan (-8,71 ± 2,68% *vs* -11,93 ± 3,74%, *p* = 0,002). Takođe, u ovoj grupi bolesnika statistički značajan bio je i porast MGLS u apikalnom preseku četiri šupljine (-10,72 ± 3,07% *vs* -13,14 ± 4,79%, *p* = 0,034). Sa druge strane, nijedna grupa bolesnika nije dostigla statistički značajan porast MGLS u apikalnom preseku dve šupljine. **Zaključak.** Dvodimenzionalna *speckle tracking* analiza miokardne deformacije sa merenjem MGLS u miru i tokom niskodoznog dobutaminskog testa sigurna je, izvodljiva i precizna metoda za određivanje longitudinalne sistolne funkcije leve komore u miru i njene kontraktilne rezerve i može doprineti boljem kliničkom rasuđivanju kod bolesnika sa hemodinamski značajnom AS.

Ključne reči:
srce, funkcija leve komore; miokard, kontrakcija; zalistak aorte, stenoza; dobutamin; srce, funkcijski testovi; ultrasonografija.

Introduction

Speckles are natural acoustic markers due to interference patterns caused by backscattered signals from small structures in myocardium [1]. Long axis systolic left ventricular (LV) function is governed by the subendocardial myocardial fibres that can be reliably quantified by the measurement of longitudinal myocardial deformation using the two dimensional, 2D speckle tracking imaging [2]. Dobutamine is a potent beta-agonist which increases heart rate and contractility of the heart, but, in low dose, the effect is more pronounced on increasing myocardial contractility than heart rate [3].

LV response to chronic pressure overload and increased wall stress in aortic stenosis (AS) is a concentric hypertrophy – an increase in mass due to increased wall thickness without chamber dilatation [4]. This mechanism enables left ventricle ejectin fraction (LVEF) to remain preserved until late in the disease course. However, revealing the progression from compensatory hypertrophy to heart failure, in timely manner, may be important because once symptoms start to occur and LVEF to decrease, outcome becomes significantly worse [5]. Thus, early detection of diminished or absent LV long-axis myocardial deformation, as a marker of LV systolic dysfunction in AS, could be helpful for better decision-making in these patients [6].

The aim of this study was to evaluate the impact of AS on LV longitudinal systolic function by using 2D-speckle tracking of myocardial deformation at rest and during low-dose dobutamine infusion, in asymptomatic patients with moderate and severe AS and preserved LVEF.

Methods

A cohort of 70 patients with AS (effective orifice area of 1.5 cm^2 or less) and preserved EF at rest (EF > 50%), as calculated by Doppler and 2D echocardiography, used to be enrolled consecutively from May 2009 to September 2010 in the clinical echocardiography laboratory of the University Clinical Center, Belgrade, Serbia. All the patients underwent coronary angiography and had no obstructive coronary disease (defined as having no stenosis greater than 50% in diameter). Due to insufficient image quality at rest and during dobutamine testing (DBT) (of the 2D-strain especially), 8 patients were excluded forming the final group of 62 patients. According to the median aortic valve area (AVA) of 0.785 cm^2 the patients were divided into two groups: below and above the median level. Exclusion criteria were atrioventricular block or bradycardia with heart rate (HR) below 50 beats per minute, other significant valvular disease and uncontrolled hypertension (> 180/100 mmHg). The Ethics Committee of the University Clinical Center approved the study, and all the patients gave written informed consent.

Echocardiography

Transthoracic echocardiography exam was performed with an General Electric, Vivid 4 cardiac ultrasound system (BTO6, 1.5–3.6 MHz; GE Healthcare Technologies, Waukesha, WI, USA). The subjects were studied in the left lateral decubitus. Left ventricular internal dimension, posterior wall thickness (PWT) and interventricular septum thickness (IVST) were measured at end-diastole, at a level immediately apical to the mitral valve leaflet tips, in two-dimensional parasternal long-axis view [7]. The LV mass was calculated using the corrected formula of the American Society of Echocardiography and was indexed for body surface area (BSA) [8]. Relative wall thickness (RWT) was calculated with the formula: RWT = (PWT + IVST)/LVEDD. Significant LV hypertrophy was defined as LV mass index > 134 g/m^2 for men and > 110 g/m^2 for women and as RWT > 0.5 [9]. AS was graded using the continuity equation[10] calculated as moderate (AVA from 1.0 cm^2 to 1.5 cm^2) or severe (AVA 1.0 cm^2 or less). The subaortic diameter was measured from inner edge to inner edge at the level of the base of the aortic cusps in a parasternal long axis frame frozen in mid-systole. Pulsed Doppler recordings were made in apical 5-chamber view with the sample volume moved axially from the aortic annulus, usually 0.5 cm to 1 cm below the valve, recording maximal velocity and velocity-time integral. Continuous wave recordings were made from the apex and right intercostal positions and the optimal signal was traced to obtain peak velocity, velocity-time integral, systolic ejection time and peak and mean pressure difference, using the on-line software.

After echocardiography exam at rest, patients underwent low dose DBT with three levels, starting from 5 μg/kg/min, than 10 μg/kg/min and peak 20 μg/kg/min, re-

spectively. Each level was lasting for 3 minutes. All standard echocardiographic measures were recorded during the last minute of each level and analysed off-line.

Strain measurement

Strain measurement was based on the speckle tracking approach: the global longitudinal myocardial deformation was evaluated from the standard 2D images. The image acquisition frame rate was 60–90 Hz (mean value 75 Hz). Peak strain was measured from acquired apical 4-chamber and 2-chamber cine loops using an EchoPac PC-workstation at rest and during 5 μg/kg/min, 10 μg/kg/min, and 20 μg/kg/min DBT. In brief, by tracing the endocardial borders on an end-systolic frame, the software automatically tracked the contour on the subsequent frames. Adequate tracking was verified in real-time and was manually corrected, if necessary. The peak global longitudinal deformation was the average of segment strains from apical 4- and 2- chamber view. The inter-observer reproducibility of measurements was tested by random selection of 10 patients. Inter-observer agreement was 90%.

Statistical analysis

The data were expressed as mean values and standard deviations or percentages, and analyzed with the paired samples *t*-test. The median split method was used to divide patients into two equal cohorts: the cohort of patients below median level representing patients with severe AS and the cohort of patients above median level, representing patients with moderate AS. A p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS statistical software (SPSS for Windows, release 17.0, SPSS, Chicago, IL).

Results

A total of 537 cine loops were analyzed in the cohort of 62 asymptomatic AS patients, mean age 66.12 ± 9.91 years; range from 33 to 83 years; 54.8% were males. Dobutamine infusion was generally well-tolerated, no adverse event was registered during or after the testing. Table 1 presents the echocardiographic data of our patient cohort. All the patients had normal end-systolic and end-diastolic LV measures, and, by definition, AVA was reduced and mean and peak gradients increased. However, the signs of LV hypertrophy and diastolic dysfunction were present, with relatively high E/E' relationship indicating increased LV end-diastolic pressure.

Heart rate was increasing under DBT ($p < 0.05$) parallel with dobutamine dose increase. All parameters describing the severity of AS and systolic LV function, and when analyzing according to AVA median level, significantly changed in all patients ($p < 0.05$) during DBT (Table 2).

Table 1

Echocardiographic parameters describing diastolic function and left ventricle (LV) hypertrophy in all the patients

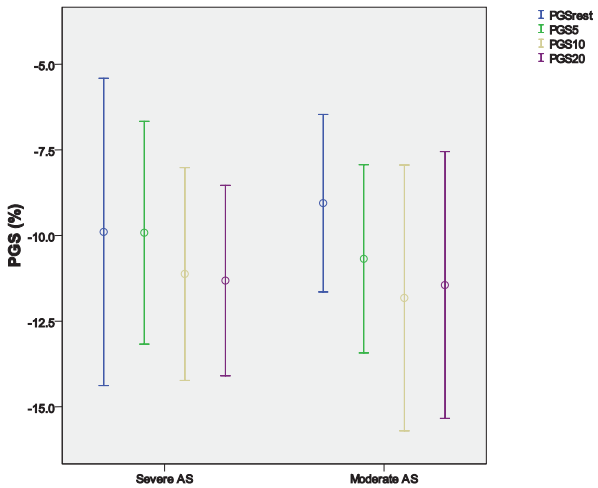
Parameters	$\bar{x} \pm SD$
LV mass index (g/m^2)	141.62 ± 33.52
Relative wall thickness	0.51 ± 0.08
Septum (cm)	1.31 ± 0.13
Posterior wall (cm)	1.24 ± 0.13
Isovolumetic relaxation time (ms)	94.00 ± 39.06
Deceleration time (ms)	243.88 ± 73.17
E/E' (index of left ventricular filling pressure) (cm/s)	14.10 ± 7.21
LV end-diastolic volume (mL)	88.07 ± 22.35
LV end-systolic volume (mL)	25.04 ± 10.14

Table 2

Clinical and echocardiographic parameters at rest and during peak dobutamine infusion for all the patients and according to aortic value area (AVA) median level

Parameters	All patients			AVA < median			AVA > median		
	rest ($\bar{x} \pm SD$)	peak dobutamine level ($\bar{x} \pm SD$)	p	rest ($\bar{x} \pm SD$)	peak dobutamine level ($\bar{x} \pm SD$)	p	rest ($\bar{x} \pm SD$)	peak dobutamine level ($\bar{x} \pm SD$)	p
Heart rate (bpm)	69.91± 11.51	100.98 ± 18.13	0.000	73.38 ± 11.11	107.58 ± 18.43	0.000	66.45 ± 11.02	94.38 ± 15.02	0.000
Systolic arterial pressure (mmHg)	147.58 ± 20.40	140.72 ± 20.18	0.001	146.12 ± 21.04	139.83 ± 21.19	0.016	149.03 ± 19.97	141.61 ± 19.42	0.002
Diastolic arterial pressure (mmHg)	88.79 ± 10.14	85.32 ± 11.76	0.014	88.70 ± 9.65	83.87 ± 10.93	0.026	88.87 ± 10.77	86.77 ± 12.55	ns
Aortic valve area (cm^2)	0.83 ± 0.23	1.01 ± 0.30	0.000	0.65 ± 0.10	0.81 ± 0.16	0.000	1.01 ± 0.19	1.20 ± 0.27	0.000
Maximal velocity (m/s)	4.28 ± 0.45	4.96 ± 0.55	0.000	4.40 ± 0.43	5.12 ± 0.53	0.000	4.16 ± 0.43	4.81 ± 0.52	0.000
Mean gradient (mmHg)	43.57 ± 10.92	57.42 ± 14.93	0.000	46.43 ± 10.49	61.84 ± 15.43	0.000	40.70 ± 9.40	52.99 ± 13.21	0.000
Indexed stroke volume (mL/m^2)	39.81 ± 10.98	45.78 ± 10.92	0.000	34.32 ± 9.62	41.31 ± 7.50	0.001	45.31 ± 9.46	50.24 ± 12.04	0.001
Ejection fraction (%)	72.24 ± 6.31	78.23 ± 8.52	0.000	71.19 ± 5.05	79.00 ± 7.68	0.000	73.29 ± 7.29	77.46 ± 9.36	0.006
S' (systolic mitral annulus tissue Doppler) (cm/s)	7.01 ± 1.49	9.63 ± 2.60	0.000	6.75 ± 1.34	8.87 ± 1.93	0.000	7.27 ± 1.61	10.40 ± 2.75	0.000
E/E' (index of left ventricular filling pressure) (cm/s)	14.10 ± 7.21	9.95 ± 6.14	p = ns	19.20 ± 5.43	14.20 ± 4.93	0.000	9.00 ± 3.22	5.60 ± 2.84	0.000

When divided according to median AVA, both groups of patients had a decreased average peak of global longitudinal strain (PGLS) at rest. No significant difference was found between them (p = ns), although patients with moderate AS had somewhat lower baseline values. However, during DBT both groups increased their average PGLS, but only the group with AVA > median level reached the statistical significance, during both 10 μg/kg/min and 20 μg/kg/min infusion (p = 0.012 and p = 0.020), while the increase during 5 μg/kg/min infusion was very close to statistical significance (p = 0.053) (Figure 1). In addition, PGLS increase was also significant in 4-chamber view in the patients with AVA above median level, but only when comparing baseline to peak 20 μg/kg/min DBT. In contrast, the patients with AVA below the median did not reach a significant increase in PGLS during DBT (Table 3). Conversely, in both groups the



AVA < median value (0.785 cm^2)		AVA > median value (0.785 cm^2)
PGSrest - PGS5;	p = ns	p = 0.053
PGSrest - PGS10;	p = ns	p = 0.012
PGSrest - PGS20;	p = ns	p = 0.020

Fig. 1 – Peak average global longitudinal strain (PGS) at rest and during dobutamine infusion according to median aortic value area (AVA) level.

Table 3

Peak global longitudinal strain (PGLS) at rest and during dobutamine infusion from apical 4- and 2- chamber view according to median aortic value area (AVA) level

AVA	x̄ ± SD (%)	p
< median value (0.785 cm^2)		
PGLS 4-chamber view at rest	-10.65 ± 3.96	ns
PGLS 4-chamber view at DBT 5 μg/kg/min	-10.33 ± 4.04	
PGLS 4-chamber view at rest	-10.94 ± 3.91	ns
PGLS 4-chamber view at DBT 10 μg/kg/min	-10.77 ± 3.42	
PGLS 4-chamber view at rest	-11.16 ± 4.09	ns
PGLS 4-chamber view at DBT 20 μg/kg/min	-11.40 ± 3.36	
> median value (0.785 cm^2)		
PGLS 4-chamber view at rest	-10.00 ± 3.05	ns
PGLS 4-chamber view at DBT 5 μg/kg/min	-9.89 ± 3.67	
PGLS 4-chamber view at rest	-9.98 ± 3.10	ns
PGLS 4-chamber view at DBT 10 μg/kg/min	-10.96 ± 4.52	
PGLS 4-chamber view at rest	-10.72 ± 3.07	0.034
PGLS 4-chamber view at DBT 20 μg/kg/min	-13.14 ± 4.79	
< median value (0.785 cm^2)		
PGLS 2-chamber view at rest	-9.55 ± 3.45	ns
PGLS 2-chamber view at DBT 5 μg/kg/min	-8.86 ± 3.57	
PGLS 2-chamber view at rest	-9.46 ± 3.63	ns
PGLS 2-chamber view at DBT 10 μg/kg/min	-10.08 ± 3.99	
PGLS 2-chamber view at rest	-9.52 ± 3.69	ns
PGLS 2-chamber view at DBT 20 μg/kg/min	-10.59 ± 3.07	
> median value (0.785 cm^2)		
PGLS 2-chamber view at rest	-9.17 ± 2.88	ns
PGLS 2-chamber view at DBT 5 μg/kg/min	-10.16 ± 3.62	
PGLS 2-chamber view at rest	-9.02 ± 2.77	ns
PGLS 2-chamber view at DBT 10 μg/kg/min	-10.16 ± 3.62	
PGLS 2-chamber view at rest	-9.48 ± 2.77	ns
PGLS 2-chamber view at DBT 20 μg/kg/min	-10.40 ± 3.56	

increase of PGLS in 2-chamber view did not reach significance. When analyzing mean LVEF at rest, we found, in contrast, that both groups have normal LVEF at rest (71.19 ± 5.05% *vs* 73.29 ± 7.29%, p = ns), and significant increase during DBT (71.19 ± 5.05% *vs* 79.00 ± 7.68%, $p < 0.01$, for AVA < median value and 73.29 ± 7.29 cm^2 *vs* 77.46 ± 9.36 cm^2, $p < 0.01$, for AVA > median level).

Discussion

The present study showed that 2D-speckle tracking analysis of myocardial deformation with measurement of PGLS during dobutamine infusion is a feasible and accurate method to determine myocardial systolic function and contractile reserve and may contribute to decision making in patients with moderate or severe AS. When compared to normal subjects, extensively investigated in the HUNT[11] study (in which authors reported normal PGLS around 16%), patients with AS have reduced longitudinal systolic function in spite of preserved LVEF at rest. This finding was recently showed by Donal et al. [6] who used exercise testing for estimating contractile reserve, and was confirmed with DBT in our study. To the best of the author's knowledge, this is the first study that used low-dose DBT for estimating longitudinal systolic function contractile reserve.

In hemodynamically significant AS, when chronically increased LV global afterload exceeds the limit of LV compensatory mechanism, intrinsic impairment of myocardial function can occur. However, despite the presence of myocardial dysfunction, often associated with disturbed myocardial architecture, LVEF is commonly normal in patients with AS. This might be due to the fact that LVEF is influenced not only by intrinsic myocardial function, but LV cavity geometry, also [2, 12, 13]. In AS, wall thickening as an adaptive mechanism to pressure overload, can thus mask subtle LV dysfunction [5]. Subclinical LV dysfunction is classically detected by a decrease in longitudinal myocardial function which, as we confirmed, can be reliably quantified by the measurement of myocardial deformation using 2D-speckle tracking analysis [14, 15]. Longitudinal function is governed by the subendocardial myocardial fibres which are aligned longitudinally and more sensitive to microvascular ischaemia [16, 17]. This may lead to progressive myocardial fibrosis that participates to reduce longitudinal myocardial function. In asymptomatic AS patients reduced subendocardial function has been showed to be associated with changes in symptomatic status during follow-up and adverce outcomes [14]. However, when the reactive subendocardial fibrosis becomes distinct, irreversible myocardial damage may occur. Therefore, early detection of intrinsic myocardial dysfunction in AS patients with preserved EF could be of help for risk assessment.

The present study showed that both patients with moderate and severe AS had impaired longitudinal myocardial function at rest. However, changes in longitudinal function during DBT were not homogenous, with the patients with AVA > 0.785 cm^2 (thus considered as moderate stenosis) having more increase (becomes more negative) in PGLS. The present observation suggest that patients with moderate stenosis better adapt to acute change in LV load, by recruiting LV contractile reserve to the increased afterload. Both inotropic contractile reserve and rise in transaortic pressure gradients are thus concomitant. Conversely, when the aortic valve is no longer compliant, or in case of a significant myocardial damage (*ie* ischaemia), mismatch between afterload and contractility can occur, which is often the case in more advanced stage of a disease [6]. Hence, limited longitudinal contractile reserve during DBT probably reflects a more advanced disease process with more extensive myocardial fibrosis, myocytes degeneration and exhausted coronary flow reserve.

Identification of subclinical LV dysfunction in hemodynamically significant AS is challenging and of clinical importance. The results of the present study show that the magnitude of DBT-induced changes in LVEF are not equal to changes in PGLS (as a measurement of LV long-axis function) and that different categories of asymptomatic AS patients can be identified according to changes in longitudinal function. In addition, PGLS, in contrast to LVEF, is decreased even during maximal DBT. This emphasizes that in AS, the assessment of myocardial contractile function by 2D-speckle tracking is more appropriate than by changes in LVEF in the setting of pressure overload. Also, the role of mitral annulus pulse tissue Doppler in distinguishing patients with limited contractile reserve, according to Van Pelt et al. [18], is less accurate.

Conclusion

2D-speckle tracking analysis of PGLS during DBT is a feasible and accurate method to determine subnormal myocardial systolic function and contractile reserve and may contribute to decision making in asymptomatic moderate and severe AS patients with preserved LVEF. However, a decrease in LV longitudinal systolic function in a significant AS cannot simply be related to the severity of valve obstruction and needs to be evaluated in comparison with control groups.

REFERENCES

1. *Geyer H, Caracciolo G, Abe H, Wilansky S, Carerj S, Gentile F*, et al. Assessment of myocacardial mechanics using speckle tracking echocardiography: fundamentals and clinical applications. J Am Soc Echocardiogr 2010; 23(4): 351−69.
2. *Donal E, Bergerot C, Thibault H, Ernande L, Loufoua J, Augeul L*, et al. Influence of afterload on left ventricular radial and longitudinal systolic function: a two-dimensional strain imaging study. Eur J Echocardiogr 2009; 10(8): 914−21.
3. *Schvammenthal E, Vered Z, Moshkowitz Y, Rabinowitz B, Ziskind Z, Smolinski AK,* et al. Dobutamine echocardiography in patients with aortic stenosis and left ventricular dysfunction: pre-

dicting outcome as a function of management strategy. Chest 2001; 119(6): 1766−77.
4. *Faggiano P, Rusconi C, Ghizzoni G.* Left ventricular remodeling in valvular aortic stenosis. Echocardiographic and clinical changes in two patients during follow up. Chest 1994; 105(4): 1273−5.
5. *Serri K*, *Reant P*, *Lafitte M*, Berhouet *M*, Le Bouffos V, Roudaut R, et al. Global and regional myocardial function quantification by two-dimensional strain: Application in hypertrophic cardiomyopathy. J Am Coll Cardiol 2006; 47(6): 1175−81.
6. *Donal E, Thebault C, O'Connor K, Veillard D, Rosca M, Pierard L*, et al. Impact of aortic stenosis on longitudinal myocardial deformation during exercise. Eur J Echocardiogr 2011; 12(3): 235−41.
7. *Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA*, et al. Recommendations for chamber quantification. Eur J Echocardiogr 2006; 7(2): 79−108.
8. *Sahn DJ, DeMaria A, Kisslo J, Weyman A***.** The committee on M-Mode Standardization of the American Society of Echocardiography: Recommendations regarding quantificationin M-mode echocardiography: Results of a survey of echocardiographic measurements. Circulation 1978; 58(6): 1072−83.
9. *Hammond IW, Devereux RB, Alderman MH, Lutas EM, Spitzer MC, Crowley JS*, et al. The prevalence and correlates of echocardiographic left ventricular hypertrophy among patients with uncomplicated hypertension. J Am Coll Cardiol 1986; 7(3): 639−50.
10. *Baumgartner H, Hung J, Bermejo J, Chambers JB, Evangelista A, Griffin BP,* et al. Echocardiographic assessment of valve stenosis: EAE/ASE recommendations for clinical practice. Eur J Echocardiogr 2009; 10(1): 1−25.
11. *Dalen H, Thorstensen A, Aase A, Ingul CB, Torp H, Vatten LJ,* et al. Segmental and global longitudinal strain and strain rate based on echocardiography of 1266 healthy individuals: the HUNT study in Norway. Eur J Echocardiogr 2010; 11(2): 176−83.
12. *Buermans HP, Paulus WJ.* Iconoclasts topple adaptive myocardial hypertrophy in aortic stenosis. Eur Heart J 2005; 26(17): 1697−9.
13. *Carabello B.* Evolution of the study of left ventricular function: everything old is new again. Circulation 2002; 105(23): 2701−3.
14. *Lafitte S*, *Perlant M*, *Reant P, Serri K, Douard H, DeMaria A*, et al. Impact of impaired myocardial deformations on exercise tolerance and prognosis in patients with asymptomatic aortic stenosis. Eur J Echocardiogr 2009; 10(3): 414−9.
15. *Vinereanu D, Lim PO, Frenneaux MP, Fraser AG.* Reduced myocardial velocities of left ventricular long-axis contraction identify both systolic and diastolic heart failure-a comparison with brain natriuretic peptide. Eur J Heart Fail 2005; 7(4): 512−9.
16. *Garcia D, Camici PG, Durand LG, Rajappan K, Gaillard E, Rimoldi OE*, et al. Impairment of coronary flow reserve in aortic stenosis. J Appl Physiol 2009; 106(1): 113−21.
17. *Delgado V, Tops LF, van Brommel RJ, van der Kley F, Marsan NA, Klautz RJ,* et al. Strain analysis in patients with severe aortic stenosis and preserved left ventricular ejection fraction undergoing surgical valve replacement. Eur Heart J 2009; 30(24): 3037−47.
18. *Van Pelt NC*, Stewart RA, Legget ME, Whalley GA, Wong SP, Zeng I, et al. Longitudinal left ventricular contractile dysfunction after exercise in aortic stenosis. Heart 2007; 93(6): 732−8.

Received on June 30, 2011.
Accepted on November 28, 2011.
OnLine-First June, 2013.

ORIGINAL ARTICLE

UDC: 614.2:616-036.22]:616.98
DOI: 10.2298/VSP1312109M

Risk factors for vancomycin-resistant *Enterococcus* colonization in hematologic patients

Faktori rizika od kolonizacije vankomicin-rezistentnog *Enterococcus*-a kod hematoloških bolesnika

Vesna Mioljević*, Ljiljana Marković-Denić[†], Ana Vidović[‡§], Milica Jovanović[¶], Tanja Tošić[¶], Dragica Tomin[‡§]

***Department of Hospital Epidemiology and Hygiene, [‡]Clinic of Hematology, [¶]Microbiology Department, Clinical Center of Serbia, Belgrade, Serbia; [†]Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [§]Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

Abstract

Background/Aim. Vancomycin-resistant *Enterococci* (VRE) is one of the most important hospital pathogens. The aim of the study was to evaluate VRE colonization in patients hospitalized at the Hematology Intensive Care Unit, as well as the associated risk factors. **Methods.** A prospective cohort study involved 70 patients hospitalized at the Intensive Care Unit (ICU), Clinic for Hematology, Clinical Center of Serbia, Belgrade, during 3 months. Baseline demographic data, data about antibiotic usage and other risk factors for VRE colonization during the present and previous hospitalizations (within 6 months) were recorded for each patient using the questionnaire. Feces or rectal swab was collected for culture from patients on admission and at discharge in case when VRE was not isolated on admission. *Enterococci* were isolated by standard microbiological methods. Isolate sensitivity was tested by disk-diffusion test using 30 μg/mL (BBL) Vancomycin plates according to the Clinical and Laboratory Standards Institute (CLSI) standard. **Results.** Analysing results showed that 7% of the patients had been already colonized with VRE upon ICU admission. The rate of VRE colonization during present hospitalization was 41.5%. Univariate logistic regression demonstrated the statistically significant differences in diagnosis, length of present stay, use of aminoglycosides and piperacillin/tazobactam in present hospitalization, duration of use of carbapenem and piperacillin/tazobactam in present hospitalization between the VRE-colonized and non-colonized patients. Acute myeloid leukemia (AML), use of carbapenem in previous hospitalization and duration of use of piperacillin/tazobactam in present hospitalization were independent risk factors for VRE-colonized patients according to multivariate logistic regression. **Conclusion.** VRE colonization rate was high among the patients admitted to hematology ICU. Rational use of antibiotics and active surveillance may be helpful preventive measures against the development of bacterial resistance to antimicrobial agents.

Key words:
enterococcus faecium; vancomycin; drug resistance, bacterial; hematologic diseases; risk factors.

Apstrakt

Uvod/Cilj. *Enterococcus* spp. rezistentan na vankomicin (VRE) jedan je od najznačajnih bolničkih patogena. Cilj rada bio je da se utvrde stope rektalne kolonizacije VRE kod bolesnika lečenih u Odeljenju za hematološku intenzivnu negu, i da se sagledaju faktori rizika od kolonizacije. **Metode.** Prospektivnom kohortnom studijom obuhvaćeno je 70 bolesnika lečenih u periodu od tri meseca u Klinici za hematologiju Kliničkog centra Srbije u Beogradu. Podaci o demografskim karatkteristikama bolesnika, upotrebi antibiotika i drugim faktorima rizika od VRE kolonizacije tokom sadašnje i prethodnih hospitalizacija (tokom 6 meseci) prikupljani su za svakog bolesnika uz pomoć upitnika. Bolesnicima je uzmana koprokultura ili rektalni bris na prijemu, a prilikom otpusta onim bolesnicima kod kojih na prijemu nije izolovan VRE. Osetljivost izolata proverena je disk-difuzionim testom sa diskovima vankomicina 30 μg/mL (BBL) u skladu sa *Clinical and Laboratory Standards Institute* (CLSI) standardima. **Rezultati.** Na prijemu je bilo 7% VRE kolonizovnaih bolesnika. Stopa VRE kolonizacije tokom tekuće hospitalizacije iznosila je 41,5%. Univarijantna logistička regresija pokazala je statistički značajne razlike u pogledu dijagnoze, dužine sadašnje hospitalizacije, primeni aminoglikozida i piperacilin/tazobaktama u sadašnjoj hospitalizaciji, dužini primene karbapenema i piperacilin /tazobaktama u sadašnjoj hospitalizaciji između bolesnika kolonizovanih VRE i nekolonizovanih bolesnika. Multivarijantnom logističkom regresijom ustanovljeno je da su akutna mijeloidna leukemija (AML), primena karbapenema u prethodnoj hospitalizaciji i dužina primene piperacilin/tazobactama u sadašnjoj hospitalizaciji bili nezavisni faktori rizika od kolonizacije bolesnika VRE. **Zaključak.** Zabeležena je visoka stopa kolonizacije pacijenata VRE. Racionalna upotreba antibiotika i aktivni nadzor mogu biti korisne mere prevencije nastanka rezistencije bakterija na antibiotike.

Ključne reči:
enterococcus faecium; vankomicin; lekovi, rezistencija mikroorganizama; hematološke bolesti; faktori rizika.

Correspondence to: Vesna Mioljević, Clinical Center of Serbia, Department of hospital epidemiology and hygiene, Belgrade, Serbia.
E-mail: vesna.mioljevic@gmail.com

Introduction

Bacteria of *Enterococcus* genus are a significant cause of hospital-acquired infections (HAI), second among urinary tract infections (UTI) and third among bacteriemias. There are many different species of *Enterococci*. The most prevalent species culturated from humans are *E. faecalis* (the most common) and *E. faecium. Enterococci* have both an intrinsic (nature) and acquired resistance to antibiotics, making them important nosocomial pathogens. They are intrinsically resistant to penicillin (low level), all cephalosporins, aztreonam, macrolides, and low levels of clindamycin. This natural resistance is present in all members of species and is chromosomally mediated. Acquired resistance to antibiotics includes resistance to glycopeptides, β lactamases fluoroquinolones, tetracycline, high aminoglycoside doses and glycopeptides (vancomycin), as a result of mutations in DNA or the acquisition of new gene(s). Glycopeptides (vancomycin) resistance has been seen in around 70–78% of the nosocomial *E. faecium* population [1–3]. The mechanism of vancomycin resistence is due to preventing the synthesis of peptidoglycan precursors of the bacterial cell wall by blocking two steps: the transglycosylation and the transpeptidation [3]. It is mediated by 5 genes referred to as *vanA,* which can induce high level resistance to both vancomycin and teicoplanin; *vanB* which is found intrinsically in non-pathogenic enterococcal species; *vanC; vanD*, and *vanE*. *VanA* is more widely distributed [4].

Vancomycin resistant *Enterococcus* spp. (VRE) was first isolated in England and France in 1986 and later on in other European and countries worldwide [5]. The proportion of enterococcal bacteremia attributable to VRE in the UK in 2007 was 8.5–12.5% for all enterococci [6]. From 2005 to 2008, a significant decrease in vancomycin resistance was observed in France (from 2 to 0.6%), Greece (from 37 to 28%), Israel (from 46 to 20%) and Italy (from 19 to 6%). Ireland, Luxembourg and Greece in 2009 reported resistance proportions above 25%, while the majority of countries (18 of 26 countries) reported resistant proportions below 7%. Several countries reported it even below 1% (Bulgaria, Estonia, Finland, France, Norway and Sweden) [3]. During the past four years, a significant increase was observed only in Austria. In 2010, 28 EU countries reported 5,577 isolates of *E. faecium*, of which 7.4% were resistant to vancomycin. During the four past years, only Latvia reported an increased trend of these enterococci [7].

According to the USA National Nosocomial Infections Surveillance (NNIS), the percentage of enterococcal isolates resistant to vancomycin increased from 12% in the period 1998–2002 to 28.5% of all isolates in 2003 [8].

VRE can cause different types of HAI, like urinary tract, surgical site, bacteriemia, meningitis, endocarditis, most commonly in immunocompromised patients. Enterococci are responsible for high morbidity and mortality rates in these patients [9].

Risk factors for VRE colonization and infections are the following: age, hepatic and renal dysfunction, hematological diseases, chronic diseases, application of the invasive diagnostic and therapeutic procedures, stay in intensive care unit (ICU), abdominal surgery, transplantation, prolonged hospitalization, and broad-spectrum antibiotic use [10, 11].

VRE may survive on dry surfaces several weeks (from 7 days to 4 months). Consequently, VRE is most commonly transmitted in hospitals from person to person by direct contact with personnel and patient's hands, either from feces, urine, or blood of a person carrying the organism. It can also be spread indirectly *via* hand contact with open wounds, or with contaminated environments (some parts of medical equipment and working surfaces contaminated by VRE) [12]. VRE colonization could persist for years. The colonized patients are significant reservoirs and sources of contamination of the environment. VRE is not transmitted through the air [13].

Methods

This prospective cohort study involved 70 patients hospitalized at the Intensive Care Unit (ICU), Clinic for Hematology, Clinical Center of Serbia, Belgrade, in the period from September to December 2011. The coproculture or rectal swab was collected from all the patients on admission and before discharge.

All the patients were daily observed during their hospitalization by an epidemiologist. Clinical charts were systematically reviewed and, when necessary, the medical staff was interviewed. Baseline demographic data, data about antibiotic usage and other risk factors for VRE colonization during present and previous hospitalization were recorded for each patient using the questionnaire. The following characteristics related to the patients, and applied diagnostic and therapeutical procedures were recorded: age, sex, underlying disease, recent prior hospitalization (within 6 months) and recent antimicrobial use, operation, inserted central venous and urinary catheter, mechanical ventilation, antibiotic prophylaxis and therapy (type of antibiotics), and VRE isolated from coproculture on admission and the discharge. Surveillance for VRE infections was carried out during the course of this study.

VRE isolation and identification

Enterococci were isolated by standard microbiological methods. Enterococcal identification was based on cultural characteristics, Gram-stained specimens, mobility in 0.5% agar, capacity of pigment production and biochemical characteristics. Isolate sensitivity was tested by the disk-diffusion test using 30 μg/mL (BBL) Vancomycin plates according to the Clinical and Laboratory Standards Institute (CLSI) standard. The plates were incubated 24 hours at 35°C. The increase in more than one colony or a part of colony was interpreted as resistance to vancomycin [14, 15]. E-test was not used in methodology given than only enterococcal isolates were recovered and identified from rectal swab or feces for the purpose of determination of the carrier state.

The descriptive and analytical methods were used for data processing: χ^2 test (for categorical data) or *t*-test (for continuous variables). The results were expressed as percentages or as mean ± standard deviations. To identify the risk

factors of VRE colonization, the univariate logistic regression and multivariate logistic regression analyses were used. Statistical data processing was carried out by SPSS program (version 10).

Results

Study population and patients characteristics

The study included 70 patients hospitalized at the Clinic for Hematology, Clinical Center of Serbia during the study period. Out of all these patients, 5 (7%) were found to have been already colonized with VRE upon ICU admission. The VRE positive patients on admission had been hospitalized at the Clinic for Hematology within the previous six months. During that hospitalization they received antibiotic therapy.

Out of 65 VRE negative patients on admission, 27 (41.53%) were colonized with VRE strains during current hospitalization. The characteristics of these patients are presented in Table 1. There were 38 (58.4%) males; the mean age of the subjects was 52.7 years (ranged from 23 to 80 years).

found a significant difference (OR: 3.06; 95%CI: 1.09–8.60; $p = 0.033$) in frequency of this diagnosis between the two groups of subjects. Furthermore, there was a significant difference in length of present hospitalization between the group of colonized patients (median days 35 ± 10.23) and non-colonized patients (median days 24.4 ± 10.6) (OR: 1.11; 95%CI: 1.04–1.19; $p = 0.002$).

Univariate logistic regresion analysis failed to find any significant difference in other characteristics between the two study groups of patients: age, sex, prior hospitalizations, antibiotic use, insertion of the urinary and central venous catheter and infection.

Antibiotic use and duration of antibiotic use

During the present hospitalization, only 15.4% patients did not receive antibiotics; 43.1% received one or two antibiotics and 41.5% three or more antibiotics. Tables 2 and 3 summarize the antibiotic use and duration of antibiotic use among the patients with and without VRE colonization. According to univariate logistic regression, the risk factors that were significantly associated with VRE colonization in-

Table 1

Characteristics of the VRE negative patients* on admission and during hospitalization

Patients' variables	n (%) of patients		OR (95%CI)	*p*
	VRE non-colonized (n = 38)	VRE colonized (n = 27)		
Age (years), mean (± SD)	52.3 (13.0)	53.3 (12.6)	1.00 (0.96–1.04)	0.175
Sex (males/females)	21 (55.26)	17 (62.96)	1.37 (0.50–3.77)	0.847
Diagnosis on admission				
acute myeloid leukemia	15 (39.47)	18 (66.66)	3.06 (1.09–8.60)	0.033
non Hodgkin lymphoma	6 (15.78)	1 (3.70)	0.20 (0.02–1.81)	0.121
acute lymphocytic leukemia	9 (23.68)	5 (18.51)	0.73 (0.21–2.49)	0.175
chronic lymphocytic leukemia	4 (10.52)	2 (7.40)	0.68 (0.11–4.00)	0.669
Length of stay (days), median ± SD				
previous hospitalization	8.26 (12.3)	8.56 (13.62)	1.00 (0.96–1.04)	0.927
present hospitalization	24.4 (10.6)	35 (10.23)	1.11 (1.04–1.19)	0.002
previous admission in other hospital	29 (76.31)	18 (66)	1.10 (0.80–1.52)	0.524
Antibiotic use				
previous hospitalization	26 (68.42)	20 (74.07)	1.10 (0.80–1.52)	0.524
present hospitalization	34 (89.47)	26 (96.29)	0.75 (0.25–2.27)	0.622
Central venous catheter			1.79 (1.43–2.24)	0.999
previous hospitalization	30 (78.94)	22 (81.48)	0.85 (0.24–2.98)	0.801
present hospitalization	21 (55.26)	19 (70.31)	1.92 (0.67–5.46)	0.220
Bladder catheter				
previous hospitalization	1 (2.63)	2 (7.40)	2.96 (0.25–34.42)	0.386
present hospitalization	11 (28.94)	8 (29.62)	1.03 (0.35–3.05)	0.952
Hospital infection				
previous hospitalization	5 (13.15)	2 (7.40)	0.52 (0.09–2.94)	0.467
present hospitalization	24 (63.15)	15 (55.55)	0.72 (0.26–1.99)	0.538

VRE – vancomycin resistent enterococci; *total number of VRE negative patients on admission = 65.

In our study groups of colonized and non-colonized patients, 18 (66.67%) and 15 (39.47%) had acute myeloid leukemia (AML), respectively. Univariate logistic regression

cluded aminoglycoside use (OR: 3.88; 95%CI: 1.14–13.1; $p = 0.030$) and piperacillin/tazobactam use (OR: 4.68; 95%CI: 1.57–13.9; $p = 0.005$) during present hospitalization (Table

2). Statistically significant difference (OR: 1.36; 95%CI: 1.94–1.69; $p = 0.006$) was also noted in the length of piperacillin/tazobactam use between colonized (median days 3.22 ± 3.93) and non-colonized patients (median days 0.87 ± 1.84) during the present hospitalization (Table 3).

Univariate logistic regresion analysis failed to show any significant difference in the frequency of use of other antibiotics (cephalosporins, quinolones, glycopeptides, antianaerobic drugs and cotrimoxazole) and the duration of antibiotic therapy between the two studied groups of patients.

Table 2

Antibiotic use during previous and present hospitalization (univariate logistic regression)

Used antibiotics	n (%) of patients		OR (95%CI)	*p*
	VRE non-colonized (n = 38)	VRE colonized (n = 27)		
Cephalosporins				
previous hospitalization	1 (2.63)	1 (3.70)	1.42 (0.08–23.7)	0.806
present hospitalization	8 (21.05)	8 (29.62)	1.57 (0.50–4.91)	0.431
Carbapenems				
previous hospitalization	2 (5.26)	6 (22.22)	5.14 (0.95–27.8)	0.057
present hospitalization	13 (34.21)	15 (55.55)	2.40 (0.87–8.61)	0.090
Quinolone				
previous hospitalization	3 (7.89)	6 (22.22)	3.33 (0.75–14.7)	0.113
present hospitalization	17 (44.73)	12 (44.44)	0.98 (0.36–2.66)	0.988
Glycopeptide				
previous hospitalization	1 (2.63)	3 (11.11)	1.05 (0.93–1.19)	0.393
present hospitalization	3 (7.89)	3 (11.11)	1.01 (0.90–1.15)	0.758
Antianaerobic agents				
previous hospitalization	1 (2.63)	0	0.97 (0.92–1.02)	0.396
present hospitalization	7 (18.42)	5 (18.51)	1.00 (0.28–3.58)	0.992
Cotrimoxazole				
previous hospitalization	1 (2.63)	2 (7.40)	2.96 (0.25–34.4)	0.386
present hospitalization	2 (5.26)	1 (3.70)	0.69 (0.60–8.04)	0.769
Aminoglycosides				
previous hospitalization	5 (13.55)	3 (11.11)	0.80 (0.18–3.79)	0.825
present hospitalization	5 (13.55)	10 (37.03)	3.88 (1.14–13.1)	0.030
Piperacilin/tazobactam				
previous hospitalization	3 (7.89)	3 (11.11)	1.45 (0.27–7.84)	0.660
present hospitalization	8 (21.05)	15 (55.55)	4.68 (1.57–13.9)	0.005

VRE – vancomycin resistent enterococci.

Table 3

Duration of antibiotic use (univariate logistic regression)

Used antibiotics	Duration (days), median ± SD		OR (95%CI)	*p*
	VRE non–colonized (n = 38)	VRE colonized (n = 27)		
Cephalosporins				
previous hospitalization	0.13 ± 8.11	0.30 ± 1.53	1.13 (0.73–1.75)	0.582
present hospitalization	2.29 ± 5.53	1.96 ± 3.70	0.98 (0.88–1.09)	0.787
Carbapenems				
previous hospitalization	0.17 ± 1.13	1.18 ± 2.74	1.39 (0.94–2.05)	0.098
present hospitalization	2.47 ± 4.39	5.04 ± 5.37	1.11 (1.00–1.23)	0.046
Quinolones				
previous hospitalization	1.71 ± 6.05	2.48 ± 5.97	1.02 (0.94–1.10)	0.609
present hospitalization	5.13 ± 7.18	3.93 ± 5.96	0.97 (0.90–1.05)	0.473
Glycopeptides				
previous hospitalization	0.91 ± 4.77	0.51 ± 1.71	1.04 (0.82–1.32)	0.719
present hospitalization	1.32 ± 3.02	1.52 ± 2.75	1.01 (0.85–1.20)	0.835
Antianaerobic agents				
previous hospitalization	0.15 ± 0.82	0.12 ± 0.38	0.70 (0.27–1.83)	0.475
present hospitalization	0.63 ± 1.55	1.07 ± 2.51	1.11 (0.86–1.43)	0.385
Cotrimoxazole				
previous hospitalization	0.15 ± 0.82	0.48 ± 1.74	1.39 (0.79–2.43)	0.243
present hospitalization	0.63 ± 1.55	0.22 ± 1.15	1.08 (0.62–1.86)	0.776
Aminoglycosides				
previous hospitalization	1.36 ± 4.20	0.44 ± 1.76	0.89 (0.73–1.10)	0.319
present hospitalization	1.45 ± 42.4	1.93 ± 2.74	1.03 (0.90–1.18)	0.605
Piperacilin/tazobactam				
previous hospitalization	0.94 ± 4.19	1.25 ± 3.83	1.01 (0.90–1.15)	0.758
present hospitalization	0.87 ± 1.84	3.22 ± 3.93	1.36 (1.94–1.69)	0.006

VRE – vancomycin resistent enterococci.

Multivariate logistic regresion analysis included all the values of $p < 0.1$ (diagnosis, number of hospital days in present hospitalization, carbapenem use in earlier and present hospitalization, use of aminoglycosides and piperacillin/tazobactam use in present hospitalization, duration of carbapenem use in previous and present hospitalization as well as length of piperacillin/tazobactam use in present hospitalization).

The results of multivariate analysis demonstrated that the diagnosis of the disease (AML), carbapenem use in earlier hospitalization and length of piperacillin/tazobactam use in present hospitalization were independent risk factors of colonization of patients with VRE (Table 4).

pneumonia) between the two study groups of patients (Table 5). In the VRE-colonized patients, vancomycin resistant *Enteroccus* spp was a cause of urinary tract infections in 4 patients, while VRE was not isolated as the cause of infection in the non-colonized patients (Table 6).

Discussion

The patients affected by malignant hemopathies are often rehospitalized and, therefore possibilly colonized by hospital pathogens including VRE. Immunosuppressed patients appear to be at special risk for VRE colonization and severe

Table 4

Risk factors for vancomycin-resistant *Enterococci* (VRE) colonization according to multivariate logistic regression analysis

Risk factors	B	SE	OR (95%CI)	*p*
Diagnosis (AML)	0.001	0.001	0.92 (0.99–1.0)	0.048
Antibiotic use				
Carbapenems – previous hospitalization	1.651	2.487	5.21 (0.04–6.81)	0.040
Duration of antibiotic use				
Piperacillin/tazobactam – present hopsitalization	1.918	0.884	6.80 (1.20–3.85)	0.030

AML – acute myeloid leukemia.

Table 5

Infection in vancomycin-resistant *Enterococci* (VRE) – colonized patients in present hospitalization

Hospital–acquired infections (HAI)	n (%) of patients		Total patients n (%)
	VRE non–colonized (n = 38)	VRE colonized (n = 27)	
Urinary tract infections	12 (31.6)	8 (29.6)	20 (30.8)
Bloodstream infections	8 (21.1)	3 (11.1)	11 (16.9)
Pneumonia	0 (0.0)	2 (7.40)	2 (3.1)
Without HAI	18 (47.3)	14 (51.9)	32 (49.2)
Total n (%)	38 (100.0)	27 (100.0)	65 (100.0)

Table 6

Cases of infection in the vancomycin-resistant *Enterococci* (VRE) – colonized and non–colonized patients in present hospitalization

Hospital acquired infection (HAI)	n (%) of patients		Total patients n (%)
	VRE non–colonized (n = 38)	VRE colonized (n = 27)	
Urinary tract infection			
Esherichia coli	5 (41.6)	0 (0.0)	5 (25.0)
Klebsiella spp.	5 (41.6)	1 (12.5)	6 (30.0)
Enterococcus spp.(vancomycin sensitive)	1 (8.3)	3 (37.5)	4 (20.0)
Enterococcus spp.(vancomycin resistent)	0 (0.0)	4 (50.0)	4 (20.0)
Providentia retgery	1 (8.3)	0 (0.0)	1 (5.0)
Total patients, n (%)	12 (60)	8 (40)	20 (100.0)
Bloodstream infection CNS*	4 (50.0)	2 (66.6)	6 (54.5)
Klebsiella spp	1 (12.5)	0 (0.0)	1 (9.09)
Pseudomonas spp.	1 (12.5)	0 (0.0)	1 (9.09)
Stenotrophomonas maltophilia	1 (12.5)	0 (0.0)	1 (9.09)
Acinetobacter spp.	1 (12.5)	0 (0.0)	1 (9.09)
Esherichia coli	0	1 (33.3)	1 (9.09)
Total patients, n (%)	8 (72.72)	3 (27.28)	11 (100.0)

***CNS – coagulasa negative staphylococci.**

Infections and pathogens

Data processing did not reveal any significant difference in the incidence of all HAI ($p > 0.05$), as well as certain HAI (urinary tract infections, bloodstream infections and VRE infections. VRE are a particular problem in the intensive care units of large hospitals where they usually occur. Our study was designed to evaluate the colonization rate during hospitalization at the Hematology ICU, colonization rate on admission, and risk factors of colonization. The re-

sults of this study showed that 7% of the patients were VRE positive on admission (VRE isolated from feces culture or rectal swab). The colonization rate during ICU hospitalization was 41.5%.

A relatively small number of articles described VRE colonization in hematological patients [10, 16–19]. However, *Enteroccoci* have recently emerged as nosocomial agents, especially in patients with hematooncological diseases. The studies from France and the Netherlands showed that 37%, and 49% of hematological patients were colonized by VRE, respectively [16, 17]. Contrary to these results, VRE colonization in the USA immunocompromised patients was reported in considerably lower percentage. It was noted that out of 2,115 hematological patients, 4.7% patients had verified rectal VRE colonization. Among all colonized patients, 5.4% were patients with leukemia, 4.9% with hematopoietic stem cell transplantation recipients, and 2.2% with lymphoma [20]. In other study which was carried out on the hematologyoncology unit, 7.7% of patients, predominantly with hematologic malignancies, were colonized or infected with VRE during the study period [21]. A much higher rate of VRE colonization in our study (41.5%) is probably the result of the lack of contact isolation measures and the increased use of antibiotics. This is supported by the fact that only 15% of patients did not receive antibiotics during present hospitalization.

Many years ago, it was demonstrated that 5–50% of all antibiotic prescriptions are considered inappropriate which can cause the emergence and dissemination of resistant organisms. However, there is not standard treatment protocol for antibiotic prescription in our country. Beside that, the antibiotics were until recently available over-the-counter in the pharmacies. Antibiotic prescription is frequently done without antibiograms or even without bacteriology isolation of pathogens. All of the above mentioned can lead to high rates of bacterial resistance to antibiotics. It is important to emphasize that there is an increasing trend of vancomycin resistance in our country [22].

Our study failed to find any association of sex and the age and development of VRE fecal colonization at discharge. Our findings are consistent with the results of similar studies conducted in Korea [23]. On the contrary, a study carried out at the Thessaloniki University Clinic confirmed that VRE colonization was significantly more frequent in patients older than 60 years of age [24].

Analysis of our results showed that VRE colonization was significantly more frequent in patients with AML. Multivariate regression analysis demonstrated that AML was an independent risk factor for VRE colonization. Similar results were found in other studies as well [21]. It can be explained by the long length of hospitalization. Namely, duration of hospital stay of our patients with AML ranged from 28 to 40 days, in distinction from the patients with acute lymphocytic leukemia (ALL), non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (HLL) who were hospitalized during significantly shorter period. From the aspect of a clinician this may be explained by the length of therapy protocol application. Besides other risk factors, the length of hospital stay is considered as one of most important risk factor [10, 11, 24]. Accordingly, AML patients are at higher risk of colonization than patients with shorter hospitalization. The results of this study are only the introduction to more comprehensive and detailed analysis of the problem not only in hematological, but also in other immunocompromised patients.

Our study failed to find any significant difference in previous hospitalization between the two studied groups of patients, contrary to the results of other studies [20, 25]. However, mean length of present hospitalization in VRE-colonized patients was significantly longer in relation to non-colonized patients, *ie* 35 *vs* 24 days.

The results of several studies showed that the use of glycopeptides, second and third generation cephalosporins and antianaerobic antibiotics are associated with the patients colonized with VRE [25, 26]. Only few studies analyzed the association of quinolone use and VRE colonization. Our study failed to establish any significant difference in the use and length of use of antibiotics from the group of quinolones (ciprofloxacin) between the two groups of subjects what is compatible with the findings obtained in other studies [25].

Several studies on the effect of carbapenem as the risk factor for VRE colonization did not show any significant difference in the use of these antibiotics between VRE-colonized and non-colonized patients [20, 26]. However, in other studies prior carbapenem use was a significant risk factor for VRE colonization [24, 25]. Our study showed that there was a difference in the duration of the use of antibiotics from carbapenem group (imipenem and meropenem) between two groups of subjects in repeated hospitalization. Mean duration of carbapenem use in the VRE-colonized and in non-colonized patients in the repeated hospitalization was 5 and 2.47 days, respectively. Moreover, carbapenem use in previous hospitalization was an independent risk factor of VRE colonization.

Vancomycin is a glycopeptide antibiotic that is used to treat infections caused by Gram-positive bacteria. For many years, it has traditionally been reserved as a drug of "last resort", used to treat severe infections for which other antibiotics had failed. Vancomycin use has increased linearly in the last decades, especially for infections related to the presence of indwelling vascular catheter which is the case with hematology-oncology patients. Vancomycin is not recommended for regular antibioprophylaxis in surgery. However, a growing number of infections caused by *Staphylococcus aureus* resistant to meticillin has led to the widespread use of vancomycin in hospitals. The results of studies on association of vancomycin use and VRE colonization and infection have been controversial. A meta-analysis of 20 studies showed that the use of vancomycin increased the risk of VRE colonization by 4.5 times [27]. Using the multivariate analysis, Ostrowsky et al. [28], in contrast, demonstrated that there was no association between the vancomycin use and VRE colonization. In addition, the results of recent systematic review did not determine a potential role for vancomycin usage reduction in controlling VRE colonization [29]. Our study did not find any significant difference in the frequency

of vancomycin use and length of its use between the two groups of patients.

Analysing a study on the effect of use of antibiotics from the group of aminoglycosides failed to find any significant difference in the frequency of use of these antibiotics between VRE-colonized and non-colonized patients [17]. On the contrary, our results showed a significant difference in the use of aminoglycoside antibiotics between the two groups of subjects during repeated hospitalization. In the VRE-colonized group, 37% of the patients were administered aminoglycoside antibiotics while in the non-colonized group 13% of the patients received these antibiotics.

Analysing of our data showed that the VRE-colonized patients received piperacillin/tazobactam in a significantly higher percentage (55%) then non-colonized patients (21%). An average length of piperacillin/tazobactam use in the VRE-colonized and non-colonized patients during the present hospitalization was 3.22 and 0.87 days, respectively. Moreover, the use of this antibiotic in present hospitalization was an independent risk factor of VRE colonization. The incidence of VRE was positively correlated with the use of piperacillin/tazobactam or beta-lactam agents in other studies as well [30].

As the part of normal fecal flora, *Enteroccoci* were not traditionally considered as important nosocomial pathogens. But, they have emerged as increasingly important pathogens with increased resistance to antibiotics. VRE become one of the leading causes of HAI, especially of urinary tract and bloodstream infections. VRE commonly colonise, but less frequently cause the infections. However, colonization precedes most infections. In our study, VRE was a cause of UTI in the colonized patients but not in the non-colonized patients. VRE infections are not more virulent than other enteroccocal infections. But, VRE infections are very problematic for treatment. *Enterococci* are the most frequent cause of UTI. During one year of surveillance, organized by the National Healthcare Safety Network in the USA there was found that *Enteroccocus* spp was third the most frequent pathogen of HAI participated with 12% in the overall number of pathogenic isolates. Regarding rank-order distribution, it was at second position for bloodstream infections and at third position for UTI. *E. faecium* and *E. faecalis* showed a high proportion of vancomycin resitance: 98.4–99.5% and 91.9–98.4%, respectively [31].

Conclusion

VRE colonization rate was high among patients admitted to hematology ICU. Rational use of antibiotics and active surveillance may be helpful preventive measures against the development of bacterial resistance to antimicrobial agents.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 175046; 2011–2014).

REFERENCES

1. *Willems RJ, Bonten MJ.* Glycopeptide-resistant enterococci: deciphering virulence, resistance and epidemicity. Curr Opin Infect Dis 2007; 20(4): 384−90.
2. *Sujatha S, Praharaj I.* Glycopeptide resistance in gram-positive cocci: a review. Interdiscip Perspect Infect Dis 2012; 2012: 781679.
3. Surveillance report. Antimicrobial resistance surveillance in Europe, 2009. Available from: www.ecdc.europa.eu/en/.../1011_SUR_annual_EARS_Net_2009
4. *Arias C, Murray B.* The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol 2012; 10(4): 266−78.
5. *Low DE, Keller N, Barth A, Jones RN.* Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis 2001; 32 Suppl 2: S133−45.
6. *Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A*, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill 2008; 13(47). pii: 19046.
7. ECDC. Antimicrobial resistance surveillance in Europe 2010. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Available from: http://repositorio.insa.pt/handle/10400.18/702
8. National Nosocomial Infections Surveillance (NNIS) System Report data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control 2004; 32(8): 470−85.
9. *Achenbach C, Flores E, Farerrell P, Pitrak D,Weber G.* Prevalence of risk factors for colonization with vancomicyn-resistant enterococcus among human immunodeficiency virus positive outpatients. Infect Control Hosp Epidemiol 2006; 27(1): 102−4.
10. *Sakka V, Tsiodras S, Galani L, Antoniadou A, Souli M, Galani I,* et al. Risk-factors and predictors of mortality in patients colonised with vancomycin-resistant enterococci. Clin Microbiol Infect 2008; 14(1): 14−21.
11. *Tacconelli E, Cataldo MA.* Vancomycin-resistant enterococci (VRE): transmission and control. Int J Antimicrob Agents 2008; 31: 99−106.
12. *Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM,* et al. SHEA Guideline for Preventing Nosocomial Transmission of Multidrug-Resistant Strains of Staphylococcus aureus and Enterococcus spp. Infect Control Hosp Epidemiol 2003; 24(5); 362−86.
13. *Yoon YK, Lee SE, Lee J, Kim HJ, Kim JY, Park DW*, et al. Risk factors for prolonged carriage of vancomycin-resistant Enterococcus faecium among patients in intensive care units: a case-control study. J Antimicrob Chemother 2011; 66(8): 1831−8.
14. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Testing; Seventeenth Informational Supplement M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
15. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; M02-A10. Approved Standard − Tenthth edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

16. *Gambarotto K, Ploy MC, Turlure P, Grélaud C, Martin C, Bordessoule D,* et al. Prevalence of vancomycin-resistant enterococci in fecal samples from hospitalized patients and nonhospitalized controls in a cattle-rearing area of France. J Clin Microbiol 2000; 38(2): 620−4.
17. *van den Braak N, Ott A, van Belkum A, Kluytmans JA, Koeleman JG, Spanjaard L,* et al. Prevalence and determinants of fecal colonization with vancomycin-resistant Enterococcus in hospitalized patients in The Netherlands. Infect Control Hosp Epidemiol 2000; 21(8): 520−4.
18. *Almyroudis NG, Lesse AJ, Hahn T, Samonis G, Hazamy PA, Wongkittiroch K*, et al. Molecular epidemiology and risk factors for colonization by vancomycin-resistant Enterococcus in patients with hematologic malignancies. Infect Control Hosp Epidemiol 2011; 32(5): 490−6.
19. *Liss BJ, Vehreschild JJ, Cornely OA, Hallek M, Fätkenheuer G, Wisplinghoff H,* et al. Intestinal colonisation and blood stream infections due to vancomycin-resistantenterococci (VRE) and extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBLE) in patients with haematological and oncological malignancies. Infection 2012; 40(6): 613−9.
20. *Matar MJ, Tarrand J, Raad I, Rolston KV*. Colonization and infection with vancomycin-resistant Enterococcus among patients with cancer. Am J Infect Control 2006; 34(8): 534−6.
21. *Suntharam N, Lankford MG, Trick WE, Peterson LR, Noskin GA.* Risk factors for acquisition of vancomycin-resistant enterococci among hematology-oncology patients. Diagn Microbiol Infect Dis 2002; 43(3): 183−8.
22. *Mirović V, Tomanović B, Konstantinović S*. The frequency of resistance to antibiotics of most frequently isolated bacteria from blood cultures during the period 1997-2002. Vojnosanit Pregl 2004; 61(4): 391−7. (Serbian)
23. *Cheong HJ, Song JY, Eom JS, Kim WJ, Choi SJ, Choi JH,* et al. Colonization rate, risk factor for acquisition, and genetic sensity of vancomicyn-resistant enterococci (VRE) isolated from culture of patients in intensive care unit from Korea. Korean J Infect Dis 2002; 34: 276−84.
24. *Metallidis S, Chatzidimitriou M, Tsona A, Bisiklis A, Lazaraki G, Koumentaki E,* et al. Vancomicyn-Resistant Enterococci, Colonizing the Intenstinal Tract of Patients in a University Hospital in Greece. Braz J Infect Dis 2006; 10(3): 179−84.
25. *Song JY, Cheong HJ, Jo YM, Choi WS, Noh JY, Heo JY,* et al. Vancomicyn-Resistant Enterococci colonization before admision to the intensive care unit: A clinico-epidemiological analysis. Am J Infect Control 2009; 37(9): 734−40.
26. *Padiglione AA, Wolfe R, Grabsch EA, Olden D, Pearson S, Franklin C,* et al. Risk Factors for New Detection of Vancomycin-Resistant Enterococci in Acute-Care Hospitals That Employ Strict Infection Control Procedures. Antimocrob Agents Chemother 2003; 47(8): 2492–8.
27. *Carmely Y, Samore MH, Huskins C.* The association between antecedent vancomicyn teatment and hospital-acquired vancomicyn-resistent eneterococci; a meta analysis. Arch Intern Med 1999; 159(20): 2461−8.
28. *Ostrovsky BE, Venkataraman L, D'Agata EM, Gold HS, De Girolami, Samore MH.* Vancomicyn-resistant enterococci intensive care units: high freguency of stool carrige during a non outbreak period. Arch Intern Med 1999; 159(13): 1467−72.
29. *de Bruin MA, Riley LW*. Does vancomycin prescribing intervention affect vancomycin-resistant enterococcus infection and colonization in hospitals? A systematic review. BMC Infect Dis 2007; 7: 24−35.
30. *Stiefel U, Paterson DL, Pultz NJ, Gordon SM, Aron DC, Donskey CJ*. Effect of the increasing use of piperacillin/tazobactam on the incidence of vancomycin-resistant enterococci in four academic medical centers. Infect Control Hosp Epidemiol 2004; 25(5): 380−3.
31. *Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA,* at al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 2008; 29(11): 996−1011.

Received on April 13, 2012.
Revised on September 6, 2012.
Accepted on October 23, 2012.

ORIGINAL ARTICLE

UDC: 616.13-007.64-07
DOI: 10.2298/VSP1312117N

Assessing the quality of angiographic display of brain blood vessels aneurysms compared to intraoperative state

Procena kvaliteta angiografskog prikaza aneurizmi krvnih sudova mozga u odnosu na intraoperativni nalaz

Igor M. Nikolić*†, Goran M. Tasić*†, Vladimir T. Jovanović*†, Nikola R. Repac*, Aleksandar M. Janićijević*, Vuk D. Šćepanović*, Branislav D. Nestorović*†

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

Abstract

Background/Aim. Aneurysms in brain blood vessels are expanding bags composed of a neck, body and fundus. Clear visibility of the neck, the position of the aneurysm and surrounding structures are necessary for a proper choice of methods for excluding the aneurysm from the circulation. The aim of this study was to evaluate the reliability of spatial reconstruction of blood vessels of the brain based on the original software for 3D reconstruction of the equipment manufacturer and a personal computer model developed earlier in the Clinic for Neurosurgery, Clinical Center of Serbia, Belgrade, compared to intraoperative identification of these aneurysms. **Methods.** This study included 137 patients of both sexes. The presence of an aneurysm was verified by angiographic methods [computed tomographic angiography (CTA), multislice computed tomography angiography (MSCTA), magnetic resonance imaging angiography (MRA), or digital subtraction angiography (DSA)]. **Results.** The quality score (0 to 5) for CTA was 3.180 ± 0.961, MSCTA 4.062 ± 0.928, and for DSA 4.588 ± 0.758 ($p < 0.01$). The results of this study favorite conventional angiography as the gold standard for diagnostic of intracranial aneurysms. **Conclusion.** The results of this study are consistent with current publications review and clearly recognize the advantages and disadvantages of diagnostic neuroradiological procedures, with DSA of brain blood vessels as a binding preoperative diagnostic procedure in cases in who it is not possible to clearly visualize the supporting blood vessel and neck of the aneurysm by using the findings of CTA, MRA and MSCTA.

Key words:
intracranial aneurysm; diagnosis; angiography; tomography, x-ray computed; magnetic resonance angiography; angiography, digital subtraction.

Apstrakt

Uvod/Cilj. Aneurizme na krvnim sudovima mozga predstavljaju vrećasta proširenja kod kojih se razlikuju vrat, telo i fundus. Jasna vizualizacija vrata, položaj aneurizme i odnos sa okolnim strukturama su uslov za adekvatnu odluku o izboru metode za isključenje aneurizme iz cirkulacije. Cilj ovog rada bio je da se proceni pouzdanost prostorne rekonstrukcije krvnih sudova mozga zasnovane na originalnom softveru za 3D rekonstrukciju proizvođača opreme i personalnom kompjuterskom modelu ranije razvijenom u Neurohirurškoj klinici Kliničkog Centra Srbije u odnosu na intraoperativnu identifikaciju ovih aneurizmi. **Metode.** Ova studija obuhvatila je 137 bolesnika oba pola (90 žena i 47 muškaraca). Prisustvo aneurizmi verifikovano je jednom od angiografskih metoda: kompjuterizovanom tomografskom angiografijom (CTA), multislajsnom kompjuterizovanom tomografskom angiografijom (MSCTA), angiografijom magnetnom rezonancom (MRA) ili digitalnom suptrakcionom angiografijom (DSA). **Rezultati.** Ocena kvaliteta (skor od 0 do 5) za CTA bila je 3,180 ± 0,961 za, MSCTA 4,062 ± 0,928, i za DSA 4,588 ± 0,758 ($p < 0.01$). Rezultati ove studije favorizuju konvencionalnu angiografiju kao zlatni standard u dijagnostici aneurizmi. **Zaključak.** Rezultati ove studije u skladu su sa pregledom aktuelnih publikacija i jasno prepoznaju dijagnostičke prednosti i nedostatke neuroradioloških procedura, pri čemu se DSA krvnih sudova mozga izdvaja kao obavezujuća preoperativna dijagnostička procedura kod bolesnika kod kojih nije moguća jasna vizualizacija nosećeg krvnog suda i vrata aneurizme na osnovu nalaza CTA, MSCTA i MRA.

Ključne reči:
aneurizma, intrakranijalna; dijagnoza; angiografija; tomografija, komjuterizovana, rendgenska; magnetna rezonanca, angiografija; angiografija, digitalna suptrakcijska.

Correspondence to: Igor M. Nikolić, Clinic of Neurosurgery, Clinical Center of Serbia, Koste Todorovića 4, 11 000, Belgrade, Serbia. E-mail: i.m.nikolic@gmail.com

Introduction

Aneurysms in brain blood vessels are expanding bags composed of a neck, body and fundus. A connection, an interaction between the neck and the body is not constant and determined, thus, there are small wide neck aneurysms, but also giant aneurysms with small necks. Clear visibility of the neck, the position of the aneurysm and surrounding structures are necessary for making a proper decision on methods for excluding an aneurysm from the circulation. In addition to conventional digital subtraction panangiography (DSA) of brain blood vessels [1], the development of sophisticated diagnostic procedures – computed tomography (CT) and magnetic resonance imaging (MRI) has enabled noninvasive imaging technology of blood vessels of the brain: CT angiography (CTA), 3D multislice (MSCT) angiography (MSCTA) and magnetic resonance angiography (MRA) [2].

Today's CTA technology allows visualization of aneurysm diameters greater than 0.7 mm, the console stand-out point of 100–120 HU, and shows only lumens of blood vessels with contrast and bone [3]. But despite this, the specified diagnosis is commonly used in many institutions as a screening method of choice, and sometimes even 3D-CTA is used as the sole diagnostic method [4–8].

Digital subtraction angiography (DSA) is based on digital elimination of bony structures so that the image shows an isolated artery in which we inject an iodine contrast agent. This is achieved by increasing the resolution until it reaches the value of 0.05 mm or by increasing the number of shoots until it reaches the number of 4–6 frames per second. 3D DSA is a software model based on rotational angiography that can show the fine structure of brain blood vessels, and this method is more accurate than standard DSA [9–11].

MRA is a noninvasive technique based on time-of-flight (TOF) sequences and contrast-enchanced MR (CEMR). There is a problem of spatial resolution, and the minimum detection volume is 3 mm [12]. It is possible to visualize aneurysm diameters less than 3 mm and is usually to detect those larger than 5 mm [13, 14]. Despite the developed software, sensitivity is less than that of DSA and is about 86–95% with a resolution of 0.2 mm^2. [8, 15–17], making it ideal as a noninvasive screening procedure [12, 14, 18].

Despite technological advances, digital DSA remains the gold standard for diagnosis. Newer methods, unfortunately, have much lower accuracy in the diagnosis [19, 20]. Microaneurysms can be visualized almost by the use of the DSA [8].

The aim of this study was to evaluate the reliability of spatial reconstruction of blood vessels of the brain based on the original software for 3D reconstruction of the equipment manufacturer and a personal computer model developed earlier in the Clinic of Neurosurgery at the Clinical Center of Serbia [21, 22] compared to the intraoperative identification of these aneurysms.

Methods

This study included 137 patients of both sexes. The presence of an aneurysm was verified by the angiographic methods (CTA, MSCT, MRA, or DSA). The analysis included patients who fulfiled the following requirements: clinically, lumbar puncture (LP) and endocranial CT verified attack of spontaneous subarachnoid hemorrhage (SAH); one or more aneurysms of the anterior cerebral artery stream of the base of the brain were verified using one or more angiographic procedures, made surgery or embolization; aneurysms of the carotid artery trunk (according to the small number of surgically treated aneurysms of the vertebrobasilar trunk).

In the analysis were used angiographic findings, spatial reconstruction of blood vessels of the brain based on the original software for 3D reconstruction of the equipment manufacturer, and based on computer models previously developed in our Clinic [21, 22].

A score of 0 to 5 was given to each angiographic finding according to the following criteria: aneurysm verification (negative findings exclude other criteria), aneurysm shape (0/1), aneurysm size (0/1), aneurysm orientation (0/1), aneurysm relationship to the carrying blood vessel (0/1), relationship of the aneurysm with perforators (0/1).

Aneurysm morphometric analysis, a comparative analysis of angiographic findings, and comparison of the quality of angiography in relation to the intraoperative findings were entered into questionnaire, followed by descriptive (measures of central tendency and dispersion measures), and analytical statistics. We used parametric (*t*-test) and nonparametric tests (χ^2 test, and median), as well as correlation tests (linear correlation and regression).

Data analysis was performed on a personal computer with Intel processor (generation of Intel Pentium III at 950 MHz, Intel QuadCore 6600 in the 2GHz Intel T6500 CoreTM2 Duo at 2.1 GHz) with a graphics card from Nvidia TNT 2 Pro with 32 MB, and Gforce Gforce G105M 8800 with 512 MB VRAM. Digitalisation of images when it was not in DICOM format was done using a scanner A4 HP ScanJet 5P (300 to 1200 dpi) and PoweShot camera Canon A710 IS (7.1 Mpixel).

Results

A total of 137 patients of both sexes (90 women and 47 men), the mean age 50.39 ± 8.25 years, were included in the study. The mean ages of the female and male patients were 52.15 ± 6.64 years, and 46.84 ± 9.96 years, respectively. The youngest patient was a 21-year-old and the oldest one a 72-year-old. There were 185 aneurysms in observed group: 164 (88.65%) were located in the carotid stream and 21 (11.35%) in the vertebrobasilar stream. The distribution of aneurysms by the carrying artery is shown in Table 1.

In 52 (37.96%) patients angiography was performed by using CTA. In 33% cases it was the only method of preoperative angiography. There were 17 diagnosis that were supplemented by DSA, and in 2 patients with MSCTA. In 9 (17.31%) patients, CTA was initially falsely negative, which was later confirmed by the subsequent diagnosis using DSA or MSCT. The largest number of false negative results was related to the internal carotid artery (ICA) (5).

Table 1

Carrying artery aneurysms distribution

Artery	Number
Internal carotid artery (ICA)	50
Anterior cerebral artery (ACA-A_1)	2
Anterior communication artery (AcoA)	41
Pericallosal artery (PA)	4
Medial cerebral artery (MCA)	67
Posterior cerebral artery (PCA)	3
Basilar artery (BA)	8
Superior cerebral artery (SCA)	4
Inferior anterior cerebral artery (IACA)	1
Posterior inferior cerebral artery (PICA)	3
Vertebral artery (VA)	2

A quality score was determined by CTA in 50 patients because ICA occlusion in the neck was found in 2 patients, and aneurysm was not visualized intraoperatively. The reliability score was 3.18 ± 0.96 with a median (Med) = 3. MSCTA was performed in 18 patients. Only in 5 patients it was the only method that was performed. A false-negative finding was observed in one patient with aneurysm on the anterior communication artery (AcoA) complex. Here, the aneurysm was confirmed with DSA and intraoperatively. In 13 patients, MSCTA was supplemented with DSA.

A quality score was determined by MSCTA in 16 patients, because in 2 patients embolization was performed, and aneurysm was no interoperatively visualized. The score was 4.06 ± 0.93 with a Med = 5

MRA was performed as initial diagnostic method in 12 patients during the acute phase of illness when they hospitalized in other centers. After diagnosing spontaneous subarachnoid hemorrhage caused by ruptured aneurysm they were referred for further treatment in the Clinic of Neurosurgery, Clinical Center of Serbia. All the patients underwent additional angiographic diagnosis by using DSA (11 patients) or CTA (4 patients).

It was shown that by using CTA significantly lower score for ICA aneurysm angiographic finding quality was obtained in comparison with the score obtained by using DSA. Statistically significant difference was not found between CTA and DSA in scores for medial cerebral artery (MCA) and AcoA aneurysm angiographic finding quality.

Due to the small number of patients submitted to MRA, which was not the only diagnostic procedure in neither case, statistical analysis of this method was not performed. Also, MRA was not performed on the same equipment in neither case, but at different magnetic fields (from 1.0 to 3.0 T).

DSA was performed in 97 patients. In 66 of the patients DSA was the only angiographic method for verification of intracranial aneurysms. In the remaining cases (n = 31), conventional angiography was the additional diagnostic procedure in preparing patients for surgery.

In all the patients angiography was performed for 3D reconstruction. In 46 patients 3D rotational angiography was performed as a part of diagnostic procedure, while the 51st patient subsequently made a spatial reconstruction based on 2D images and protocol for reconstruction in MatLab.

The quality score of DSA was determined in 71 patients submitted to direct intracranial aneurysm surgery. For other patients, occlusion was performed by endovascular procedure. The aneurysm was not visualized interoperatively. The quality score was 4.59 ± 0.76 with a Med = 5. If we compared DSA scores for MCA, ACA and ACI aneurysm visualization finding no statistically significant differences were found.

After evaluating reliability analysis for each of the diagnostic procedures used, comparative analysis among the differnet diagnostic procedures was done, with the exception of MRA. Table 3 shows the results of testing comparative reliability quality scores of CTA, MSCTA and DSA.

Table 2

Mean quality score of computed tomography angiography (CTA) and digital subtraction angiography (DSA) for carotid aneurysms

Carrying artery	CTA		DSA	
	mean	SD	mean	SD
MCA	3.48	1.40	4.65	0.80
AcoA	3.41	1.003	4.63	0.83
ICA	2.33	1.37	4.41	0.71

MCA – medial cerebral artery; ICA – internal carotid artery; AcoA – anterior communication artery.

Table 3

The results of the *t*-test for comparison of angiographic methods

Angiographic method	*t*	DF	*p*
CTA/MSCTA	3.222	64	< 0.01
CTA/DSA	8.310	119	< 0.01
MSCTA/DSA	2.080	85	< 0.05

CTA – computed tomography angiography; MSCTA – multislice computed tomography angiography; DSA – digital subtraction angiography.

Discussion

In the observed group of patients standardized CTA was performed in 52 patients, while MSCTA was performed in 18 patients. MSCTA was performed in a much smaller number of cases because the equipment was purchased later (it entered in the second half of the study). False negative findings on CTA were found by the subsequent additional diagnostics (DSA or MSCTA) in 9 (17.31%) patients, while 5 of them had the ICA aneurysms. When evaluating the reliability of methods for intracranial aneurysms detection and its relationship with the supporting blood vessel and perforators, CTA assessment received score of 3.18 ± 0.96, and MSCTA 4.06 ± 0.93 (found only one false-negative finding). Despite of almost equal quality of CTA and DSA for detection of middle cerebral artery aneurysms, the grades observed sample was considerably different and there was a statistically significant difference in Korst DSA as a better method [23]. On the other hand, Dehdasti et al. [24] in comparative analysis indicate that CTA is still insufficient compared to DSA.

Because of the fact that the accuracy in the diagnosis of intracranial aneurysms according to literature data is about 84.6%, we emphasize that our findings are consistent with

the literature [25]. This lack of precision primarily relates to the detection of small aneurysms, subtraction bone in infraclinoid aneurysms, the lack of the knowledgable of radiologists, and artifacts that give previously placed clips or coil [3, 26–31]. The problem with small aneurysms detection, and increased accuracy compared to standard CTA that works on a four- or 16-slice aparatus 64MSCTA somewhat beyond, and it can be almost comparable with the standard 2D DSA [26, 32]. But still for aneurysms less than 4 mm a combination of DSA with 3D rotational angiography (3DRA) is the method of choice [33]. Also, the development of "dual-energy direct bone removal" CTA technique (DE-BR-CTA) was started in the late 70s, has enabled great progress in subtraction bone structures in MSCT, thus the image quality is much closer to conventional angiography [32, 34]. 64MSCTA can be used as an initial diagnostic procedure and to assess whether it is suitable for direct aneurysm surgery or embolization, given the possibility of 3D reconstruction in subtraction [32]. A significant progress in subtraction resulted in orbital synchronized helical scan (OSHST) technique that allows a piston or a subtraction coil on postoperative recordings [35]. However, despite the development of all CT, angiography is limited in diagnosing aneurysms of the posterior stream [36].

Despite the shortcomings CTA and MSCT are not only used as initial diagnostic procedure and the screening method of choice, but in some establishments 3D-CTA is used as the sole diagnostic method for preoperative treatment of patients with spontaneous subarachnoid hemorrhage [4–8, 37, 38]. We should not ignore the cost of diagnostic procedures, because the cost of 3D CTA is significantly lower than the cost of DSA, and can be considered as the method of choice in screening [39]. Due to lower prices and a small number of complications, this method is very suitable for postoperative monitoring and evaluation, as well as the only diagnosis in elderly patients with degenrative altered blood vessels, where catheter placement is difficult [40, 41].

Conventional angiography is an invasive method for radiological detection of blood vessels and their pathological changes. The biggest positive step forward is made by the introduction of digital substraction and computer models with which we eliminate the bone structure. DSA with spatial reconstruction is the gold standard in diagnosing intracranial aneurysms [42–44]. Also, van Rooij et al. [45, 46], in their studies emphasize the advantage of rotational angiography over conventional angiography for detection of small aneurysms. They believe that negative DSA angiographic findings should be done by the 3D-DSA, because in a great number of cases small aneurysms can be detected with this method [45]. According to Hai et al. [47] it is of great benefit in planning 3DRA embolization of small aneurysms, the procedure itself reduces the radiation dose in comparison to conventional DSA.

However, Hirai et al. [48] stress that the lack of methods is false pseudostenosis of the intracranial blood vessels. This phenomenon is related to the angle at which it is rotational angiography and length of blood vessel [48]. A reduction in artifacts of pseudostenosis solved by the introduction of flat panel detector (FPD) system [49].

In more than 2/3 (70.8%) of the patients in the observed group DSA was performed. Study on comparative analysis of biplanar DSA and 3D angiography demonstrated the advantages of 3-dimensional images, suggesting that all patients should be subjected to it for spatial reconstruction of brain blood vessels and intracranial aneurysms. It is done either in the form of 3DRA as a part of standard diagnostic apparatus using the Siemens Axiom Artis, or in the form of computer reconstructions based on MatLab biplanarnih shots (PA and lateral projections) [21, 22, 50–52].

False negative findings were not found in any case, reliability score was performed in 71 patients with direct aneurysm surgery. DSA with spatial reconstruction in the observed group received a high score of 4.59 ± 0.76. This relatively higher score of reliability is consistent with literature. DSA with 3D angiography is still considered to be a gold standard, because of the fact that microaneurysms can be visualized almost by the use of the DSA [8, 42, 45, 53, 54]. Imperfections in the diagnosis among other things can be explained by Jou et al. [55]. Artifacts can be created by the effect of pulsations, gravity and unequal density and contrast levels.

Aneurysms of various localization were slightly less noticeable than aneurysms in ICA, but if we compare the grades among themselves there were no statistically significant differences ($p > 0.05$). It is not a small problem in the conversion of pixels as the relative size in millimeters, *ie.* real size. Fox et al. [56] conducted a study on comparative analysis of digital angiography in different picture archiving and communication systems (PACS). Kawashima et al. [57] emphasize that there are differences in morphometric measurements between biplanar DSA and 3D subtraction angiography. In this study comparative analysis of the intraoperative findings was not performed, thus the conclusion about a more realistic value could not be drawn. Beck et al. [58] find that the dimensions of aneurysms by 3DRA are not less than by biplanar DSA and are close to their actual sizes.

Several studies demonstrated the application of 3DRA within the system neuronavigation. Raabe et al. [59] carried out a number of operations of the intracranial aneurysms using BrainLab's apparatus for neuronavigation, Vector Vision 2 and the Philips Integris Allura System angiographic. Previously it was used during diagnostic frame for registration. During the work it was shown that the maximum error is in the angulation of 90° and 90° in the rotation. According to them, and despite the fact that such a system requires further improvement it provides a useful topographical information about the vascular anatomy [59]. Willems et al. [60] developed the integrated 3DRA neuronavigation system and the first phantom, and then applied it in the course of operations. They used a Philips Integris BV5000 angiographic apparatus and Medtronic's neuronavigation StealthStation system. Impossibility of direct transfer of data from the angiographic system console to the console of the neuronavigation system, they overcame by using a dynamic reference frame (DRF). By this way they were enabled to enhance visualization of the operation of complex giant aneurysms and arteriovenous malformations [60].

MRI, or nuclear magnetic resonance (NMR) imaging, is a noninvasive diagnostic procedure without the use of ionizing radiation. Because of that it is ideal as a noninvasive screening procedure [12, 14, 18]. Also it is grateful for its noninvasive and long-term monitoring of patients after treatment [61–63]. With magnetic field intensification and matrix size recording, resolution and sensitivity increase as indicated by phantom studies [64, 65]. Clinical study on diagnosing unruptured aneurysms using DSA of 1.5T and 7.0T as the reference standard by Mönninghoff et al. [66] showed the advantage of a stronger magnetic field in the detection of intracranial aneurysms. Gibbs et al. [67] obtained similar results. They also reduced magnetic field amplification and recording time.

Sensitivity, despite the developed software, is always lower than in DSA and is about 86–95% with a resolution of 0.2 mm^2 [20]. Schwab et al. [53] did a comparative analysis of MRA and DSA findings in 133 patients with aneurysms and found deviation of MRA findings with regard to DSA in as much as 59%. These differences relate not only to the location and number of aneurysms, but also to the type of aneurysm. Therefore, they recommend screening before using CTA as a less invasive method of DSA [53]. In order to assess the quality of MRA as a screening method, Hiratsuka et al. [68] worked comparative analysis of 3T MRA 64MDCTA with 3D-DA as a reference standard. Their study of 38 patients with nonruptured aneurysms showed no statistically significant difference between MRA and MDCT. In both methods we used volume rendering, and the advantage of it is contrary to Schwab et al. [53] because MRA is completely noninvasive and does not use contrast agents [68].

Conclusion

The results of this study, consistent with the review of current publications, clearly recognize the advantages and disadvantages of diagnostic neuroradiological procedures, with DSA of brain vessels as a binding preoperative diagnostic procedure in case that findings on CT, MRA and MSCT are not enough for clear visualization of the supporting blood vessel and the neck of aneurysm.

REFERENCES

1. *Kato Y, Nair S, Sano H, Sanjaykumar MS, Katada K, Hayakawa M,* et al. Multi-slice 3D-CTA - an improvement over single slice helical CTA for cerebral aneurysms. Acta Neruochir (Wien) 2002; 144(7): 715−22.
2. *Morhard D, Ertl-Wagner B.* MDCT in Neuro-Vascular Imaging. In: *Reiser MF, Becker, Nikolaou K, Glazer G,* editors. Multi-Slice CT. Berlin: Springer-Verlag; 2009. p. 123−36.
3. *Wolf RL.* CT Imaging and Physiologic Techniques in Interventional Neuroradiology. In: *Hurst RW, Rosenwasser RH,* editors. Interventional neuroradiology. New York: Informa Healthcare; 2008. p. 87−111.
4. *Kato Y, Sano H, Katada K, Ogura Y, Hayakawa M, Kanaoka N,* et al. Application of three-dimensional CT angiography (3D-CTA) to cerebral aneurysms. Surg Neurol 1999; 52(2): 113−21.
5. *Kato Y, Hayakawa M, Katada K.* Three-dimensional multislice helical CT angiography of cerebral aneurysms. Nippon Rinsho 2004. 62(4): 715−21. (Japanese)
6. *Matsumoto M, Sato M, Nakano M, Endo Y, Watanabe Y, Sasaki T,* et al. Three-dimensional computerized tomography angiography-guided surgery of acutely ruptured cerebral aneurysms. J Neurosurg, 2001; 94(5): 718−27.
7. *Matsumoto M, Endo Y, Sato M, Sato S, Sakuma J, Konno Y*, et al. Acute aneurysm surgery using three-dimensional CT angiogrphy without conventional catheter angiography. Fukushima J Med Sci 2002. 48(2): 63−73.
8. *Kornienko VN, Pronin IN.* Diagnostic Neuroradiology. Berlin: Springer-Verlag; 2009:
9. *Sugahara T, Korogi Y, Nakashima K, Hamatake S, Honda S, Takahashi M.* Comparison of 2D and 3D digital subtraction angiography in evaluation of intracranial aneurysms. AJNR Am J Neuroradiol 2002; 23(9): 1545−52.
10. *Manabe H, Takemura A, Hasegawa S, Nagahata M, Iko Y.* Extravasation from Rupturing Aneurysm Demonstrated by 3D Digital Subtraction Angiography. AJNR Am J Neuroradiol, 2005; 26(6): 1370−1.
11. *Asir A, Guven G, Tekin T, Kutlay M, Colak A, Simsek H,* et al. Three-dimensional versus conventional digital subtraction angiography in the diagnosis of intracranial aneurysms: case report. Balkan Military Medical Review 2007; 10: 147−9.
12. *Huston J 3rd.* Magnetic Resonance Angiography. In: *Winn RH*, editor. Youman`s Neurological Surgery. Philadelphia: Saunders; 2004. p. 1575−99.
13. *Huston J 3rd, Torres VE, Sulivan PP, Offord KP, Wiebers DO.* Value of magnetic resonance angiography for the detection of intracranial aneurysms in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 1993. 3(12): 1871−7.
14. *Hughes DG.* Neuroradiology and Ultrasound. In: *Moore AJ, Newell DV*, editor. Neurosurgery: Principles and Practice. London: Springer; 2005. p. 23−8.
15. *Anzalone N, Scomazzoni F, Cirillo M, Righi C, Simionato F, Cadioli M,* et al. Follow-up of coiled cerebral aneurysms at 3T: comparison of 3D time-of-flight MR angiography and contrast-enhanced MR angiography. AJNR Am J Neuroradiol 2008; 29(8): 1530−6.
16. *Huston J 3rd, Lewis BD, Wiebers DO, Meyer FB, Riederer SJ, Weaver AL.* Carotid artery: prospective blinded comparison of two-dimensional time-of-flight MR angiography with conventional angiography and duplex US. Radiology 1993; 186(2): 339−44.
17. *Huston J 3rd, Ehman RL.* Comparison of time-of-flight and phase-contrast MR neuroangiographic techniques. Radiographics 1993; 13(1): 5−15.
18. *Berisavac I, Bojović V, Bizjak B, Ateljević M, Bićanin G.* Intracranial aneurysmal bleeding. Subotica: Biografika; 1998. (Serbian)
19. *Satoh T.* Delineation of cerebral aneurysms with fly-through imaging of 3D-MRA using perspective volume rendering. No Shinkei Geka 2001; 29(2): 181−6. (Japanese)
20. *Watanabe Z, Kikuchi Y, Izaki K, Hanzu N, Gotou H, Koizumi J*, et al. The usfulness of 3D MR angiography in surgery for ruptured cerebral aneurysms. Surg Neurol 2001; 55(6): 359−64.
21. *Nikolić IM.* Computer-assisted spatial reconstruction of cerebral blood vessels and intra cranial aneurysms. Med Pregl 2006; 59(1−2): 24−7. (Serbian)
22. *Nikolić IM.* The application of mathematical modeling in the preoperative preparation of patients with cerebrovascular malformations - aneurysms. Belgrade: Faculty of Medicine, University of Belgrade; 2003. (Serbian)

23. *Villablanca JP, Hooshi P, Martin N, Jahan R, Duckwiler G, Lim S*, et al. Three-dimensional helical computerized tomography angiography in the diagnosis, characterization, and management of middle cerebral artery aneurysms: comparison with conventional angiography and intraoperative findings. J Neurosurg 2002; 97(6): 1322−32.
24. *Dehdashti AR, Rufenacht DA, Delavelle J, Reverdin A, de Tribolet N.* Therapeutic decision and management of aneurysmal subarachnoid haemorrhage based on computed tomographic angiography. Br J Neurosurg 2003; 17(1): 46−53.
25. *Schuknecht B.* High-concentration contrast media (HCCM) in CT angiography of the carotid system: impact on therapeutic decision making. Neuroradiology 2007; 49(Suppl 1): S15−26.
26. *Li Q, Lv F, Li Y, Li K, Luo T, Xie P.* Subtraction CT angiography for evaluation of intracranial aneurysms: comparison with conventional CT angiography. Eur Radiol 2009; 19(9): 2261−7.
27. *González-Darder JM, Pesudo-Martínez JV, Feliu-Tatay RA.* Microsurgical management of cerebral aneurysms based in CT angiography with three-dimensional reconstruction (3D-CTA) and without preoperative cerebral angiography. Acta Neruochir (Wien) 2001; 143(7): 673−9.
28. *Meyer RE, Nickerson JP, Burbank HN, Alsofrom GF, Linnell GJ, Filippi CG.* Discrepancy rates of on-call radiology residents' interpretations of CT angiography studies of the neck and circle of Willis. AJR Am J Roentgenol 2009; 193(2): 527−32.
29. *Livingston RR.* Regarding the Risk of Death from CT Angiography in patients with Subarachnoid Hemorrhage. AJNR Am J Neuroradiol 2008; 29(6): e44; author reply e46−7.
30. *Karamessini MT, Kagadis GC, Petsas T, Karnabatidis D, Konstantinou D, Sakellaropoulos GC,* et al. CT angiography with three-dimensional techniques for the early diagnosis of intracranial aneurysms. Comparison with intra-arterial DSA and the surgical findings. Eur J Radiol 2004; 49(3): 212−23.
31. *Romijn M, Gratama van Andel HA, van Walderveen MA, Sprengers ME, van Rijn JC, van Rooij WJ*, et al. Diagnostic accuracy of CT angiography with matched mask bone elimination for detection of intracranial aneurysms: comparison with digital subtraction angiography and 3D rotational angiography. AJNR Am J Neuroradiol 2008; 29(1): 134−9.
32. *Li Q, Lv F, Li Y, Luo T, Li K, Xie P.* Evaluation of 64-section CT angiography for detection and treatment planning of intracranial aneurysms by using DSA and surgical findings. Radiology 2009; 252(3): 808−15.
33. *McKinney AM, Palmer CS, Truwit CL, Karagulle A, Teksam M.* Detection of aneurysms by 64-section multidetector CT angiography in patients acutely suspected of having an intracranial aneurysm and comparison with digital subtraction and 3D rotational angiography. AJNR Am J Neuroradiol 2008; 29(3): 594−602.
34. *Watanabe Y, Uotani K, Nakazawa T, Higashi M, Yamada N, Hori Y,* et al. Dual-energy direct bone removal CT angiography for evaluation of intracranial aneurysm or stenosis: comparison with conventional digital subtraction angiography. Eur Radiol 2009; 19(4): 1019−24.
35. *Watanabe Y, Kashiwagi N, Yamada N, Higashi M, Fukuda T, Morikawa S*, et al. Subtraction 3D CT angiography with the orbital synchronized helical scan technique for the evaluation of postoperative cerebral aneurysms treated with cobalt-alloy clips. AJNR Am J Neuroradiol 2008; 29(6): 1071−5.
36. *Amagasaki K, Takeuchi N, Sato T, Kakizawa T, Shimizu T.* Current usage of three-dimensional computed tomography angiography for the diagnosis and treatment of ruptured cerebral aneurysms. J Clin Neurosci 2004; 11(5): 481−5.
37. *Agid R, Willinsky RA, Farb RI, Terbrugge KG.* Life at the end of the tunnel: why emergent CT angiography should be done for patients with acute subarachnoid hemorrhage. AJNR Am J Neuroradiol 2008. 29(6): e45; author reply e46−7.
38. *Fox AJ, Symons SP, Aviv RI.* CT angiography is state-of-the-art first vascular imaging for subarachnoid hemorrhage. AJNR Am J Neuroradiol 2008; 29(6): e41−2.
39. *Erdem Y, Yilmaz A, Ergün E, Koşar U, Karatay M, Bayar MA.* Bilateral internal carotid artery hypoplasia and multiple posterior circulation aneurysms. Importance of 3DCTA for the diagnosis. Turk Neurosurg 2009; 19(2): 168−71.
40. *Pechlivanis I, Harders A, Tuttenberg J, Barth M, Schulte-Altedorneburg G, Schmieder K.* Computed tomographic angiography: diagnostic procedure of choice in the management of subarachnoid hemorrhage in the elderly patient? Cerebrovasc Dis 2009; 28(5): 481−9.
41. *Chen W, Yang Y, Qiu J, Peng Y, Xing W.* Clinical application of 16-row multislice computed tomographic angiography in the preoperative and postoperative evaluation of intracranial aneurysms for surgical clipping. Surg Neurol 2009; 71(5): 559−65.
42. *Weyerbrock A, Woznica M, Rosahl SK, Berlis A.* Aneurysmal and Non-Aneurysmal SAH - Is Initial Computed Tomography Predictive? Rofo 2009; 181(9): 881−7.
43. van Rooij SB, van Rooij WJ, Sluzewski M, Sprengers ME. Fenestrations of intracranial arteries detected with 3D rotational angiography. AJNR Am J Neuroradiol 2009; 30(7): 1347−50.
44. *de Gast AN, van Rooij WJ, Sluzewski M.* Fenestrations of the anterior communicating artery: incidence on 3D angiography and relationship to aneurysms. AJNR Am J Neuroradiol 2008; 29(2): 296−8.
45. *van Rooij WJ, Peluso JP, Sluzewski M, Beute GN.* Additional Value of 3D Rotational Angiography in Angiographically Negative Aneurysmal Subarachnoid Hemorrhage: How Negative is Negative? AJNR Am J Neuroradiol 2008; 29(5): 962−6.
46. *van Rooij WJ, Sprengers ME, de Gast AN, Peluso JP, Sluzewski M.* 3D rotational angiography: the new gold standard in the detection of additional intracranial aneurysms. AJNR Am J Neuroradiol 2008; 29(5): 976−9.
47. *Hai J, Deng DF, Chen ZQ, Pan QG.* Endovascular embolization of small ruptured intracranial aneurysms using a biplane angiographic system with three-dimensional rotational digital subtraction angiography. J Clin Neurosci 2009; 16(8): 1028−33.
48. *Hirai T, Korogi Y, Ono K, Yamura M, Uemura S, Yamashita Y.* Pseudostenosis phenomenon at volume-rendered three-dimensional digital angiography of intracranial arteries: frequency, location, and effect on image evaluation. Radiology 2004; 232(3): 882−7.
49. *Kakeda S, Korogi Y, Ohnari N, Hatakeyama Y, Moriya J, Oda N,* et al. 3D digital subtraction angiography of intracranial aneurysms: comparison of flat panel detector with conventional image intensifier TV system using a vascular phantom. AJNR Am J Neuroradiol 2007; 28(5): 839−43.
50. *Nikolić I, Milovanović D, Antunović V, Stanković S.* The Evaluation of Computer Based 3-D Reconstruction of MCA Aneurysms. J Automatic Control 2005; 15(Suppl): 35−7.
51. *Nikolić IM, Nagulić M, Antunović V.* Significance of the spatial reconstruction based on mathematical modeling in the surgical treatment of giant intracranial aneurysms. Vojnosanit Pregl 2006; 63(1): 65−8. (Serbian)
52. *Nikolić IM, Rakić MLj, Slavik EE, Tasić GM, Đurović BM, Jovanović VT,* et al. Space reconstruction of the aneurysms of the vertebrobasilary confluence based on conventional angiography-our experience. Acta Chir Iugosl 2008; 55(2): 75−8. (Serbian)
53. *Schwab KE, Gailloud P, Wyse G, Tamargo RJ.* Limitations of magnetic resonance imaging and magnetic resonance angiography in the diagnosis of intracranial aneurysms. Neurosurgery 2008; 63(1): 29−35.
54. *Piotin M, Gailloud P, Bidaut L, Mandai S, Muster M, Moret J,* et al. CT angiography, MR angiography and rotational digital subtraction angiography for volumetric assessment of intracranial

aneurysms. An experimental study. Neuroradiology 2003; 45(6): 404−9.
55. *Jou LD, Mohamed A, Lee DH, Mawad ME*. 3D rotational digital subtraction angiography may underestimate intracranial aneurysms: findings from two basilar aneurysms. AJNR Am J Neuroradiol 2007; 28(9): 1690−2.
56. *Fox AJ, Millar J, Raymond J, Pryor JC, Roy D, Tomlinson GA*, et al. Dangerous Advances in Measurements from Digital Subtraction angiography: When Is a Milimeter Not a Milimeter? AJNR Am J Neuroradiol 2009; 30(3): 459−61.
57. *Kawashima M, Kitahara T, Soma K, Fujii K.* Three-dimensional digital subtraction angiography vs two-dimensional digital subtraction angiography for detection of ruptured intracranial aneurysms: A study of 86 aneurysms. Neurol India 2005; 53(3): 287−9; discussion 290.
58. *Beck J, Rohde S, Berkefeld J, Seifert V, Raabe A.* Size and location of ruptured and unruptured intracranial aneurysms measured by 3-dimensional rotational anegiography. Surg Neurol 2006; 65(1): 18−25.
59. *Raabe A, Beck J, Rohde S, Berkefeld J, Seifert V.* Three-dimensional rotational angiography guidance for aneurysm surgery. J Neurosurg 2006; 105(3): 406−11.
60. *Willems PW, van Walsum T, Woerdeman PA, van de Kraats EB, de Kort GA, Niessen WJ*, et al. Image-guided vascular neurosurgery based on three-dimensional rotational angiography. Technical note. J Neurosurg 2007; 106(3): 501−6.
61. *Pierot L., Delcourt C, Bouquigny F, Breidt D, Feuillet B, Lanoix O*, et al. Follow-up of intracranial aneurysms selectively treated with coils: Prospective evaluation of contrast-enhanced MR angiography. AJNR Am J Neuroradiol 2006; 27(4): 744−9.
62. *Majoie CB, Sprengers ME, van Rooij WJ, Lavini C, Sluzewski M, van Rijn JC,* et al. MR angiography at 3T versus digital subtraction angiography in the follow-up of intracranial aneurysms treated with detachable coils. AJNR Am J Neuroradiol 2005; 26(6): 1349−56.
63. *Thornton J, Debrun GM, Aletich VA, Bashir Q, Charbel FT, Ausman J.* Follow-up angiography of intracranial aneurysms treated with endovascular placement of Guglielmi detachable coils. Neurosurgery 2002; 50(2): 239−50.
64. *Kakeda S, Korogi Y, Hiai Y, Sato T, Ohnari N, Moriya J,* et al. MRA of intracranial aneurysms embolized with platinum coils: a vascular phantom study at 1.5T and 3T. J Magn Reson Imaging 2008; 28(1): 13−20.
65. *Hiai Y, Kakeda S, Sato T, Ohnari N, Moriya J, Kitajima M,* et al. 3D TOF MRA of intracranial aneurysms at 1.5 T and 3 T: influence of matrix, parallel imaging, and acquisition time on image quality - a vascular phantom study. Acad Radiol 2008; 15(5): 635−40.
66. *Mönninghoff C, Maderwald S, Theysohn JM, Kraff O, Ladd SC, Ladd ME,* et al. Evaluation of intracranial aneurysms with 7 T versus 1.5 T time-of-flight MR angiography - initial experience. Rofo 2009; 181(1): 16−23.
67. *Gibbs GF, Huston J 3rd, Bernstein MA, Riederer SJ, Brown RD Jr.* Improved image quality of intracranial aneurysms: 3.0-T versus 1.5-T time-of-flight MR angiography. AJNR Am J Neuroradiol 2004; 25(1): 84−7.
68. *Hiratsuka Y, Miki H, Kiriyama I, Kikuchi K, Takahashi S, Matsubara I,* et al. Diagnosis of unruptured intracranial aneurysms: 3T MR angiography versus 64-channel multi-detector row CT angiography. Magn Reson Med Sci 2008; 7(4): 169−78.

Received on April 25, 2012.
Revised on July 13, 2012.
Accepted on September 27, 2012.

ORIGINAL ARTICLE

UDC: 611.847::617.77-089
DOI: 10.2298/VSP1312124D

Surgical anatomy and histology of the *levator palpebrae superioris* muscle for blepharoptosis correction

Hirurška anatomija i histologija mišića podizača gornjeg kapka u korekciji blefaroptoze

Boban Djordjević*, Marijan Novaković†‡, Milan Milisavljević§, Saša Milićević*, Aleksandar Maliković§

***Clinic for Plastic Surgery and Burns, ‡Head Office, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia; §Institute of Anatomy, Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

Abstract

Background/Aim. The detailed knowledge of the architecture of the upper eyelid is very important in numerous upper eyelid corrective surgeries. The article deals with the detailed anatomy of the major components of the upper lid, which are commonly seen in surgical practice. **Methods.** This study was conducted on 19 human cadavers (12 adults and 7 infants) without pathologic changes in the orbital region and eyelids. Anatomic microdissection of the contents of the orbita was performed bilaterally on 12 orbits from 6 unfixed cadavers (3 male and 3 female). Micromorphologic investigations of the orbital tissue were performed on 8 *en bloc* excised and formalin-fixed orbits of infant cadavers. Specimens were fixed according to the Duvernoy method. An intra-arterial injection of 5% mixture of melt formalin and black ink was administered into the carotid arterial system. Using routine fixation, decalcination, dehydration, illumination, impregnation and molding procedures in paraplast, specimens were prepared for cross-sections. **Results.** The measurement of the muscle length and diameter *in situ* in 6 nonfixed cadavers (12 orbits) showed an average length of the *levator palpbrae superioris* (LPS) muscle body of the 42.0 ± 1.41 mm on the right, and 40.3 ± 1.63 mm on the left side. In all the cases, the LPS had blood supply from 4 different arterial systems: the lacrimal, supratrochlear, and supraorbital artery and muscle branches of the ophthalmic artery. The LPS muscle in all the specimens was supplied by the superior medial branch of the oculomotor nerve. The connective tissue associated with the LPS muscle contains two transverse ligaments: the superior (Whitnall's) and intermuscular transverse ligaments (ITL). The orbital septum in all the specimens originated from the arcus marginalis of the frontal bone, and consisted of two layers – the superficial and the inner layer. In addition, a detailed histological analysis revealed that the upper eyelid's crease was formed by the conjoined fascia including the fascia of the orbicularis muscle, the superficial layer of the orbital septum, and the aponeurosis of the LPS muscle, as well as the pretarsal fascia. **Conclusion.** The conducted study provided a valuable morphological basis for biomechanical and clinical considerations regarding blepharoptosis surgery.

Key words:
oculomotor muscles; blepharoptosis; microdissection; oculomotor nerve.

Apstrakt

Uvod/Cilj. Detaljno poznavanje građe gornjeg kapka veoma je važno za mnogobrojne korektivne hirurške zahvate na gornjem kapku. Ovaj članak bavi se detaljnom anatomijom glavnih struktura gornjeg kapka koji se obično susreću u hirurškoj praksi. **Metode.** Studija je sprovedena na 19 ljudskih kadavera (12 odraslih i 7 odojčadi) bez patoloških promena u orbitalnoj regiji i kapcima. Anatomska mikrodisekcija sadržaja orbite sprovedena je na 12 orbita, obostrano na 6 svežih kadavera odraslih (3 muška i 3 ženska). Mikromorfološka ispitivanja struktura orbite izvedena su na 8 *en bloc* orbita kadavera odojčadi fiksiranih formalinom. Preparati su fiksirani Duvernoy metodom. U karotidni sistem intraarterijski je ubrizgavana mešavina 5% rastvora formalina i crnog tuša. Rutinskom procedurom, koja obuhvata fiksaciju, dekalcifikaciju, dehidrataciju, prosvetljavanje, impregnaciju i kalupljenje u paraplastu, uzorci su pripremani za pravljenje preseka. **Rezultati.** Merenje dužine i širine mišića na šest svežih kadavera (12 orbita) pokazalo je prosečnu dužinu tela mišića *levator palpebrae superioris* (LPS) od 42,0 ± 1,41 mm na desnoj strani, a 40,3 ± 1,63 mm na levoj strani. U svim slučajevima, LPS je bio vaskularizovan iz četiri različita arterijska sistema: *a. lacrimalis*, *a. supratrochlearis*, *a. supraorbitalis* i mi-

Correspondence to: Boban Djordjević, Milovana Šaranovića 14, 11 000 Belgrade, Serbia. Phone: +381 64 2 096 096.
E-mail: bobandjordjevic68@gmail.com

šićnih grana *a. ophthalmicae*. Mišić LPS u svim slučajevima inervisala je gornja medijalna grana *n. oculomotoriusa*. Ustanovljeno je da je vezivno tkivo povezano sa LPS mišićem, sastavljeno od dva poprečna ligamenta: gornjeg (Whitnallovog) i intermuskularnog poprečnog ligamenta (ITL). Orbitalni septum je u svim slučajevima polazio od supraorbitalne ivice čeone kosti i sastojao se od dva sloja – površnog i dubokog. Osim toga, detaljna histološka analiza pokazala je da gornji kapačni žleb formira fascija mišića *orbicularis oculi*, površni sloj orbitalnog septuma, aponeuroza mišića *levator palpebrae superioris* i pretarzalna fascija. **Zaključak.** Istraživanjem je obezbeđena važna morfološka osnova za biomehanička i klinička ispitivanja u hirurgiji blefaroptoza.

Ključne reči:
mišići, okulomotorni; blefaroptoza; mikrodisekcija; n. oculomotorius.

Introduction

A wider knowledge of the *levator palpebrae superioris* (LPS) muscle and the suspensory fibrous tissue related to the LPS muscle is essential for the eyelid surgery, especially for the blepharoptosis correction. Even though, there are several papers providing the description of the eyelid anatomy, anatomic features of the *orbicularis oculi* and the LPS muscle, there are various anatomical details that are still controversial. In particular, the importance of the orbital connective tissue and the role they play in pathogenesis or the blepharoptosis treatment are still unclear.

In most patients with congenital and acquired blepharoptosis, palpebral creases are not so distinctive. In addition, Anderson et al. [1] described an atrophic and dehiscent superior transverse ligament (Whitnall's ligament-WL [2]) in those patients. Fink et al. [3] first reported the presence of the connective tissue which underlines the LPS muscle, the tissue that was called the intermuscular transverse ligament (ITL) by Lukas et al. [4]. However, the functional role of these ligaments in the LPS muscle is still unclear.

The aim of this study was to reinvestigate the detailed anatomy of the connective tissues related to the LPS muscle, and to establish their role in the suspension and creation of normal upper eyelid contours in the occidental race.

Methods

The anatomic microdissection study and histological analysis were conducted on 19 human cadavers (12 adults and 7 infants). We used microdissection techniques for the orbits from formalin-unfixed/fixed cadavers, as well as micromorphologic investigation methods.

The anatomic microdissection of the contents of the orbita through the anterior cranial fossa (transcranial approach) was performed bilaterally on 12 orbits from six unfixed cadavers (3 male and 3 female). The cadavers, aged 42–75 years (the mean age was 60 years) had no pathologic changes in the orbital region and eyelids. We used the standard technique for the brain extraction, and the customary abduction technique to remove the orbital bony roof. The periorbita was then incised, and turned laterally so that the levator muscle of the upper eyelid could be seen. Anteriorly, we found the transverse fibrous strands of Whitnall's ligament, and, after cutting the LPS muscle proximally, an intramuscular transverse ligament with connections to the bone of the orbital roof. The space under the aponeurosis of the LPS muscle was dissected to expose the tarsal plate. Microdissection of the orbital subject was performed using a stereo magnifying glass (4×).

The method of standard dissection of 12 orbits from 6 formalin fixed cadavers (3 male and 3 female) was performed by the combined transcutaneous and transcranial approach. Incisions were made on the skin, the orbicularis oculi muscle was exposed, and a strip of *orbicularis oculi* muscle was turned aside. After the junction of the orbital septum and levator aponeurosis was observed, a blunt dissection was carried out through the outer layer of the orbital septum, and continued to the Whitnall's ligament between the levator aponeurosis and the inner layer of the orbital septum, which covers the posterior surface of the orbital fat.

All the specimens were photographed by a Sony Cyber Shot DCS P8 digital camera.

Micromorphologic investigations of the orbital tissue were performed on 8 *en bloc* excised and formalin-fixed orbits of infant cadavers with no pathologic changes in the orbital region and eyelids. An intra-arterial injection of 5% mixture of melt formalin and black ink was administered in the carotid arterial system, and rinsed out using a saline solution and 4% neutral buffered solution of formaldehyde. The specimens were fixed according to the method of Duvernoy. We used the standard technique for the brain removal, and the specific transcranial approach. Microdissections (using micro instruments) of injected orbital blood vessels, nerves and muscles were analyzed under the Leica MZ6 stereomicroscope.

All the specimens were photographed by Sony Cyber Shot DCS P8 digital camera. To get more easily visible details, we used the Leica DC 300 digital camera (magnifications 6.3×, 10× and 20×).

Histological analysis was carried out on 6 *en bloc* excised and formalin-fixed infant orbits. We used the material from the Anatomic Institute of the Faculty of Medicine, Belgrade. The aim was to establish the exact micromorphologic architecture of the upper eyelid. Orbital and frontal region specimens were perfunded with the saline solution through a cannula placed in one of the carotid arteries, and the 4% neutral buffered formaldehyde solution was used afterwards for the same purpose. All the specimens were fixed in the 4% neutral buffered formaldehyde solution in volume 20 times larger than the tissue volume prepared for fixation. Using routine fixation, decalcination, dehydration, illumination, impregnation and molding procedures (Bio-Plas plus, Bio-Optica, Italy), paraplast specimens were prepared for sections At the distance of 15 μm, we cut off a series of tissue

midsagital sections of 4–5 μm in thickness using a Reichert-Jung microtome. Sections were placed on special high-adhesive glass plates (Super Frost Plus, DAKO, Denmark), dried for 60 minutes in thermostat (56°C), and dyed afterwards. Out of standard hematoxilin-eosin (H&E) dyeing procedures, we used advanced histochemical dyeing methods: periodic acid-Schiff (PAS), Masson trichrome and elastica Martius scarlet blue (MSB).

This study was conducted in accordance with the Serbian laws and regulations. The methods for securing the human tissue were humane, proper consents and approvals were obtained, and the tenets of the Declaration of Helsinki were followed.

Results

Anatomic investigations

Levator palpebrae superioris (LPS) muscle

The measurement of the muscle length and diameter *in situ* on 6 nonfixed cadavers (12 orbits) showed the average length of the LPS muscle body to be 42.0 ± 1.41 mm on the right, and 40.3 ± 1.63 mm on the left side. In its origin part, the muscle width was 3.93 ± 0.37 mm on the right, and 3.60 ± 0.40 on the left side, while at the point of the muscle transition to aponeurosis (LPS tendon), it was 4.97 ± 0.29 mm and 4.78 ± 0.27 mm, respectively. The transition of the LPS muscle body to its aponeurosis was 14–17 mm [15.83 ± 0.75 mm (right side), and 14.83 ± 0.75 mm (left side)] from the superior edge of the tarsus (Table 1).

Distribution of the oculomotor nerve terminal branches in the m. levator palpebrae superioris (LPS)

The *levator palpebrae superioris* muscle in all dissections (8 orbits) was supplied by the upper medial branch of the oculomotor nerve. In one case, the LPS muscle was supplied by 2 divided branches of the upper medial branch. After excising the lateral wall of the cavernous sinus and entering the orbit through its upper medial part, just behind the annulus of Zinn, the oculomotor nerve divided into two branches: the superior and the inferior one. The upper branch (*ramus superior*) was directed upward, and after the short travel was divided into two divisions: the lateral division that supplied the superior rectus muscle, and the medial division for inervation of the LPS muscle. Medial division ran continuously to the LPS muscle along the medial border of the superior rectus in 7 of 8 specimens (87.5 %), while in 1 specimen, it passed through the rectus (12.5%) and entered the inferior surface of the LPS muscle. The number of terminal branches of the medial division in the LPS muscle was 2.93 ± 0.45 (mean ± standard deviation) (Figure 1d).

The terminal branches of the upper medial division traveling to the LPS muscle were traced. We classified the pattern of distribution of the superior division of the oculomotor nerve course as follows: type I – terminal branches extending to the proximal third of the LPS; type II – terminal branches extending to the middle third of the LPS; type III – terminal branches extending to the distal third of the LPS.

Table 1

Measurements of the *levator palpebrae superioris* muscle (LMS)

Parameter		Values						x̄	SD
The length of muscle bodies LPS	Right (mm)	42	44	40	41	43	42	42.00	1.41
	Left (mm)	41	42	38	39	42	40	40.33	1.63
The width of origin part muscle bodies LPS	Right (mm)	4.0	4.4	3.4	3.6	4.2	4.0	3.93	0.37
	Left (mm)	3.7	4.1	3.0	3.3	3.9	3.6	3.60	0.40
The width of LPS at the place of the muscle transition to aponeurosis	Right (mm)	5.0	5.4	4.6	4.7	5.1	5.0	4.97	0.29
	Left (mm)	4.8	5.2	4.4	4.6	4.9	4.8	4.78	0.27
The length of aponeurosis LPS	Right (mm)	16	17	15	15	16	16	15.83	0.75
	Left (mm)	15	16	14	14	15	15	14.83	0.75

Blood supply of levator palpebrae superioris (LPS) muscle

Our anatomical investigation of the LPS muscle blood supply revealed that the muscle got the blood from four different arterial systems: the lacrimal, supratrochlear, and supraorbital artery and muscle branches of the ophthalmic artery (fellows of *n. oculomotorius* and its branches), in all our cases. The anterior half of the LPS muscle had the blood supply from the lacrimal artery branches (the lateral part) and branches of the supratrochlear and supraorbital artery (the medial part), while the blood into the posterior half of the LPS muscle came from the muscle branch of the ophthalmic artery (a fellow artery of the superior branch of the oculomotor nerve). All the arterial branches entered the muscle from its superior surface (Figures 1 a–c).

In our investigation, the type I distribution of terminal branches was seen in 1 of 8 (12.5%) specimens, type II in 1 (12.5%) specimen, and type III in 5 (62.5%) specimens. There were 2 separate medial branches in 1 dissection, 1 ending in the proximal third, and the other terminating in distal third of the LPS muscle.

Type III was further reclassified as follows: type IIIa – terminal braches running along the medial third of the LPS; type IIIb – terminal braches running along the central third of the LPS; type IIIc – terminal braches running along the lateral third of the LPS.

In our study, the type IIIa was seen in 1 of 5 (20%), type IIIb in 3 of 5 (60%) specimens, while 1 of 5 (20%) dissections showed the type IIIc.

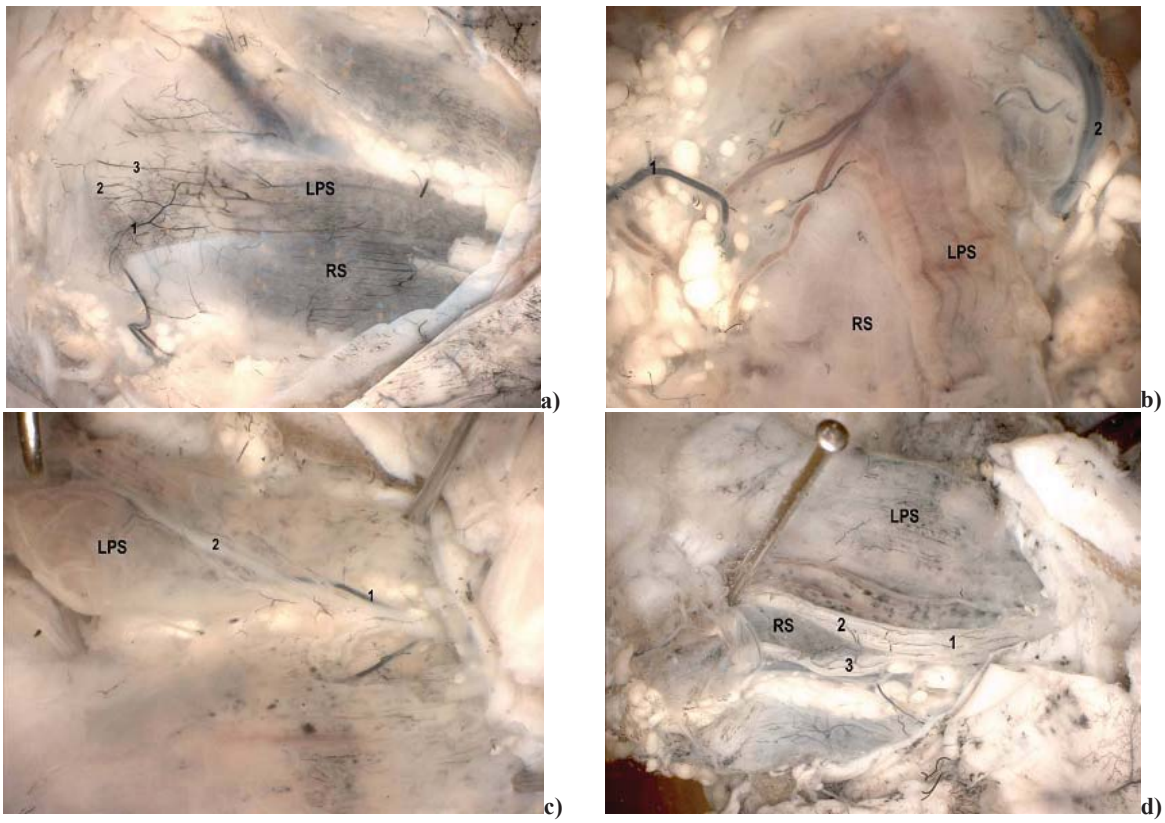


Fig. 1 – Microdissection – intraarterial injection of 5% mixture of melt formalin and black ink

a) 1 – branches of the lacrimal artery; 2 – branches of the supratrochlear artery; 3 – branches of the supraorbital artery, the *levator palpebrae superioris* (LPS), the *rectus superior* (RS) muscle; b) 1 – the lacrimal artery; 2 – the supraorbital artery of the *levator palpebrae superioris* (LPS) muscle the *rectus superior* (RS) muscle; c) 1 – branches of the ophthalmic artery; 2 – the upper medial branch of the oculomotor nerve of the *levator palpebrae superioris* (LPS) muscle; d) 1 – the upper medial branch of the oculomotor nerve; 2 – the branch of the upper medial branch of the oculomotor nerve for the *levator palpebrae superioris* (LPS) muscle; 3 – the branch of the upper medial branch of the oculomotor nerve for the *rectus superior* (RS) muscle and *levator palpebrae superioris* (LPS) muscle.

Transverse ligaments related to the levator palpebrae superioris (LPS) muscle

In our study we found 2 transverse ligaments (thickening of the muscle sheath) related to the LPS muscle: the superior transverse ligament (Figure 2) and the intermuscular transverse ligament (ITL) (Figure 3).

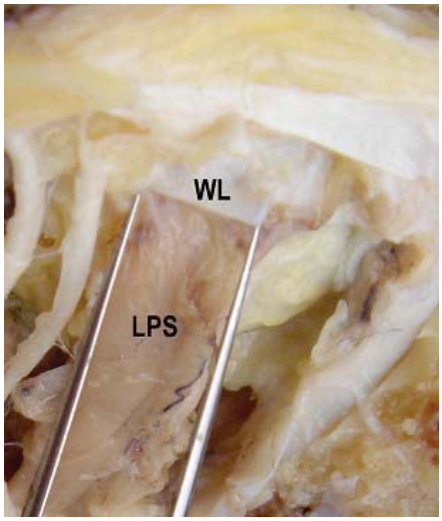


Fig. 2 – The Whitnall's (WL) ligament.

LPS – *levator palpebrae superioris*.

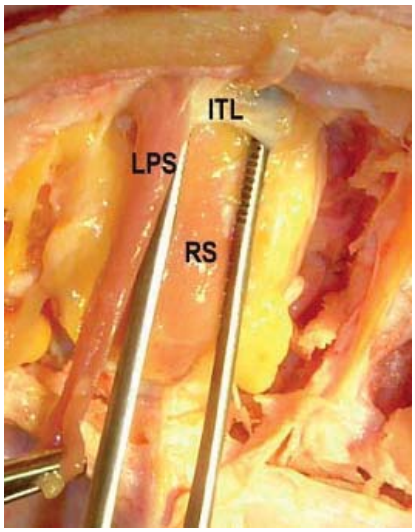


Fig. 3 – The intermuscular transverse ligament (ITL).

LPS – *levator palpebrae superioris* muscle;
RS – *rectus superior* muscle.

As it could be seen from the above said, the Whitnall's ligament (Table 2) assumed spindle shape showing its maximum anterior-posterior extension over the LPS muscle. The 60% of specimens in our study showed the WL bundles fused with the medial and lateral horn of the LPS muscle.

Table 2

Measurements of the Whitnall's (WL) ligament and the intermuscular transverse ligament (ITL) (n = 20)

Parameter	WL ($\bar{x} \pm SD$)		ITL ($\bar{x} \pm SD$)	
	right orbit	left orbit	right orbit	left orbit
Anterio-posterior extension (mm)	11.4 ± 4.2	11.5 ± 4.0	15.7 ± 4.8	15.4 ± 4.7
Mediolateral extension (mm)	36.6 ± 5.6	36.4 ± 5.1	34.6 ± 7.2	35.0 ± 6.2
Thickness (mm)	1.4 ± 0.4	1.6 ± 0.5	1.2 ± 0.3	1.4 ± 0.4
Angle with the LPS muscle (°)	84.4 ± 5.4	83.3 ± 4.8	84.7 ± 9.6	83.8 ± 8.9
Distance from the superior posterior tarsal border (mm)	12.1 ± 2.7	11.9 ± 3.2	15.7 ± 4.1	15.6 ± 4.8

LPS – *levator palpebrae superioris*

Fine connective tissue fibers occurred between the orbital roof periost and the WL. Due to this connective tissue was consistently observed between the LPS muscle and the superior rectus (SR) muscle, this ligament was named ITL by Lucas et al. [4] (Table 2) to ensure clear differentation from the WL. As already mentioned, the trapezoidal-shaped ITL with a nearly transverse anterior border continued posteriorly as a thin transparent layer (the intermuscular septum). A sleeve for the LPS muscle was formed by the fusion of the WL and the ITL near their medial and lateral insertions. Originating from the outer and, mainly, from the inner fascia of the lacrimal gland or the lateral orbital wall, the ITL extended transversally below the LPS muscle to insert at the superomedial orbital notch, and continued posteriorly to the trochlea and the connective tissue around the superior oblique. The main ligament insertions were slightly lower in the orbit than in the middle of both ligaments connected with the LPS muscle. The connections of the ITL with the upper conjunctival fornix, and the presence of the sagittal connective tissue strands between the ITL and the SR and its pulley were revealed by microscopic preparations.

Orbital septum

In all the specimens, the orbital septum originated from the *arcus marginalis* of the frontal bone, and consisted of two layers (Figure 4). The outer (superficial) whitish layer, containing vertically running vessels, descended just posteriorly to the *orbicularis oculi* muscle and the submuscular fibrofatty layer. Slightly above the tarsal upper edge (3 to 5 mm), there was a junction of the orbital septum, the levator aponeurosis and deep aponeurosis of the *orbicularis oculi* muscle reflecting downward without the attachment of the surrounding connective tissue, and ending in the deep layer of the pretarsal skin. The inner (deep) layer ran closely abreast, reflected at the levator aponeurosis and continued superiorly and posteriorly to the levator sheath. Tracing of the deep layer led to the superior transverse ligament of Withnall above the levator aponeurosis. In the space between the palpebral part of the *orbicularis oculi* muscle and the orbital septum, we found the (loose) areolar tissue. Most commonly, this space contains the preseptal fatty tissue.

Our study of orbits and the upper eyelid revealed 3 different compartments of the fatty tissue: lateral, medial and pre-aponeurotic fatty pads. The lateral fatty pad (light yellow) was placed between the lateralis rectus and the SR muscle, based anteriorly-superiorly to the orbital part of the lacrimal gland. It did not reach the upper eyelid in any of the specimens. The medial fatty pad (light yellow, too), between the medial rectus and the oblique superior muscle reached the orbital septum at the level of the medial part of the eyelid. The pre-aponeurotic fat is a cephalic portion of the orbital fat. It is located below the orbital roof, behind the orbital septum, and in front of the LPS muscle aponeurosis. The pre-aponeurotic fat, light yellow in color, extended laterally behind the lacrimal gland, and medially, to the oblique superior tendon, which separated it from the medial fat. Fat formations of the upper eyelid were separated by fascial extensions from the WL. Medial and central fatty pads were completely separated in few cases (16.7%), while, in most

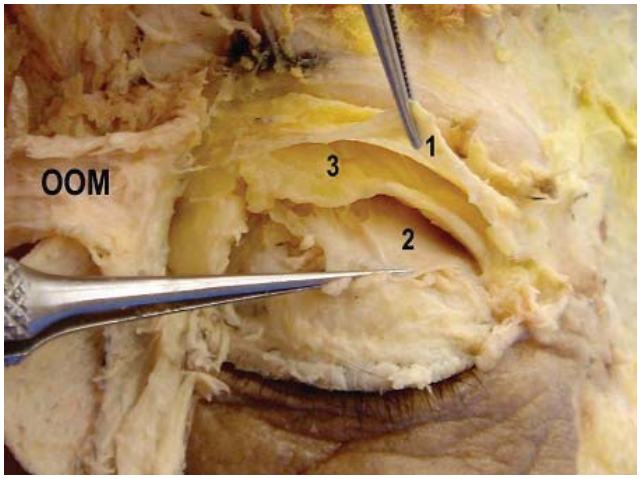


Fig. 4 – Microdissection of the orbital septum.

1 – the superficial layer; 2 – the submuscular fibrofatty layer; 3 – the pre-aponeurotic fatty pad; OOM – *orbicularis oculi* muscles.

cases, they were connected partially. The blood supply and the innervation of fatty pads came from terminal branches of the supraorbital artery and nerve.

Histological analysis

In our upper eyelid investigation, we identified a formation that creates the palpebral crease called the conjoined fascia by Siegel. In all the cases, it was placed in front of the tarsus at the level of the upper palpebral crease, and consisted of *orbicularis oculi fascia*, the superficial layer of the orbital septum, the LPS muscle aponeurosis and the pretarsal fascia. At the level of the upper palpebral crease, the LPS muscle aponeurosis showed a junction to the orbital septum and the orbicularis oculi fascia. These fibers proceeded downward, and met the pretarsal fascia. The conjoined fascia was firmly attached to the tarsus and the pretarsalis part of the orbicularis oculi muscle. Some fibers of the LPS muscle aponeurosis continued forward and was inserted in the deep surface of the skin. Behind the *orbicularis oculi* muscle, as well as in the front of the orbital septum, we found the lose fibrofat layer, mostly avascular, and called it the suborbicular fascial plan (Figure 5a).

The dense, thick collagen fibers of both layers of the orbital septum enclosed the pre-aponeurotic fat in the midsagital line. Collagen fibers of the deep layer proceeded to the WL and sheath of the LPS muscle. At the level of the upper palpebral crease, the deep layer of the orbital septum was intimate with and directly adjacent to the LPS muscle aponeurosis. The deep layer of the orbital septum near the supraorbital edge terminated in the periorbital periostium, without a connection to the superficial layer (Figure 5b).

In addition, we found that the superficial layer of the orbital septum ran cephalad as a galeal layer, without a direct or indirect connection to the frontal muscle. We identified fibrous connections between the orbital septum and the suborbicular fascial layer near the eyebrow. These connections indirectly limited the frontal muscle in the eyebrow elevation (Figure 5c).

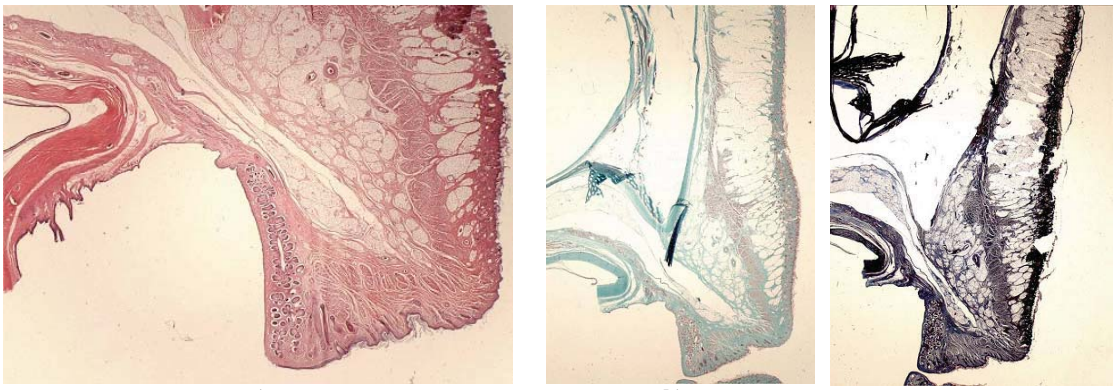
In all the cases, the anterior half of the LPS muscle was supplied by the branches of the lacrimal, the supratrochlear, and the supraorbital arteries, while the posterior half was supplied by the muscular branch of the ophthalmic artery (fellow artery of the superior division of the oculomotor nerve). George [9] found that the posterior half of the LPS muscle had the blood supply by the posterior ethmoid artery that we did not find in any case.

We consider that injuries to the blood vessels supplying the anterior part of the muscle might be indirectly involved in muscle activity, that is the inadequate hemostasis of these vessels, especially in surgical procedures involving the LPS muscle, might result in hematoma, and interfere with postoperative results.

The LPS muscle in all the specimens was supplied by the superior medial branch of the oculomotor nerve. The upper medial branch of the oculomotor nerve extended to the middle third of the LPS muscle (Type III distribution according to the study of Hwang et al. [10]).

These nerve endings were exposed and vulnerable, so their injuries could cause permanent postoperative blepharoptosis. They were also sensitive to local anesthetics, and could be disturbed during the eyelid surgery under local anesthesia. Due to that, exceptional care should be taken during the infiltration of anesthetic near the LPS muscle in order to avoid its transient paralysis postoperatively.

The microarchitecture of the orbital connective tissue system has a significant role in extraorbicular muscles functioning [11–15]. The connective tissue associated to the LPS muscle contains two transverse ligaments: the superior transverse (Whitnall's) ligament and the intermuscular transverse ligament. The Whitnall's ligament has been described as a condensed sheet of fascia underlying the LPS on the superior surface of the muscle, localized in the transitional zone between the muscle body and the aponeurosis. In spite of several descriptions, the function of the WL is still controversial. Whitnall has considered its function as a check ligament. Lemke et al. [16] have mentioned that the WL shows no tension with the open eyelid, while Dutton [17] believes that the WL plays no role in the suspensory activity of the LPS. Anderson and Dixon [18] think that the WL has the role in



a) **b)** **c)**

Fig. 5 – Phistological analysis of the orbital septum.

a) Van Gieson's methods (×15); b) Masson trichrome methods (×15); c) Martius scarlet blue (MSB) methods (×15).

Discussion

Our analysis of anatomical and topographic features of the LPS muscle has not shown any statistically significant differences compared to previous studies [5–8].

changing the anterior-posterior traction power of the LPS into vertical one, relieving the eyebrow elevation. In addition, they have found the WL dehiscence and atrophy in some patients with congenital blepharoptosis.

Our study revealed that the superior transverse ligament (WL) and the intermuscular transverse ligament surrounding the LPS muscle in all cases were located at the junction of the muscle body and its aponeurosis. Both transverse ligaments had their origin from the medial and lateral orbital wall. The Withnall's ligament had firm attachments to the LPS on the outer parts of the muscle. The main and dominant origin of the WL was the lateral one, under the LPS muscle, thus, it could be of a great importance for the suspensory activity of the LPS. Under the upper surface of the WL, there were brands of the loose connective tissue reaching periostium of the orbital roof. The ITL is the condensed fascia of the LPS muscle, between the LPS and the rectus superior muscle, connected by brands to the superior conjunctival fornix. This ligament showed a greater anterior-posterior extension than the WL. The main insertion was placed under the middle of both transverse ligaments. We assume that the LPS muscle runs freely over the ITL thanks to condensing of the connective tissue in the anterior parts of the ligament.

According to the conclusions of a separate examination, we think that these two ligaments could be inadequate. Anatomically, both ligaments create an annular formation containing the fibroelastic and fibromuscular tissue surrounding the LPS muscle near its musculoaponeurotic junction. We consider that, despite the fact that the dominant origin of both ligaments is lower, it could be the morphological substratum of suspension action of the LPS muscle during the passive lowering of the eyelid with the upward view.

In the Gray's anatomy, the upper eyelid elevation is to be checked by the orbital septum [19]. In 1910 Whitnall described how the superficial part of the levator sheath forms a conspicuous band above the LPS muscle in a vertical section through the orbit, just behind the aponeurosis. Moreover, he recognized a delicate layer of the connective tissue running over the aponeurosis and posteriorly to the orbital septum. Fink [3] reported that the fascial sheath of the LPS muscle continued anteriorly from the WL as a continuous membrane that could be traced up to the supraorbital rim, where it blends with the orbital septum.

Conclusion

Our study showed that the superficial layer of the orbital septum, 3 to 5 mm above the superior tarsal edge, had its origin from the LPS fascia, and continued upward as a deep galeal layer in the frontal region, without connections to the frontal muscle, and exhibited no blending with the superficial layer of the orbital septum. Behind and above the origin of the superficial layer of the orbital septum, the aponeurosis of the LPS muscle deep layer of the orbital septum exhibited, the junction to aponeurosis by the connection to an undefined integrity. We consider that the junction of the orbital septum, fascia of the LPS muscle, and the deep fascia of the *orbicularis oculi* muscle plays a supporting role for the periorbital fat, and is placed more medially than laterally in the upper eyelid [16, 20–22].

In addition, this detailed histological analysis revealed that the upper eyelid's crease was formed by the conjoined fascia containing the fascia of the *orbicularis* muscle, the superficial layer of the orbital septum, and the aponeurosis of the LPS muscle, as well as the pretarsal fascia. These findings suggest that in creating the upper eyelid's crease, the skin suture should be placed through the aponeurosis and the superficial layer of the orbital septum, as to protect the integrity of the crease (levator-dermal fixation).

Acknowledgments

This work was supported by the grant No. 175030 from the Ministry of Education, Science and Technological Devolopment of the Republic of Serbia.

REFERENCES

1. *Anderson RL, Jordan DR, Dutton JJ*. Whitnall's sling for poor function ptosis. Arch Ophthalmol 1990; 108(11): 1628−32.
2. *Whitnall SE*. On a ligament acting as a check to the action of thee levator palpebrae superioris muscle. J Anat Physiol 1910; 45: 131−9.
3. *Fink WH*. An anatomic study of the check mechanism of the vertical muscles of the eyes. Am J Ophthalmol 1957; 44(6): 800−9.
4. *Lukas JR, Priglinger S, Denk M, Mayr R*. Two fibromuscular transverse ligaments related to the levator palpebrae superioris: Whitnall's ligament and an intermuscular transverse ligament. Anat Rec 1996; 246(3): 415−22.
5. *Plock J, Contaldo C, von Ludinghausen M*. Levator palpebrae superioris muscle in human fetuses: anatomical findings and their clinical relevance. Clin Anat 2005; 18(7): 473−80.
6. *Kakizaki H, Zako M, Nakano T, Asamoto K, Miyaishi O, Iwaki M*. The Levator Aponeurosis Consists of Two Layers That Include Smooth Muscle. Ophthal Plast Reconstr Surg 2005; 21(5): 379−82.
7. *Ettl A, Daxer A, Priglinger S, Kramer J, Koornneef L*. Dynamic magnetic resonance imaging of the levator palpebrae superioris muscle. Ophthalmic Res 1998; 30(1): 54−8.
8. *Tian J, Yu C, Sun T*. Transcranial approach to the orbit: a microanatomical study. Zhonghua Yi Xue Za Zhi 1999; 79(6): 435−8. (Chinese)
9. *George JL*. Arterial vascularization of the levator muscle of the upper eyelid. J Fr Ophtalmol 1984; 7(2): 143−9. (French)
10. *Hwang K, Lee DK, Chung IH, Lee SI*. Patterns of oculomotor nerve distribution to the levator palpebrae superioris muscle, and correlation to temporary ptosis after blepharoplasty. Ann Plast Surg 2001; 47(4): 381−4.
11. *Ettl A, Priglinger S, Kramer J, Koornneef L*. Functional anatomy of the levator palpebrae superioris muscle and its connective tissue system. Br J Ophthalmol 1996; 80(8): 702−7.
12. *Moreno JJ, González CH*. La ptosis palpebral: fundamentos y técnica quirúrgica. Madrid: Allergan; 1995.
13. *Ettl A, Zonneveld F, Daxer A, Koornneef L*. Is Whitnall's ligament responsible for the curved course of the levator palpebrae superioris muscle. Ophthalmic Res 1998; 30(5): 321−6.

14. *Goldberg RA, Wu JC, Jesmanowicz A, Hyde JS*. Eyelid anatomy revisited: dynamic high-resolution magnetic resonance images of Whitnall's ligament and upper eyelid structures with the use of surface coil. Arch Ophthalmol 1992; 110(11): 1598−600.
15. *Shin HH, Kim JH, Kwon IT*. Anatomic charcteristics of upper eyelid structures important in ptosis surgery. Korean J Ophthalmol 1993; 34: 599−605.
16. *Lemke BN, Stasior OG, Rosenberg PN*. The surgical relations of the levator palpebrae superioris muscle. Ophthal Plast Reconstr Surg 1988; 4(1): 25−30.
17. *Dutton JJ*. Clinical and Surgical Orbital Anatomy. 2nd ed. Philadelphia: Saunders; 1994. p. 96−7.
18. *Anderson RL, Dixon RS*. Aponeurotic ptosis surgery. Arch Ophthalmol 1979; 97(6): 1123−8.
19. *Standring S*. Gray's Anatomy. The Anatomical Basis of Clinical Practice. 40th ed. Philadelphia: Churchill Livingstone Elsevier; 2008.
20. *Hwang K, Kim DJ, Chung RS, Lee SI, Hiraga Y*. An anatomical study of the junction of the orbital septum and the levator aponeurosis in Orientals. Br J Plast Surg 1998; 51(8): 594−8.
21. *Hoffmann KT, Hosten N, Lemke AJ, Sander B, Zwicker C, Felix R*. Septum orbitale: high-resolution MR in orbital anatomy. AJNR Am J Neuroradiol 1998; 19(1): 91−4.
22. *Meyer DR, Linberg JV, Wobig JL, McCormick SA*. Anatomy of the orbital septum and associated eyelid connective tissues: implications for ptosis suregery. Ophthal Plast Reconstr Surg 1991; 7(2):104−13.

Received on April 28, 2012.
Revised on July 3, 2012.
Accepted on July 4, 2012.

ORIGINAL ARTICLE

UDC: 617-089.5::616.1-089
DOI: 10.2298/VSP1312132N

High thoracic epidural anesthesia in patients with synchronous carotid endarterectomy and off-pump coronary artery revascularization

Visoka torakalna epiduralna anestezija kod bolesnika sa istovremenom karotidnom endarterektomijom i *off-pump* revaskularizacijom miokarda

Vojislava Nešković*, Predrag Milojević†, Dragana Unić-Stojanović†, Ivan Ilić‡, Zoran Slavković*§

***Clinic for Anesthesiology and Intensive Care, Military Medical Academy, Belgrade, Serbia; †Dedinje Cardiovascular Institute, Belgrade, Serbia; ‡Clinical Hospital Center Zemun, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. In order to reduce the risk of cerebrovascular insults (CVI), the latest recommendations suggest that carotid endarterectomy (CEA) is strongly indicated in patients scheduled for coronary surgery when significant carotid artery stenosis is symptomatic and/or bilateral. The best results are obtained in small studies with CEA performed immediately prior to off-pump coronary bypass (OPCAB). We present 16 consecutive patients who underwent synchronous CEA and OPCAB under general anesthesia combined with high thoracic epidural anesthesia (TEA) in order to evaluate the safety and potential benefits of such anesthetic management. **Methods.** A total of 16 consecutive patients scheduled for simultaneous CEA and OPCAB with no contraindication for TEA were enrolled in the study. All the patients were anesthetized with TEA combined with general anesthesia. Early extubation was planed in all the patients for early assessment of neurological outcome. Demographics, comorbidity, quality of postoperative recovery, duration of mechanical ventilation, successful early extubation, outcome, length of Intensive Care Unit (ICU) and hospital stay were recorded. **Results.** Only two patients did not fulfill the criteria for early extubation. The average duration of mechanical ventilation for patients who fulfilled criteria for early extubation was 87.9 ± 85.0 (0–255) min. Five (31.25%) patients were extubated in the operating theater at the end of surgery. There were no deaths, nor neurological complications of TEA. Seven (43.7%) patients had at least one of the postoperative complications considered significant. None of them had CVI. None of the early extubated patients was reintubated or had postoperative respiratory failure. **Conclusion.** Our study revealed that a combination of general anesthesia with TEA appears to be good choice in synchronous CEA and OPCAB due to advantages of early extubation and early neurological assessment. Larger studies are necessary to determine real benefits on both short and long-term outcomes of such anesthetic management in synchronous CEA and OPCAB.

Key words:
endarterectomy, carotid; coronary artery bypass; comorbidity; anesthesia, epidural; postoperative complications.

Apstrakt

Uvod/Cilj. Najnovije preporuke ukazuju da je kod bolesnika planiranih za hiruršku revaskularizaciju miokarda koji imaju značajnu bilateralnu i/ili simptomatsku stenozu karotidnih arterija potrebno uraditi karotidnu endarterektomiju (CEA) pre revaskularizacije miokarda. Ovim bi se moglo postići sniženje rizika od nastanka perioperativnog cerebrovaskularnog inzulta (CVI). Najbolji rezultati su postignuti u studijama sa malim brojem bolesnika, kod kojih je urađena sinhrona operacija CEA i revaskularizacija miokarda bez ekstrakorporalne, cirkulacije, *off-pump* (OPCAB). Ovde je prikazana grupa od 16 uzastopnih bolesnika kod kojih je urađena sinhrona operacija CEA i OPCAB koji su bili anestezirani visokom torakalnom epiduralnom anestezijom (TEA), kombinovanom sa opštom anestezijom, sa ciljem da se ocene efekti ovakvog anesteziološkog pristupa. **Metode.** Ukupno 16 uzastopnih bolesnika kod kojih je bila indikovana sinhrona operacija CEA i OPCAB, bez kontraindikacije za TEA, bili su uključeni u studiju. Svi bolesnici anestezirani su kombinovanom TEA i opštom anestezijom. Rana ekstubacija planirana je kod svih bolesnika sa ciljem da se rano proceni postoperativna neurološka funkcija. Kod ispitivanih bolesnika praćeni su sledeći

Correspondence to: Vojislava Nešković, Clinic for Anesthesiology and Intensive Care, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia, Phone: +381 11 1775 320. E-mail: vojkan43@gmail.com

parametri: demografske karakteristike, komorbiditet, kvalitet postoperativnog oporavka, dužina mehaničke ventilacije, uspešnost rane ekstubacije, ishod, kao i dužina boravka u jedinici intenzivnog lečenja i bolnici. **Rezultati.** Samo dva bolesnika nisu ispunila kriterijume za ranu ekstubaciju. Prosečno trajanje mehaničke ventilacije rano ekstubiranih bolesnika bilo je 87,9 ± 85,0 min (0–255 min). Pet (31,25%) bolesnika ekstubirano je na kraju operacije u operacionoj sali. Nije bilo smrtnih ishoda. Nije bilo neuroloških komplikacija vezanih za TEA. Sedam (43,7%) bolesnika imalo je bar jednu od postoperativnih komplikacija koje su praćene. Nijedan bolesnik nije imao CVI. Nijedan rano ekstubiran bolesnik nije bio reintubiran, niti imao respiratornu insuficijenciju. **Zaključak**. Rezultati naše studije ukazuju da je kombinacija TEA i opšte anestezije dobar izbor anestezije za bolesnike sa sinhronom operacijom CEA i OPCAB. Osnovne prednosti su mogućnost rane ekstubacije i rane procene neurološke funkcije. Potrebne su studije sa većim brojem bolesnika da bi se pokazale sve prednosti i uticaj na kratkoročne i dugoročne rezultate sinhrone operacije CEA i OPCAB.

Ključne reči:
endarterektomija; aa. carotis; aa. coronariae, premošćavanje; komorbiditet; anestezija, epiduralna; postoperativne komplikacije.

Introduction

Cerebrovascular insult (CVI) is among grim complications following cardiac surgery with the overall incidence of 2% [1]. Significant stenosis of the carotid artery has been recognized as the most powerful predictor of perioperative stroke after cardiac surgery [2, 3]. There is a 14% risk of perioperative stroke in patients with severe carotid artery disease undergoing coronary artery bypass grafting (CABG), and the stroke rate remains 4% per year for the first 4 years after coronary revascularization [4, 5]. Significant carotid artery stenosis is present in near 20% of patients scheduled for CABG [6]. The incidence can be even higher in those having left main coronary artery disease [7]. Despite being significant risk factor for developing perioperative CVI, carotid artery disease is estimated to be direct cause in only 40% of the cases [1]. Also, CEA performed in nonrevascularized patients with severe coronary artery disease has been shown to be associated with mortality rate as high as 20%; myocardial infarction has been found to be responsible for 50% to 75% of all late deaths in this patient population [3].

Many studies have identified risk factors for stroke during coronary atery operation, including hypertension, age, diabetes, carotid bruit, previous transient ischemic attack, and prior stroke [8, 9]. In addition, prolonged cardiophylmonary bypass (CPB) time also has been reported to be associated with a higher risk of stroke [8, 10]. In addition to CPB time, also, surgery technique brings some additional risk factors, and most of them are related to the use of extracorporeal circulation. Of note, CVI may be a consequence of the embolization due to cardiac or aortic manipulation during the procedure [11].

Current recommendations for carotid revascularization in patients undergoing CABG surgery are based on expert opinion, since there is a lack of strong evidence to guide decision-making [12, 13]. The latest recommendations suggest that CEA should be strongly considered in patients scheduled for CABG surgery with concomitant significant symptomatic and/or bilateral carotid artery stenosis [14–16].

Up to now, encouraging results have been shown in small series of patients, where CEA had been performed immediately prior to off-pump coronary bypass (OPCAB), when compared to synchronous CEA with standard CABG surgery [17]. This may be related to the fact that OPCAB by definition avoids CPB, as one of the most important contributing factors for stroke [3]. However, available data are scarce on which surgical or anesthetic technique is superior for this specific patient population at high risk and individual approach has been strongly suggested.

We presented a series of 16 consecutive patients with simultaneous CEA and OPCAB, operated on under general anesthesia combined with high thoracic epidural anesthesia (TEA). We sought to determine the safety and potential benefits of such anesthetic management.

Methods

From February 2002 until October 2005, after local Ethical Committy approval, a total of 16 consecutive patients scheduled for simultaneous carotid endarterectomy and off-pump coronary bypass surgery (OPCAB) were included in the observational study at the Dedinje Cardiovascular Institute.

All the patients indicated for simultaneous carotid endarterectomy and CABG, with no contraindication for TEA were considered eligible for the study.

All the patients had significant carotid stenosis of more than, or equal to 70% on preoperative carotid duplex scanning. They were either symptomatic, had bilateral significant carotid stenosis, or were classified as having an unstable atherosclerotic plaque.

All the patients were anesthetized with high TEA combined with general anesthesia. Early extubation was planed in all the patients for early assessment of neurological outcome.

Exclusion criteria for the study were: acute infections, myocardial infarction within one month before surgery, coagulation disorders, and emergency surgery.

Preoperative assessment and medication were standard for all the patients. Induction of anesthesia was done intravenously, with midazolam (up to 5 mg), bolus doses of propofol, fentanyl and pancuronium. For the maintenance of adequate depth of general anesthesia sevoflurane was used, together with intravenous bolus doses of fentanyl and pancuronium.

Epidural catheter was placed at Th2-Th3 or Th3-Th4 level, 30 min before surgery, or at least 2 h before the first dose of heparin was used. After the test dose, bolus of bupivacaine with fentanyl in different concentrations (0.125%, 0.25%, or 0.5%) was used followed by continuous infusion of the same local anesthetic with fentanyl, usually at the 0.125% or 0.25% concentration. The rate of continuous infusion was adjusted accordingly; usually, it was 5–10 mL per hour during the operation.

Postoperative analgesia was mainly based on continuous epidural infusion of local anesthetic (0.125% bupivacaine with fentanyl). According to the study protocol, it was planned to take out epidural catheter at the end of the second postoperative day, two hours before the prescribed dose of the low-molecular weight heparin.

Antithrombotic therapy was stopped before elective surgery (ticlopidine and clopidogrel 10 days before the operation). Aspirin was not stopped in all patients, and was not considered as the contraindication for TEA.

Coagulation studies – prothrombin time (PT), partial thromboplastin time (PTT), international normalized (INR) and platelet count, were performed in all the patients preoperatively. Any detected abnormality was considered as contraindication for TEA and exclusion criteria for the study.

Heparin was used in standard intraoperative doses, including 50 mg *i.v.* bolus dose just before carotid artery was clamped, and was neutralized with protamine after finishing the last proximal anastomosis.

Demographics, comorbidity, pre- and intraoperative therapy, operation time, ischemic time and number of grafts were noted.

Quality of postoperative recovery, duration of mechanical ventilation, as well as successful early extubation, outcome, length of Intensive Care Unit (ICU) and hospital stay, were also noted. Any postoperative complication, including cardiovascular, respiratory, neurological, gastrointestinal, as well as possible infections, were followed.

Surgery technique

Vascular surgeon performed CEA before sternotomy. No shunt was used. The neck wound was left open until heparin was reversed with protamine after CABG. The wound was closed after CABG and after reversing heparin, with or without drainage.

Cardiac team performed coronary artery surgery. OPCAB was performed through median sternotomy. Different mechanical stabilizers were used (The Octopus or Starfish). No intracoronary shunts were used during the procedure.

Results

All except one patient included in this study were man, 61.9 ± 7.3 years of age (range from 50–70 years).

Demographic characteristics of the patients are shown in Table 1.

Table 1

Demographic characteristics of the patients

Characteristics of the patients	Mean value ± SD	Range
Weight (kg)	81.3 ± 14.7	60–106
Height (cm)	171.0 ± 8.3	150–187
BMI (kg/m^2)	27.7 ± 4.1	20.8–32.7
EF (%)	43.1 ± 12.0	15–60
Euroscore	5.3 ± 1.8	3–9
NYHA class	2.8 ± 0.6	2–4

BMI – body mass index; EF – ejection fraction; NYHA – New York Heart Association.

Preoperative risk factors for all the patients are shown in Table 2.

Table 2

Preoperative risk factors of the patients

Risk factors	Number of patients (%)
Hypertension	12 (75)
Angina	15 (93.8)
MI	7 (43.8)
Diabetes mellitus	5 (31.3)
Hyperlipidemia	6 (40.0)
Smoking	8 (53.3)
COPD	2 (13.3)
CVI	3 (18.8)
CEA (previous)	4 (24.8)
Preoperatively symptomatic	8 (50.0)

MI – Myocardial infarction; CEA – Carotid endarterectomy; COPD – chronic obstructive pulmonary disease; CVI – cerbrovascular incident.

Preoperative β-blocker therapy was present in 9/16 (56.3%) of patients, while 8/16 (50%) were on ACE-inhibitors.

Aspirin was not stopped preoperatively in 10/16 (62.5%) of the patients.

Epidural anesthesia was adequate in all the patients. Local anesthetic in low concentration (0.25% or 0.125% bupivacaine) mixed with fentanyl, either as i.v. bolus and continuous i.v. infusion was given in 9/16 (56.3%) of the patients. The rest of the patients, 7/16 (43.7%), were given bupivacaine in higher concentration (0.5% bupivacaine as i.v. bolus, followed by 0.25% bupivacaine in i.v. continuous infusion). General anesthesia was maintained with sevoflurane in 15/16 (93.8%) of the patients.

Intraoperative hypotension treated with inotropes or vasopressors was present in 4/16 (25%) of the patients. Symptomatic bradycardia was present in 6/16 (37%) of the patients and was treated either with atropine or ephedrine.

Eversion CEA, which employs a transverse arteriotomy and reimplantation of the carotid artery, was done in 13/16 (81.3%) of the patients. Left CEA was performed in 12/16

(75%), and right CEA in 4/16 (25%) of the patients. Myocardial revascularization was done simultaneously in all the patients. Triple bypass grafts were done most frequently, in 8/16 (50%) patients. Four (25%) patients had two grafts, while double or single bypass was performed in two (12.5% each) patients each.

The average duration of the surgery was 204.1 ± 41.4 min (range from 125–300 min).

Only two patients did not fulfill the criteria for early extubation (within 6 hours from the end of the surgery). One of them had slow recovery from anesthesia and was extubated 450 min after the end of surgery. The other patient developed neck hematoma that required reexploration for bleeding in the first 2 postoperative hours. Although aspirin was not stopped preoperatively and that could contribute to hematoma development, surgical bleeding was found and hemostasis performed. After that he also developed two major postoperative complications: myocardial infarction and low cardiac output. He was mechanically ventilated for 1170 min. In the same patient neurological deficit was noticed after the extubation: speech deficit and dysphagia as well as suspected lesion of *n. hypoglosus*. The patient received antiinflammatory and antiedematous therapy and recovered significantly in the next few days.

The average duration of mechanical ventilation was 178.1 ± 290.5 min. For the patients that fulfilled criteria for early extubation, average duration of mechanical ventilation was 87.9 ± 85.0 minutes (range from 0–255 min). Five (31.25%) patients were extubated in the operating theater, at the end of surgery.

There were no deaths. There were no neurological complications of TEA. Seven (43.7%) of the patients had at least one of the postoperative complications considered significant (Table 3).

Table 3

Incidence of the postoperative complications in the study group

Complications	Number of patients (%)
Agitation	1 (6.3)
Infection*	3 (18.8)
Myocardial infarction	2 (12.5)
Low CO	2 (12.5)
Respiratory failure	1 (6.3)
Neurological deficit	3 (18.8)
Arrhythmias	1 (6.3)
Pneumothorax	1 (6.3)

***Presence of signs of systemic, respiratory and urinary infections**

The patient with respiratory failure was reintubated and mechanically ventilated between the third and sixth postoperative day. He had preoperative chronic renal failure and diabetes mellitus poorly regulated postoperatively, which led to severe metabolic acidosis and respiratory insufficiency. This was the patient that had slow postoperative recovery from anesthesia and late postoperative extubation (after 450 minutes from the end of surgery).

None of the early extubated patients was reintubated or had postoperative respiratory failure.

The average stay in the ICU was 61.6 ± 43.9 hours (range 18–168 hours) and length of hospital stay was 10.5 ± 6.3 days (range 7–29 days).

Discussion

Management of patients with severe combined carotid and coronary artery disease is controversial, thus a balanced and individualized approach is needed [13].

CEA performed in conjunction with CABG surgery carries increased perioperative risk [18]. Although the latest recommendations suggest that CEA should be strongly considered in patients scheduled for CABG surgery with concomitant significant symptomatic and/or bilateral carotid artery stenosis [14–16], these cases are not the most commonly seen in everyday practice. The most frequent, severe carotid artery stenosis in this patient population is asymptomatic and unilateral [15, 16]. The real clinical challenge is to manage this combined arterial disease with the lowest possible adverse outcome rate.

Although carotid stenosis is an important risk factor for development of perioperative stroke, increasing evidence suggests that the most common single cause of post-CABG stroke is embolisation of atherosclerotic material from the atheromatous aortic arch during surgical manipulation [19]. As a consequence, a number of surgical strategies have been developed to reduce the risk of macroembolisation during aortic cannulation, cross clamping and finishing proximal anastomoses. OPCAB may avoid many of these perioperative risks, but these potential benefits have to be balanced with the possible drawbacks [3, 20, 21].

Available data confirm that the 30-day reported death/stroke rate is significantly lower when CEA is performed immediately prior to OPCAB surgery (1.0%) [22, 23], as compared to reported risks for patients undergoing synchronous CEA and CABG (30-day death/stroke 8.7%). One possible interpretation may be that the lower stroke risk in patients undergoing CEA and OPCAB may be attributable to a minimal manipulation with aorta without cannulation (less potential for embolisation), rather than to effects of the prophylactic CEA. However, very low procedural risk observed following synchronous CEA and OPCAB might simply reflect small numbers and selective reporting.

Importantly, little data exist on anesthetic techniques that would be the most beneficial for this high risk and particular patient population.

The landmark general anesthesia *versus* local anesthesia for carotid surgery, (GALA) trial (3,526 patients, enrolled in 95 centers in 24 countries), randomized patients with CEA, to general anesthesia or local anesthesia [24]. This study revealed that the applied anesthetic technique itself was not associated with a significant difference in the trial endpoint, which was a composite of perioperative death, myocardial infarction, and stroke (4.8% for general and 4.5% for local anesthesia, respectively).

Additionally, a recent large single-center "real-world experience" trial failed to demonstrate any outcome advantage of local anesthesia [25].

To our knowledge, there are no studies examining effects of different anesthetic techniques in simultaneous CEA and coronary artery surgery, including OPCAB.

Chakravarthy et al. [26], published two case reports of CEA and OPCAB, performed in regional anesthesia for both CEA and OPCAB, during the simultaneous procedure. Authors believe in the advantage of having conscious patients during carotid surgery, that offers possibility of making rapid diagnosis of transient cerebral ischemia and avoiding any further damage by prompt intervention. At the same time, TEA is an alternate technique in which the benefits of epidural anesthesia are retained without the adverse effects of general anesthesia [27]. Although performed without problems in our series of patients, this the anesthetic choice may be demanding and not without limitations. According to available published literature, 16 consecutive patients presented in our study are the only group of patients with simultaneous CEA and OPCAB surgery operated on under combined general and TEA, in order to achieve early extubation and early neurological assessment. Almost 90% of our patients were, due to anesthetic choice, extubated early, within two hours from the end of the surgery. Five of them were fully awake and extubated in the operating theater. None of the early extubated patients experienced respiratory failure and reintubation.

Our group of patients was assessed preoperatively as the high-risk group (Euroscore for the group was over 5), with expected mortality rate of more than 10% [28]. Also, all the patients scheduled for simultaneous procedure had significant carotid stenosis of more than or equal to 70%. Half of them had symptoms and the rest bilateral significant carotid stenosis, or were classified as having an unstable atherosclerotic plaque. It is well known that these patients' characteristics correlate with bad outcome [1]. In spite of these risks, there were neither deaths nor cerebrovascular insults in our study. Only two patients were diagnosed myocardial infarction, and only one of them was experiencing prolonged recovery. We believe that these results can be considered as much better than it was anticipated.

TEA proved to be good choice of anesthesia for this patient population. The patients were hemodynamically stable; anesthesia was adequate, early recovery was achieved in the majority despite of high perioperative risk. There were no complications related to the anesthesia itself.

Although there was no mortality, seven patients had at least one of the postoperative complications considered as significant. This explains rather long ICU and hospital stay (62 h and nearly 10 days, respectively).

The incidence of postoperative atrial fibrillation in patients with simultaneous CEA and OPCAB was as high as 46% in one study [21], while the expected incidence of this complication after CABG was around 20%. In our study, only 3 (18.8%) of the patients had postoperative atrial fibrillation, which is the expected incidence in general cardiac surgery population. Nevertheless, this incidence is still higher in comparison with data published in studies referring to positive effects of TEA in cardiac surgery [1]. However, the number of patients in our study is too small for drawing out any firm and general conclusions regarding the occurrence of postoperative atrial fibrillation.

There are several limitations of our study that should be discussed. This study was observational, with a small number of included patients. However, these patients were consecutive and enrollment lasted for 4 years, because the indication for simultaneous carotid and OPCAB was not made often. Also, these are the results of only one cardiac surgery team, which was involved in the OPCAB program. Secondly, neuroprotective effects of our approach cannot be assumed, due to a small number of patients and incomplete postoperative neurologic evaluation. According to our study protocol, only obvious, significant neurologic deficits could be noted. Again, a limited number of patients prevents drawing out more conclusions on the effects of the surgical technique, as well as the choice of anesthesia on patient outcome. However, we presumed that combining two techniques, both in surgery and anesthesia, that have known advantages in this patient population, may lead to better outcome for a high-risk group of patients involved in this study. Lastly, since the patients were followed-up until the end of their hospital stay, long-term complications and outcome related to the OPCAB procedure (graft closure, cerebrovascular incidents, long term mortality) could not be determined.

Conclusion

Our study revealed that a combination of general anesthesia with TEA appears to be good choice for simultaneous CEA and OPCAB. This anesthetic approach offers several advantages, including early extubation and early neurological assessment, the possibility of early re-exploration if necessary, but also, hemodynamic stability, comfort for the patients, and possibility for the implementation of the fast track protocol. Even in this high-risk group of patients, the results were encouraging. Larger studies are necessary to determine real benefits on both short and long-term outcome of such anesthetic management in simultaneous CEA and OPCAB.

REFERENCES

1. *Naylor AR.* A critical review of the role of carotid disease and the outcomes of staged and synchronous carotid surgery. Sem Cardiothoracic Vasc Anesth 2004; 8(1): 37−42.
2. *Ricotta JJ, Faggioli GL, Castilone A, Hasset JM.* Risk factors for stroke after cardiac surgery. Buffalo Cardiac Cerebral Group. J Vasc Surg 1995; 21(2): 359−63.
3. *Meharwal ZS, Mishra A, Trehan N.* Safety and efficacy of one stage off-pump coronary artery operation and carotid endarterectomy. Ann Thorac Surg 2002; 73(3): 793−7.
4. *Lees CD, Hertzer NR.* Postoperative stroke and late neurologic complications after carotid endarterectomy. Arch Surg 1981; 116(2): 1561−8.

5. *Hertzer NR, Loop FD, Beven EG, O'Hara PJ, Krajewski LP.* Surgical staging for simultaneous coronary and carotid disease: a study including prospective randomization. J Vasc Surg 1989; 9(3): 455−63.
6. *Bridgman AH, Kieffer RW, Wilcox RA, McCann RL, Tawil MP,* et al. Asymptomatic carotid stenosis and stroke in patients undergoing cardiopulmonary bypass. J Vasc Surg 1995; 21(1): 146−53.
7. *Homick P, Smith PL, Taylor KM.* Cerebral complications after coronary artery bypass grafting. Curr Opin Cardiol 1994; 9(6): 670−9.
8. *McKhann GM, Goldsborough MA, Borowicz LM Jr, Mellits ED, Brookmeyer R, Quaskey SA,* et al. Predictors of stroke risk in coronary artery bypass patients. Ann Thorac Surg 1997; 63(2): 516−21.
9. *Mickelborough L, Walker P, Takagi Y, Ohashi M, Ivanov J, Tamariz M.* Risk factors for stroke in patients undergoing coronary artery bypass grafting. J Thorac Cardiovasc Surg 1996; 112(5): 1250−9.
10. *Craver JM, Bufkin BL, Weintraub WS, Guyton RA.* Neurologic events after coronary artery bypass grafting: further observations with warm cardioplegia. Ann Thorac Surg 1995; 59(6): 1429−34.
11. *Wolman RL, Nussmeier NA, Aggarwal A, Kanchuger MS, Roach GW, Newman MF,*et al. Cerebral injury after cardiac surgery: identification of a group at extraordinary risk. Stroke 1999; 30(3): 514−22.
12. *Mortaz HS, Mostafazadeh DB, Sahraian MA.* Carotid endarterectomy for carotid stenosis in patients selected for coronary artery bypass surgery. Cochrane Database Syst Rev 2009; 4: CD006074.
13. *Augoustides JG.* Advances in the management of carotid artery disease: focus on recent evidence and guidelines. J Cardiothorac Vasc Anesth 2012; 26(1): 166−71.
14. *Naylor RA.* Managing patients with symptomatic coronary and carotid artery disease. Perspect Vasc Surg Endovasc Ther 2010; 22(2): 70−6.
15. *Venkatachalam S, Gray BH, Mukherjee D, Shishehbor MH.* Contemporary management of concomitant carotid and coronary artery disease. Heart 2011; 97(3): 175−80.
16. *Venkatachalam S, Shishehbor MH.* Management of carotid disease in patients undergoing coronary artery bypass surgery: Is it time to change our approach. Curr Opin Cardiol 2011; 26(6): 480−7.
17. *Fareed KR, Rothwell PM, Mehta Z, Naylor AR.* Synchronous carotid endarterectomy and off-pump coronary bypass: an updated, systematic review of early outcomes. Eur J Vasc Endovasc Surg 2009; 37(4): 375−8.
18. *Cywinski JB, Koch CG, Krajewski LP, Smedira N, Li L, Starr NJ.* Increased risk associated with combined carotid endarterectomy and coronary artery bypass graft surgery: a propensity-matched comparison with isolated coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 2006; 20(6): 796−802.
19. *Djaiani GN.* Aortic Arch Atheroma: Stroke Reduction in Cardiac Surgical Patients. Sem Cardiothoracic Vasc Anesth 2006; 10(2): 143−57.
20. *Lytle BW.* On-pump and off-pump coronary bypass surgery. Circulation 2007; 116(10): 1108−9.
21. *Youssuf AM, Karanam R, Prendergast T, Brener B, Hertz S, Saunders CR,* et al. Combined off-pump myocardial revascularization and carotid endarterectomy: early experience. Ann Thorac Surg 2001; 72(5): 1542−5.
22. *Naylor AR, Cuffe RL, Rothwell PM, Bell PR.* A systematic review of outcomes following staged and synchronous carotid endarterectomy and coronary artery bypass. Eur J Vasc Endovasc Surg 2003; 25(5): 380−9.
23. *Naylor R, Cuffe RL, Rothwell PM, Loftus IM, Bell PR.* A systematic review of outcome following synchronous carotid endarterectomy and coronary artery bypass: Influence of surgical and patient variables. Eur J Vasc Endovasc Surg 2003; 26(3): 230−41.
24. *Lewis SC, Warlow CP, Bodenham AR, Colam B, Rothwell PM, Torgerson D,* et al. General anaesthesia versus local anaesthesia for carotid surgery (GALA): a multicentre, randomised controlled trial. Lancet 2008; 372(9656): 2132−42.
25. *Sideso E, Walton J, Handa A.* General or local anesthesia for carotid endarterectomy: the "real-world" experience. Angiology 2011; 62(8): 609−13.
26. *Chakravarthy M, Jawali V, Manohar MV, Patil T, Jayaprakash K, Kalligudd P,* et al. Combined carotid endarterectomy and off-pump coronary artery bypass surgery under thoracic epidural anesthesia without endotracheal general anesthesia. J Cardiothorac Vasc Anesth 2006; 20(6): 850−2.
27. *Chakravarthy M, Jawali V, Patil TA, Jayaprakash K, Shivananda NV.* High thoracic epidural anesthesia as the sole anesthetic for performing multiple grafts in off-pump coronary artery bypass surgery. J Cardiothorac Vasc Anesth 2003; 17(2): 160−4.
28. *Nashef SA, Roques F, Michael P, Gauducheau E, Lemeshow S, Salamon R.* The EuroSCORE study group. European system for cardiac operative risk evaluation (EuroSCORE). Eur J Cardiothorac Surg 1999; 16(1): 9−13.

Received on September 3, 2012.
Revised on October 21, 2012.
Accepted on November 7, 2012.

GENERAL REVIEW

UDC: 613.86:616.379-008.64
DOI: 10.2298/VSP1312138S

Psychological problems in patients with type 2 diabetes – Clinical considerations

Psihološki problemi bolesnika sa dijabetesom tipa 2 – klinička razmatranja

Žana Stanković*, Miroslava Jašović-Gašić†, Dušica Lečić-Toševski†‡

***Clinic for Psychiatry, Clinical Centre of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ‡Institute of Mental Health, Belgrade, Serbia**

Key words:
diabetes mellitus, type 2; comorbitidy; mental health; mental disorders; quality of life; therapeutics.

Ključne reči:
dijabetes melitus, insulin-nezavisni; komorbiditet; mentalno zdravlje; psihički poremećaji; kvalitet života; lečenje.

Introduction

Diabetes mellitus is a major health and social problem. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 [1]. Type 2 diabetes mellitus (DMT2) (adult-onset diabetes) is the most common form of this disease, which is present in 90–95% of patients [2]. In Serbia, the prevalence of DMT2 in the year 2000 was 4.5%, both in men and women [3]. Diabetes has negative impact on physical, psychological and social functioning [4]. DMT2 is a disease related to stress and stressful life events which may have an important role in its pathogenesis, course and outcome.

The interactions between behaviour, central nervous and endocrine systems that might cause immunosuppression is the most fascinating finding in modern medicine, and its implications are important for the prevention and treatment of somatic illnesses [5]. The patophysiological mechanisms related to the role of stressful life events on the emergence of DMT2 include hypothalamic arousal syndrome, with parallel activation of the hypothalamic-pituitary-adrenal axis (HPA) and the central sympathetic nervous system, leading to development of endocrine abnormalities, insulin resistance, and DMT2 [6].

DMT2 is a chronic, self-managed disease that significantly affects the lives of patients and their families. According to the biopsychosocial model originally proposed by Engel [7], the mind and body are two important systems that are interconnected. The model offers additional insights into how chronic illnesses such as diabetes can affect daily life. This model makes a distinction between pathological processes that cause disease, which includes the individual's perception of his or her health and its subsequent impact on health. Stress and coping with diabetes can affect the severity of disease directly, through pathophysiological processes or indirectly, through patients' own perception of illness by deteriorating adherence to therapy and daily functioning.

The results of numerous studies indicated the presence of difficulties of many patients with diabetes living with their disease [8–10], such as the threat of serious complications (renal disease, amputation, blindness) and the potential for reduced life expectancy [11]. The constant stress of maintaining tight glycemic control can result in two types of psychological distress: subclinical emotional distress and diagnosable psychological disorders [10].

Comorbid mental disorders in patients with type 2 diabetes

According to the results of a number of studies, anxious disorders and depression are the most frequent psychiatric comorbid conditions in DMT2 patients [12, 13]. People with diabetes of any type have a 20% higher prevalence of lifetime diagnosis of anxiety than those without it [14]. Generalized anxiety disorder (GAD) appears to be the most common anxiety disorder in this patients' population, with prevalence rate ranging from 13% to 14% [15]. Panic disorder (PD), posttraumatic stress disorder (PTSD) and social phobia are also more frequent in patients with DMT2 than in general population.

Depression occurs two to three times more frequently in DMT2 patients in relation to the general population [16]. Depression is also more common in patients with undiagnosed [17] and newly diagnosed DMT2 [18]. The relationship

Correspondence to: Žana Stanković Clinic for Psychiatry, Clinical Centre of Serbia, 11000 Belgrade, Serbia. Phone: +381 11 2683 093. E-mail: stankovic.zana@gmail.com

between DMT2 and depression appears to be bidirectional in terms of causes, influences and treatment outcomes [19]. Comorbidity of these two conditions is associated with suboptimal adherence to pharmacological therapy and dietary regimen, resulting in unfavorable clinical outcome [20, 21]. Antidepressant treatment of comorbid depression in DMT2 patients is associated with reduction of depressive symptoms and improvement of quality of life. However, results referring to the effect of antidepressant treatment on improvement of glycemic control are inconsistent [22–24].

Diabetes is clearly associated with impaired health-related quality of life, in comparison with the population without diabetes, independently of psychological factors and presence of somatic comorbid diseases [25], which was shown in many studies [13, 26–28]. In one of our studies, the severity of self-rated depressive symptoms in patients with DMT2 significantly inversely correlated with both social adjustment and quality of life [29].

Along with anxious disorders and depression, various other psychiatric disorders are present among DMT2 patients: adjustment disorder [30], substance and/or alcohol use disorders [31, 32], bipolar I disorder [33] and schizophrenia [34]. DMT2 also increases the risk of developing dementia, mostly Alzheimer's disease and vascular dementia [35].

Diabetes-related distress

Diabetes-related distress (DD), a measure of health-related quality of life, includes negative emotional reactions to the diagnosis, threat of complications, self-management demands (testing and monitoring blood glucose level, compliance with dietary regimen and engaging in regular physical activity), treatment and social support. The results of a large cross-national DAWN study (The Diabetes Attitudes, Wishes, and Needs Study), examining the experiences of patients and health professionals in dealing with diabetes, have shown that DD was frequently present and interfered with their self-management efforts [36, 37]. The majority of patients (85.2%) reported feelings of shock, guilt, anger, anxiety, depression and helplessness at the time of diagnosis. The distress remained common in the long period of time after diagnosis (15 years in average) [36]. Of particular clinical importance are adaptation problems associated with repeated hypoglycaemic episodes and the implementation of insulin therapy.

Although emotional problems related to diabetes in majority of patients are not severe enough to fulfil criteria for mental disorders, they are associated with worsening of quality of life and self-management of disease [38]. DD may differ by country and ethnicity due to cultural influences and health care system factors.

Clinical, behavioural and biological correlates of diabetes–related distress

DD was found to be an important contributor both to poor metabolic control and suboptimal adherence to treatment. One of important barrier to optimal adherence in DMT2 patients is the presence of multiple somatic comorbid illnesses, each having its own set of guidelines. That could further complicate adherence to treatment and self-management of the disease [39, 40].

Several cross-sectional studies have shown that DD is associated with glycemic control [41–45], obesity, depressive symptoms, and quality of life [43]. The psychosocial factors directly influenced diabetes self-care habits [42] and women reported significantly higher DD [44]. According to the results of prospective studies, DD predicted glycemic control [46, 47], indirectly through self-efficacy [47]. Reduction of the intensity of DD, not a change in depressive symptoms, was associated with improvement of glycemic control [48–50]. A diabetes self-management education intervention has proven successful in patients of different ethnic origin [49]. Fisher et al. [51] have shown that DD was significantly associated with glycemic control and physical activity and that both DD and severity of depression were significantly and independently associated with diet and adherence to medication.

There are several studies that clearly showed an association between DD and type of antidiabetic therapy. The patients treated with insulin experienced higher DD in comparison with those who were treated with oral hypoglicemic therapy or diet [52]. A negative appraisal of insulin therapy was significantly associated with higher level of DD, higher severity of depression and low education [53, 54].

Hypoglycemia is often associated with unpleasant symptoms, such as tremors, profuse sweating, cognitive dysfunction, and irritability. Risk factors for the emergence of hypoglycemia would be the following: a history of hypoglycemia length of time since the first insulin treatment and a higher level of variability in blood glucose level. Severe hypoglicemic reactions can lead to unconsciousness, coma and death. Patients may manifest subclinical symptoms of anxiety related to hypoglycemia that may also adversely affect diabetes self-management. A fear of hypoglicemia is linked to both state and trait anxiety, although this relationship is complex [55].

"Psychological insulin resistance" (PIR) (patients' reluctance to both initiate and intensify insulin therapy) represents a complex of beliefs about the meaning of insulin therapy, poor self-efficacy concerning the skills needed for this, a lack of accurate information, the fear of unwanted effects and complications from insulin use, as well as lifestyle adaptations, restrictions required by insulin use and social stigma [56, 57]. Worry about efficacy was the factor most strongly correlated with delay of insulin therapy [54], and self-blame has been identified as the attitude most predictive of patients' unwillingness to begin insulin therapy [58]. PIR is the most frequent among females and ethnic minorities [59]. Approximately 9% of diabetic patients on insulin treatment reported anxiety symptoms related to self-injecting [60].

Psychological factors and psychiatric disorders associated with diabetes–related distress

The results of 18-months study showed that risk factors for subsequent DD over time were female gender, previously

having major depression, experiencing more negative events or more chronic stress, having more complications, poor diet and low exercise. Negative life events increased the negative effects of both poor glycemic control and complications on the emergence of distress over time [61].

Personal characteristics of patients with DMT2, such as coping style and temperament could also significantly impact the intensity of DD. Poor coping with anger may cause poorer glycemic control by provoking greater DD [62]. A clear majority of patients with diabetes of different nationalities and cultural characteristics reported to accept their disease [63, 64]. Denial and/or mental disengagement and resignation were present only in a small minority of patients. In a Norwegian survey 40% of the respondents reported that they often blamed themselves. Self-blame correlated significantly with both active and passive coping styles [63]. The most frequently used coping strategies in Turkish patients were acceptance, religion, planning, positive reframing, instrumental support, emotional support, self-distraction and venting. The effect of certain coping strategies on patient's level of anxiety may be indicative of cultural differences in how patients from various cultures distract or vent their DD [64]. A recent study [65] showed a greater variance in emotional distress accounted for by coping styles, and perceived support than by clinical factors.

There are few studies of temperament and metabolic control in patients with DMT2. Patients with excessive depressive and anxious temperaments had worse psychological adjustment to diabetes, more depressive symptoms and worse metabolic control. Only depressive temperament was independently associated with metabolic control [66]. These findings may indicate that healthcare providers should pay more attention to non-clinical factors such as personality traits, coping styles and social support, when addressing DD.

A number of studies have shown an association between DD and depression [67]. Diabetes-specific emotional problems were most common in patients with a comorbid depressive disorder [68]. DD was shown to be significantly related to the severity of depressive symptoms, independent of physical complications and glycemic control [69]. DD, severity of baseline depression and a degree to which depression disrupted patients' quality of life were shown to be independent predictors of 1-year depression outcomes [70]. DD was associated with higher levels of depression and poor emotional well-being [71] and mediated the relation between depression and glycemic control [72, 73]. Our recent cross-sectional study [74] indicated that the level of DD was significantly higher in patients with a comorbid major depression in comparison to those without, as well as that DD, extra-disease stressful life events and polineuropathy were significant predictors of depression.

Incomplete therapeutic adherence, reduced self-efficacy, negative attitudes toward treatment (introduction of insulin treatment) are the components of DD that can result in poor glycemic control. The influence of sociodemographic factors (gender, education level, socio-economic status, cultural environment) as well as psychological characteristics of individuals and the presence of symptoms of depression were significantly associated with DD and glycemic control. Better glycemic control can be achieved by appropriate interventions (person-centred, i.e. adjusted to the personal and social characteristics of the patients) to overcome DD. It is obvious that the course and outcome of the illness depend not only on the application of optimal hypoglycemic therapy, but also on the timely detection of DD, and the implementation of interventions aimed at changing attitudes and behavior of patients and alleviating fears associated with the illness and its treatment.

Recognition of diabetes-related distress

Peyrot et al. [37] have shown that most health providers of the countries studied are aware of the level of patient's distress secondary to diabetes. In spite of this general awareness, many providers had a lack of confidence in their ability to identify and evaluate psychological problems and to provide support for their patients. Thus, these factors remain considerable barriers to managing negative emotions more effectively and improving quality of life in this population of patients. Recognition of DD and application of effective strategies to reduce its intensity, even if diabetes self-care is adequate might be a key health care intervention in patients with diabetes.

Discussion about distress may be the most effective clinical approach and the first step in detection of psychological problems related to diabetes. Many diabetic patients hesitate to talk to their physician about emotional distress and prefer to report medical symptoms and complaints. Some patients spontaneously express their DD, often in terms of demoralization about their ability to manage their diabetes and an unwillingness or inability to engage in active self-management despite recognition of the need for change [75]. The next step in identifying patients with DD would be asking questions about specific sources and intensity of the distress (having trouble accepting diabetes, feeling overwhelmed or burned out by the demands of diabetes management, getting support from family and worry about getting complications).

The ability of depression screening measures to identify DD is modest [76]. The Problem Areas in Diabetes (PAID) questionnaire [39], that takes less than 5 minutes, could be useful. Shorter versions of the PAID, PAID-5 and PAID-1 (only one question referring to worrying about the future and serious complications), appear to be psychometrically robust measures of DD [77].

Recognition of DD and mental disorders associated with diabetes is very important for primary care physicians, who participate in the implementation of prevention and treatment of DMT2 as well as other chronic somatic diseases [36]. Since detection of patients with severe mental disorders by primary care physicians is better than detection of subthreshold symptoms, mild form of anxiety and other factors making distress related to disease [78], it is necessary to provide special training for primary care physicians by mental health care specialists, which would significantly advance this important area of clinical practice. Introduction of clini-

cal guidelines for screening and application of necessary interventions for alleviation of emotional reactions and improvement of health behavior and compliance with treatment would be very useful. Education should be held continuously, in other to provide the newest methods and results in that field of clinical practice.

The symptoms of several psychiatric conditions such as adjustment disorder, depression and anxiety disorders could overlap with emotional and behavioural problems that make DD. Distinguishing DD from these mental disorders is important due to necessity to implement appropriate interventions that would be more efficient than treatment specifically directed at clinical depression and other mental disorders [79].

Depression is related to, but distinct from, diabetes distress. There has been considerable confusion among major depressive disorder (MDD), diabetes distress, and depressive symptoms. The physical symptoms associated with diabetes could also complicate distress assessment because they may be mistaken for symptoms of MDD [80, 81]. It is important to know that the connection between chronic illness and depressive symptoms diminishes with age, as does the association between functional disability and depressive symptoms. Expectations of functioning in important roles appear crucial for explaining the link between disease and significant emotional distress.

Interventions to reduce the intensity of diabetes-related distress

The intensity of DD can vary considerably over time, depending on diabetes status, and should be regularly evaluated as part of a comprehensive diabetes care. Because of the bidirectional relationship between distress and diabetes management, interventions that focus on addressing both DD and diabetes management are likely to have maximal effects.

Initiating discussion about DD by health provider could act positively on patients. Even brief conversations that address feelings and link them to difficulties with self-management could normalize emotional reactions related to disease. The patient's verbalization and expression of emotional experiences of having diabetes can be therapeutic. Because of the reciprocal influences between emotional distress and diabetes self management, integrative approach (psychological treatment and changes in health behaviour) that target both of these problems are likely to have stronger effects on diabetes health outcome than those that focus on either in isolation [81]. It includes participation of nurses, nutritionists, health psychologists and psychiatrists. Taking into account that patients on insulin therapy represent group with more severe form of DMT2 requiring more demanding self-management, interventions leading to both reduction of distress and better health behaviour would be of particular importance.

A recent randomized study of the new developed program for the initiation of intensive insulin therapy in DMT2 patients (MEDIAS 2 ICT: More Diabetes Self-management for Type 2 Diabetes – Intensive Conventional Insulin Therapy) conducted as group sessions in comparison with an established education program (a combination of two older education programs regarding initiating mealtime insulin and treating hypertension) as an active comparator condition, has shown that this program was as effective in lowering HbA1c as the control education program, but superior in reducing DD. A key element of that program is shared decision-making between patients and diabetes educators referring to realistic treatment goals. The patients discuss individual problems and barriers to achieving the treatment goals and methods to overcome the barriers as well as attitudes and personal perceptions about certain aspects of diabetes treatment. An important issue of that program is social support with an active participation of family members, partners or friends of patients with diabetes [82].

Ethnic minorities with low socio-economic status are the group of patients with risk for unfavourable diabetes outcome. The treatment adapted to educational level and social characteristics of the patients appear to be successful. A pilot study [83] demonstrated that literacy-adapted, intensive, problem-solving-based diabetes self-management training was effective for key clinical and behavioural outcomes in a sample of patients with lower income.

In a review by Plack et al. [84] summarizing the effects of interventions on metabolic control and other medical variables, as well as diabetes self-management and psychological outcomes it was concluded that behavioral interventions are effective in diabetes treatment, especially in patients with a high level of DD, difficulty in coping, or insufficient blood glucose awareness.

Novel approaches to the emotional and self-management problems related to diabetes are clearly needed for the far larger population of patients struggling with disease-related distress.

Patients who experience long-standing and profound diabetes distress and those who have any mental disorder may require a referral for specialized care. Efficient consultation-liaison services (with psychiatrists and psychologists who are integrated into the multidisciplinary diabetes care team) and education of endocrinologists in recognition of diabetes distress as well as psychiatric disorders associated with diabetes would be necessary for the implementation of integrative treatment of these patients.

To our mind, the main limitations of previous studies on disease related distress in DMT2 patients would be methodological problems related to the proper diagnosis of comorbid depression and its differentiation from the disease related distress. It is therefore necessary to use structured diagnostic interviews for mental disorders, because it has been shown [38] that a considerable number of DMT2 patients had high levels of depressive symptoms on self-report measures and were not clinically depressed when structured interviews were used. In addition, more prospective studies on the relationship between personal characteristics, distress and depression, as well as the impact of distress on clinical and metabolic parameters of the disease would be of special clinical significance. Taking into account the increasing number of DMT2 patients, further studies on the impact of sociodemographic and clinical factors on the effectiveness of

different interventions to reduce DD are needed, as well as the application of new developed programs and integrated, multidisciplinary care.

Conclusion

Distress related to disease, a non-psychiatric, subclinical emotional distress is present in many patients with DMT2. Diabetes-specific distress corresponds to a complex set of repetitive thoughts regarding feeling of being overwhelmed by diabetes, worries about access to care, concerns about diet, physical activity, medications, and not receiving understanding and appropriate support from others. Distress secondary to diabetes is a significant contributor to unfavourable disease course and outcome due to its relationship to both poor metabolic control, suboptimal adherence to treatment and impairment of quality of life. High level of diabetes distress is related to a negative appraisal of insulin therapy and patients' reluctance to both initiate and intensify treatment with insulin ("psychological insulin resistance"). Personal characteristics of patients with diabetes, such as coping style and temperament could also contribute to the intensity of disease distress.

Recognition of distress related to disease and application of effective strategies to reduce its intensity is a key health care intervention in patients with diabetes. Special training is needed for primary care physicians in mental health issues in order to acquire necessary knowledge and skills in identifying diabetes distress and implement interventions for its alleviation.

Interventions that aim to alleviate psychological problems in patients with diabetes, even those that may not meet diagnostic thresholds, have the potential to not only improve mental health and quality of life of patients, but may also have important impact on treatment outcome. In order to achieve maximal efficacy of these interventions, a comprehensive approach is necessary, that integrates the treatment aimed at reducing fears related to the illness, decreasing social stigma, improving health behavior and compliance with dietary regimen. The development of efficient consultation-liaison services and person-centred medicine in general hospitals with providing education about the psychological aspects of diabetes would allow an effective collaboration between endocrinologists and mental health care professionals which would lead to the improvement of both the patient's psychological functioning and disease outcome.

R E F E R E N C E S

1. *Wild D, von Maltzahn R, Brohan E, Christensen T, Clauson P, Gonder-Frederick L.* A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. Patient Educ Couns 2007; 68(1): 10−5.
2. American Diabetes Association: American Diabetes Association Complete Guide to Diabetes. Alexandria, Va: American Diabetes Association; 1999.
3. *Atanacković-Marković Z, Bjegović J, Janković S, Kocev N, Laaser U, Marinković J.* The Burden of Disease and Injury in Serbia. Serbian Burden of Disease Study. Belgrade: Ministry of Health of the Republic Serbia; 2003.
4. *Koopmanschap M.* CODE-2 Advisory Board. Coping with Type II diabetes: the patient's perspective. Diabetologia 2002; 45(7): S18–22.
5. *Lecic Tosevski D, Pejovic Milovancevic M.* Stressful life events and physical health. Curr Opin Psychiatry 2006; 19(2): 184–9.
6. *Björntorp P, Holm G, Rosmond R.* Hypothalamic arousal, insulin resistance and Type 2 diabetes mellitus. Diabet Med 1999; 16(5): 373–83.
7. *Engel GL.* The need for a new medical model: a challenge for biomedicine. Science 1977; 196(4286): 129–36.
8. *Polonsky WH, Welch G.* Listening to our patients' concerns: understanding and addressing diabetes-specific emotional distress. Diabetes Spectrum 1996; 9(1): 8–11.
9. *Black SA, Ray LA, Markides KS.* The prevalence and health burden of self-reported diabetes in older Mexican Americans: findings from the Hispanic established populations for epidemiologic studies of the elderly. Am J Public Health 1999; 89(4): 546–52.
10. *Rubin RR, Payrot M.* Psychological Issues and Treatments for People with Diabetes. J Clin Psychol 2001; 57(4): 457−78.
11. *American Diabetes Association.* Diabetes 1996 vital statistics. Alexandria, VA: American Diabetes Association, 1996.
12. *Almawi W, Tamim H, Al-Sayed N, Arekat MR, Al-Khateeb GM, Baqer A,* et al. Association of comorbid depression, anxiety, and stress disorders with Type 2 diabetes in Bahrain, a country with a very high prevalence of Type 2 diabetes. J Endocrinol Invest 2008; 31(11): 1020–4.
13. *Das-Munshi J, Stewart R, Ismail K, Bebbington PE, Jenkins R, Prince MJ.* Diabetes, common mental disorders, and disability: findings from the UK National Psychiatric Morbidity Survey. Psychosom Med 2007; 69(6): 543–50.
14. *Li C, Barker L, Ford ES, Zhang X, Strine TW, Mokdad AH.* Diabetes and anxiety in US adults: Findings from the 2006 behavioral risk factor surveillance system. Diabet Med 2008; 25(7): 878–81.
15. *Grigsby AB, Anderson RJ, Freedland KE, Clouse RE, Lustman PJ.* Prevalence of anxiety in adults with diabetes: A systematic review. J Psychosom Res 2002; 53(6): 1053–60.
16. *Anderson RJ, Freedland KE, Clouse RE, Lustman PJ.* The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care 2001; 24(6): 1069–78.
17. *Gale CR, Kivimaki M, Lawlor DA, Carroll D, Phillips AC, Batty GD.* Fasting glucose, diagnosis of type 2 diabetes, and depression: the Vietnam experience study. Biol Psychiatry 2010; 67(2): 189–92.
18. *Perveen S, Otho MS, Siddiqi MN, Hatcher J, Rafique G.* Association of depression with newly diagnosed type 2 diabetes among adults aged between 25 to 60 years in Karachi, Pakistan. Diabetol Metab Syndr 2010; 2(1): 17.
19. *Mezuk B, Eaton WW, Albrecht S, Golden SH.* Depression and type 2 diabetes over the lifespan: a meta-analysis. Diabetes Care 2008; 31(12): 2383–90.
20. *Ciechanowski PS, Katon WJ, Russo JE.* Depression and diabetes: impact of depressive symptoms on adherence, function, and costs. Arch Intern Med 2000; 160(21): 3278–85.
21. *Katon WJ.* The comorbidity of diabetes mellitus and depression. Am J Med 2008; 121(11): 8–15.
22. *Lustman PJ, Williams MM, Sayuk GS, Nix BD, Clouse RE.* Factors influencing glycemic control in type 2 diabetes during acute- and maintenance-phase treatment of major depressive disorder with bupropion. Diabetes Care 2007; 30(3): 459–66.

23. *Abrahamian H, Hofmann P, Prager R, Toplak H*. Diabetes mellitus and co-morbid depression: treatment with milnacipran results in significant improvement of both diseases (results from the Austrian MDDM study group). Neuropsychiatr Dis Treat 2009; 5: 261–6.
24. *Georgiades A, Zucker N, Friedman KE, Mosunic CJ, Applegate K, Lane JD,* et al. Changes in depressive symptoms and glycemic control in diabetes mellitus. Psychosom Med 2007; 69(3): 235–41.
25. *Choi YJ, Lee MS, An SY, Kim TH, Han SJ, Kim HJ,* et al. The Relationship between Diabetes Mellitus and Health-Related Quality of Life in Korean Adults: The Fourth Korea National Health and Nutrition Examination Survey (2007-2009). Diabetes Metab J 2011; 35(6): 587–94.
26. *Aikens JE, Perkins DW, Piette JD, Lipton B.* Association between depression and concurrent Type 2 diabetes outcomes varies by diabetes regimen. Diabet Med 2008; 25(11): 1324–9.
27. *Egede LE, Ellis C.* The effects of depression on metabolic control and quality of life in indigent patients with type 2 diabetes. Diabetes Technol Ther 2010; 12(4): 257–62.
28. *Stanković Ž.* Psycho-social, clinical and neurobiological correlates of depresion in patients with type 2 diabetes [dissertation]. Belgrade: Faculty of Medicine, University of Belgrade; 2011. (Serbian).
29. *Stanković Ž, Nikolić-Balkoski G, Leposavić Lj, Popović Lj.* Perception of quality of life and social adjustment of patients with recurrent depression. Srp Arh Celok Lek 2006; 134 (9−10): 369–74. (Serbian)
30. *Dalvi M, Feher M, Caglar E, Catalan J.* Liaison psychiatrist in a specialist diabetes centre. The Psychiatrist 2008; 32: 461–3.
31. *Banerjea R, Sambamoorthi U, Smelson D, Pogach, L.* Expenditures in mental illness and substance use disorders among veteran clinic users with diabetes. J Behav Health Serv Res 2008; 35(3): 290–303.
32. *Prisciandaro JJ, Gebregziabher M,* Grubaugh AL, Gilbert GE, Echols C, Egede LE. Impact of psychiatric comorbidity on mortality in veterans with type 2 diabetes. Diabetes Technol Ther 2011; 13(1): 73–8.
33. *Regenold WT, Thapar RK, Marano C, Gavirneni S, Kondapavuluru PV*. Increased prevalence of type 2 diabetes mellitus among psychiatric inpatients with bipolar I affective and schizoaffective disorders independent of psychotropic drug use. J Affect Disord 2002; 70(1):19–26.
34. *Lin PI, Shuldiner AR*. Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes. Schizophr Res 2010; 123(2−3): 234–43.
35. *Luchsinger JA*. Type 2 diabetes, related conditions, in relation and dementia: an opportunity for prevention? J Alzheimers Dis 2010; 20(3): 723–36.
36. *Skovlund SE, Peyrot M.*. The Diabetes Attitudes, Wishes, and Needs (DAWN) program: a new approach to improving outcomes of diabetes care. Diabetes Spectrum 2005; 18(3): 136–42.
37. *Peyrot M, Rubin RR, Lauritzen T, Snoek FJ, Matthews DR, Skovlund SE*. Psychosocial problems and barriers to improved diabetes management: results of the cross-national Diabetes Attitudes, Wishes, and Needs study. Diabet Med 2005; 22(10): 1379–85.
38. *Fisher L, Skaff MM, Mullan JT, Arean P, Mohr D, Masharani U,* et al. Clinical Depression Versus Distress Among Patients With Type 2 Diabetes: Not just a question of semantics. Diabetes Care 2007; 30(3): 542–8.
39. *Polonsky WH, Anderson BJ, Lohler PA, Welch G, Jacobson AM, Aponte JE,* et al. Assessment of diabetes related distress. Diabetes Care 1995; 18(6):754–60.
40. *Gary TL, Crum RM, Cooper-Patrick L, Ford D, Brancati FL*. Depressive symptoms and metabolic control in African Americans with type 2 diabetes. Diabetes Care 2000; 23(1): 23–9.
41. *Polonsky WH, Anderson BJ, Lohrer PA, Aponte JE, Jacobson AM, Cole CF*. Insulin omission in women with IDDM. Diabetes Care 1994; 17(10): 1178–85.
42. *Ogbera A, Adeyemi-Doro A*. Emotional distress is associated with poor self care in type 2 diabetes mellitus. J Diabetes 2011; 3(4): 348–52.
43. *Ting RZ, Nan H, Yu MV, Kong AP, Ma RC, Wong RY*, et al. Diabetes-Related Distress and Physical and Psychological Health in Chinese Type 2 Diabetic Patients. Diabetes Care 2011; 34(5):1094–6.
44. *Graue M, Haugstvedt A, Wentzel-Larsen T, Iversen MM, Karlsen B, Rokne B*. Diabetes-related emotional distress in adults: Reliability and validity of the Norwegian versions of the Problem Areas in Diabetes Scale (PAID) and the Diabetes Distress Scale (DDS). Int J Nurs Stud 2012; 49(2):174–8.
45. *Shakibazadeh E, Rashidian A, Larijani B, Shojaeezadeh D*. Psychometric Properties of the Iranian Version of Resources and Support for Chronic Illness Self-management Scale in Patients with Type 2 Diabetes. Int J Prev Med 2012; 3(2): 84–90.
46. *Nozaki T, Morita C, Matsubayashi S, Ishido K, Yokoyama H, Kawai K,* et al. Relation between psychosocial variables and the glycemic control of patients with type 2 diabetes: a cross-sectional and prospective study. Biopsychosoc Med 2009; 3: 4.
47. *Nakahara R, Yoshiuchi K, Kumano H, Hara Y, Suematsu H, Kuboki T*. Prospective study on influence of psychosocial factor on glycemic control in Japanese patients with type 2 diabetes. Psychosomatics 2006; 47(3): 240–6.
48. *Zagarins SE, Allen NA, Garb JL, Welch G*. Improvement in glycemic control following a diabetes education intervention is associated with change in diabetes distress but not change in depressive symptoms. J Behav Med 2012; 35(3):299–304.
49. *Leyva B, Zagarins SE, Allen NA, Welch G*. The relative impact of diabetes distress vs. depression on glycemic control in Hispanic patients following a diabetes self-management education intervention. Ethn Dis 2011; 21(3): 322–7.
50. *Fisher L, Mullan JT, Arean P, Glasgow RE, Hessler D, Masharani U*. Diabetes distress but not clinical depression or depressive symptoms is associated with glycemic control in both cross-sectional and longitudinal analyses. Diabetes Care 2010; 33(1): 23–8.
51. *Fisher L, Glasgow RE, Strycker LA*. The relationship between diabetes distress and clinical depression with glycemic control among patients with type 2 diabetes. Diabetes Care 2010; 33(5):1034–6.
52. *Delahanty LM, Grant RW, Wittenberg E, Bosch JL, Wexler DJ, Cagliero E,* et al. Association of diabetes-related emotional distress with diabetes treatment in primary care patients with Type 2 diabetes. Diabet Med 2007; 24(1): 48–54.
53. *Makine C, Karşidağ C, Kadioğlu P, Ilkova H, Karşidağ K, Skovlund S,* et al. Symptoms of depression and diabetes-specific emotional distress are associated with a negative appraisal of insulin therapy in insulin-naïve patients with Type 2 diabetes mellitus. A study from the European Depression in Diabetes [EDID] Research Consortium. Diabet Med 2009; 26(1): 28–33.
54. *Peyrot M, Rubin RR, Lauritzen T, Skovlund SE, Snoek FJ, Matthews DR,* et al. Resistance to insulin therapy among patients and providers: results of the cross-national Diabetes Attitudes, Wishes, and Needs (DAWN) study. Diabetes Care 2005; 28(11): 2673–9.
55. *Wild S, Roglic G, Green A, Sicree R, King H*. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27(5): 1047–53.
56. *Polonsky WH, Fisher L, Guzman S, Villa-Caballero L, Edelman SV*. Psychological insulin resistance in patients with type 2 diabetes: the scope of the problem. Diabetes Care 2005; 28(10): 2543–5.

57. *Brod M, Kongsø JH, Lessard S, Christensen TL.* Psychological insulin resistance: patient beliefs and implications for diabetes management. Qual Life Res 2009;18(1): 23–32.
58. *Peyrot M, Rubin RR, Siminerio LM.* Physician and nurse use of psychosocial strategies in diabetes care: results of the cross-national Diabetes Attitudes, Wishes and Needs (DAWN) study. Diabetes Care 2006; 29(6):1256–62.
59. *Nam S, Chesla C, Stotts NA, Kroon L, Janson SL.* Factors associated with psychological insulin resistance in individual with type 2 diabetes. Diabetes Care 2010; 33(8):1747–9.
60. *Mollema ED, Snoek FJ, Ader HJ, Heine RJ, van der Ploeg HM.* Insulin-treated diabetes patients with fear of self-injecting or fear of self-testing: Psychological comorbidity and general well-being. J Psychosom Res 2001; 51(5): 665–72.
61. *Fisher L, Mullan JT, Skaff MM, Glasgow RE, Arean P, Hessler D.* Predicting diabetes distress in patients with Type 2 diabetes: a longitudinal study. Diabet Med 2009; 26(6): 622–7.
62. *Yi JP, Yi JC, Vitaliano PP, Weinge K.* How Does Anger Coping Style Affect Glycemic Control in Diabetes Patients? Int J Behav Med 2008; 15(3): 167–72.
63. *Karlsen B, Bru E.* Coping styles among adults with type 1 and type 2 diabetes. Psychol Health Med 2002; 7(3): 245–59.
64. *Tuncay T, Musabak I, Gok DE, Kutlu M.* The relationship between anxiety, coping strategies and characteristics of patients with diabetes. Health Qual Life Outcomes 2008; 6: 79.
65. *Karlsen B, Oftedal B, Bru E.* The relationship between clinical indicators, coping styles, perceived support and diabetes-related distress among adults with type 2 diabetes J Adv Nurs 2012; 68(2): 391−401.
66. *Gois C, Barbosa A, Ferro A, Santos AL, Sousa F, Akiskal H,* et al. The role of affective temperaments in metabolic control in patients with type 2 diabetes. J Affect Disord 2011; 134(1−3): 52–8.
67. *Stanković Ž, Jašović-Gašić M.* Comorbidity of depression and type 2 diabetes–risk factors and clinical significance. Vojnosanit Pregl 2010; 67(6): 493–500. (Serbian)
68. *Kokoszka A, Pouwer F, Jodko A, Radzio R, Mućko P, Bieńkowska J,* et al. Serious diabetes-specific emotional problems in patients with type 2 diabetes who have different levels of comorbid depression: a Polish study from the European Depression in Diabetes (EDID) Research Consortium. Eur Psychiatry 2009; 24(7): 425–30.
69. *Hosoya T, Matsushima M, Nukariya K, Utsunomiya K.* The relationship between the severity of depressive symptoms and diabetes-related emotional distress in patients with type 2 diabetes. Intern Med 2012; 51(3): 263–9.
70. *Pibernik-Okanovic M, Begic D, Peros K, Szabo S, Metelko Z.* Psychosocial factors contributing to persistent depressive symptoms in type 2 diabetic patients: a Croatian survey from the European Depression in Diabetes Research Consortium. J Diabetes Complications 2008; 22(4): 246–53.
71. *Huis In 't Veld EM, Makine C, Nouwen A, Karşıdağ C, Kadıoğlu P, Karşıdağ K,* et al. Validation of the Turkish version of the problem areas in diabetes scale. Cardiovasc Psychiatry Neurol 2011; 2011: 315068.
72. *van Bastelaar KM, Pouwer F, Geelhoed-Duijvestijn PH, Tack CJ, Bazelmans E, Beekman AT,* et al. Diabetes-specific emotional distress mediates the association between depressive symptoms and glycaemic control in Type 1 and Type 2 diabetes. Diabet Med 2010; 27(7): 798–803.
73. *Welch G, Zagarins SE, Feinberg RG, Garb JL.* Motivational interviewing delivered by diabetes educators: Does it improve blood glucose control among poorly controlled type 2 diabetes patients. Diabetes Res Clin Pract 2011; 91(1): 54–60.
74. *Stanković Ž, Jašović-Gašić M, Zamaklar M.* Psycho-social and clinical variables associated with depression in patients with type 2 diabetes. Psychiatr Danub 2011; 23(1): 34–44.
75. *Peyrot M, Rubin RR.* Behavioral and Psychosocial Interventions in Diabetes: A conceptual review. Diabetes Care 2007; 30(10): 2433–40.
76. *Hermanns N, Kulzer B, Krichbaum M, Kubiak T, Haak T.* How to screen for depression and emotional problems in patients with diabetes: comparison of screening characteristics of depression questionnaires, measurement of diabetes-specific emotional problems and standard clinical assessment. Diabetologia 2006; 49(3): 469–77.
77. *McGuire BE, Morrison TG, Hermanns N, Skovlund S, Eldrup E, Gagliardino J,* et al. Short-form measures of diabetes-related emotional distress: the Problem Areas in Diabetes Scale (PAID)-5 and PAID-1. Diabetologia 2010; 53(1): 66–9.
78. *Borowsky SJ, Rubenstein LV, Meredith LS, Camp P, Jackson- Triche M, Wells K.* Who is at Risk of Nondetection of Mental Health Problems in Primary Care? J Gen Intern Med 2000; 15(6): 381–8.
79. *Casey P, Bailey S.* Adjustment disorders: the start of the art. World Psychiatry 2011; 10(1): 11–8.
80. *Fisher L, Glasgow RE, Mullan JT, Skaff MM, Polonsky WH.* Development of a Brief Diabetes Distress Screening Instrument. Ann Fam Med 2008; 6(3): 246–52.
81. *Gonzalez JS, Fisher L, Polonsky WH.* Depression in diabetes: have we been missing something important? Diabetes Care 2011; 34(1): 236–9.
82. *Hermanns N, Kulzer B, Maier B, Mahr M, Haak T.* The effect of an education programme (MEDIAS 2 ICT) involving intensive insulin treatment for people with type 2 diabetes. Patient Educ Couns 2012; 86(2): 226–32.
83. *Hill-Briggs F, Lazo M, Peyrot M, Doswell A, Chang YT, Hill MN,* et al. Effect of problem-solving-based diabetes self-management training on diabetes control in a low income patient sample. J Gen Intern Med 2011; 26(9): 972–8.
84. *Plack K, Herpertz S, Petrak F.* Behavioral medicine interventions in diabetes. Curr Opin Psychiatry 2010; 23(2): 131–8.

Received on April 18, 2012.
Revised on August 2, 2012.
Accepted on August 6, 2012.

CURRENT TOPIC

UDC: 615.322:[615.035:615.22
DOI: 10.2298/VSP120613012G

Cardiovascular effects of resveratrol

Kardiovaskularni efekti rezveratrola

Ljiljana Gojković Bukarica*, Dragana Protić*, Vladimir Kanjuh†, Helmut Heinle‡, Radmila Novaković*, Radisav Šćepanović§

***Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Serbian Academy of Sciences and Arts, Belgrade, Serbia; ‡Institute of Physiology, University of Tuebingen, Germany; §Clinical Hospital Center "Dr Dragiša Mišović", Belgrade, Serbia**

Key words:
phytotherapy; stilbenes; risk assesment; cardiovascular diseases; diet.

Ključne reči:
fitoterapija; stilbeni; rizik, procena; kardiovaskularne bolesti; ishrana.

Introduction

Resveratrol, trans-3, 5, 4'-trihydroxy stilbene is a naturally occurring phytoalexin present in many different types of nutrients which we consume on daily basis. Resveratrol was first isolated from dried roots of *Polygonum cuspidatum*, as the principal active ingredient. *Polygonum cuspidatum* and its extract have been used in Japanese and Chinese traditional medicine for treatment of various skin inflammations, cardiovascular and liver diseases, and fungal infections [1, 2].

There are two isoforms of resveratrol: cis- and trans–resveratrol. Trans–resveratrol is biologically active isoform. The main source of resveratrol is grape skin. Also, resveratrol is present in fruits such as cranberry, lingonberry, bilberry, mulberry, deer berry, blueberry, sparkleberry, partridgeberry, jackfruit, and in a peanut orchid tree, scots pine, corn lily, white hellebore, eucalyptus, spruce etc. (Table 1) [3, 4]. Resveratrol has anti-cancer and anti-inflammatory effects and beneficial cardiovascular effects [4]. There were around 800 published articles about biological properties of resveratrol and its health benefits, from 1940 until 2005. From 2005 until nowadays, there are more than 4,000 new studies with resveratrol on cells, isolated animal organs, animals and humans.

It is well known that resveratrol has beneficial effects on the cardiovascular system. It plays the most important role in the epidemiological phenomenon called "French paradox" (existence of cardiovascular risk factors with low incidence/mortality rates which may attribute to moderate consumption of red wine) [12, 13]. The following will describe the most important effects of resveratrol on the cardiovascular system.

Table 1

The amount of resveratrol found in natural food

Source	Amount of resveratrol
Bilberries	~0.65 μg/g [5]
Blueberries	~0.32 ng/g [6]
Dry grape skin	~24.06 μg/g [7]
Grapes	0.16–3.54 μg/g [8]
Peanuts	0.02–1.92 μg/g [9]
Pistachios	0.09–1.67 μg/g [10]
Red wines	01–14.3 mg/L [11]

Bioavailability of resveratrol

After oral administration, resveratrol absorbs rapidly (75%) by transepithelial diffusion. It is detected in a 15-min post-administration and reaches peak concentrations after 30 min. Values returned to baseline within 4 h [14]. Previously, we showed that different bile acids micellar solutions improved resveratrol solubilization [15]. The metabolism of resveratrol is extensive in the intestine and liver. Because of intense metabolism, an oral bioavailability of resveratrol is less than 1%. The major active metabolites of resveratrol are glucuronides (trans-resveratrol-3-O-glucuronide) and sulfates (trans-resveratrol-3-sulfate). Also, colonic bacterial metabolism plays an important role in resveratrol metabolism [16]. Metabolites of resveratrol are eliminated by kidneys.

Correspondence to: Ljiljana Gojković Bukarica, Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, P.O. Box 38, University of Belgrade, 11 129 Belgrade, Serbia. Phone: +381 11 3643 395; Fax: +381 11 3643 397.
E-mail: l.g.bukarica@sezampro.rs

How much?

The best source of resveratrol is considered to be red wine and it is generally believed that resveratrol is responsible for cardioprotective effects related to red wine consumptionn [11, 17]. Approximately 300 mL of red wine for man and up to 200 mL for women is the average recommended dose (equate to a dose of 15 mg and 10 mg of resveratrol, repectively). It is well known that resveratrol produces beneficial effects to human health in a dose-dependent manner, by diverse mechanisms. Data about dose-dependency of resveratrol in the cardiovascular system are shown in Table 2 [4].

Table 2

Effects of different doses of resveratrol on the cardiovascular system

Doses	Effects on cardiovascular system
50 nM	Induces eNOS activity and activates ERK [18]
0.15/0.25 μM	Inhibits platelet activation [19]
10 μM	Improves cardiac functions after ischemia/reperfusion injury [20]
	Protects the heart from ischemia/reperfusion related injury by making the heart pharmacologically preconditioned [20]
25 μM	Exerts lesser degree of cardioprotection [20]
2.5 mg/kg bw	Alleviates cardiac dysfunction in streptozotocin-induced diabetes [21]
2.5–5 mg/kg	Improves post ischemic cardiac functions [3]
	Reduces myocardial infarction, cardiomyocytes apoptosis [22]
22.4 mg/kg	Extands the life span, in case of high-calorie diets induce mice, by overexpressing sirtuin 1 (SIRT1) [23]
50–100 μM	Inhibition of metabolic activity and cell proliferation [24]
1–100 μM	Vasodilatation [25]

eNOS – endothelial nitric oxide synthase; ERK – extracellular signal regulated kinase.

Resveratrol-induced vasorelaxation

Resveratrol relaxes isolated human internal mammary artery, rat aorta and mesenteric artery (Figure 1) [25–27]. Also, there are evidences that resveratrol relaxes mesenteric and uterine arteries of guinea pig and porcine coronary and retinal artery [28–30]. Rakici et al. [31] have described that resveratrol effects vascular tone and endothelial function of the human saphenous vein and internal mammary artery. In addition, resveratrol significantly improves vascular response in streptozotocin-induced diabetic rats [32].

Mechanism of vasodilatation by resveratrol is not well defined. Resveratrol induces endothelium-dependent and endothelium-independent vasorelaxation. Endothelium-dependent mechanisms of relaxation by resveratrol include stimulation of endothelial nitric oxide (NO) production by SIRT1-dependent endothelial NO synthase (eNOS) upregulation and SIRT1-dependent eNOS deacetylation. Estrogen receptor (Erα)-dependent, ERK1/2-mediated eNOS phosphorylation is stimulated by resveratrol [33]. Resveratrol lowers superoxide-mediated NO inactivation by different mechanisms. It decreases expression and activity of vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) and stimulates expression of superoxide dismutases (SOD), catalase and glutathione peroxidases [34]. Thus, oral treatment with resveratrol results in endothelium-dependent relaxation.

In the clinical study (double-blind, randomized cross-over study) which included 19 obese men and post-menopausal women with untreated borderline hypertension, acute consumption of resveratrol (30, 90 or 270 mg) increases resveratrol concentration in plasma and flow-mediated dilation of the brachial artery [35]. According to this, it seems that resveratrol improves endothelial (dys)function.

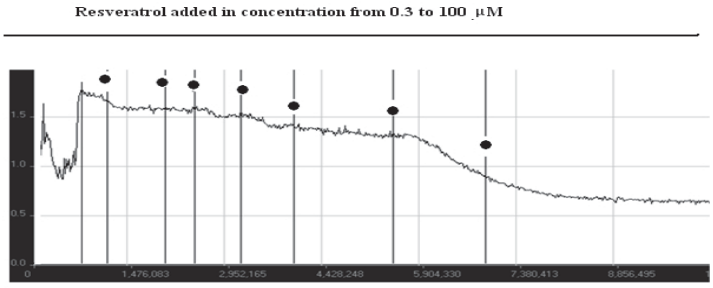


Fig. 1 – Endothelium-independent relaxation of rat mesenteric artery induced by resveratrol precontracted by phenylephrine (PE, 1 μM).

Endothelium was removed mechanically by rubbing with a steel wire. Cumulative concentrations of resveratrol were added to the organ-bath (from 0.3 to 100 μM).

The endothelium-independent relaxation is probably mediated by different ion channels in the membrane of vascular smooth muscle cells, including big Ca^{2+}-activated K^+ (BK_{Ca})-channels or voltage-gated calcium channels [36]. Also, there are evidences that margatoxin-sensitive smooth muscle voltage-sensitive K^+ (Kv) channels play important role in va-

sorelaxation induced by resveratrol (Figure 2) [25–27]. All studies suggest tissue and species selectivity for resveratrol.

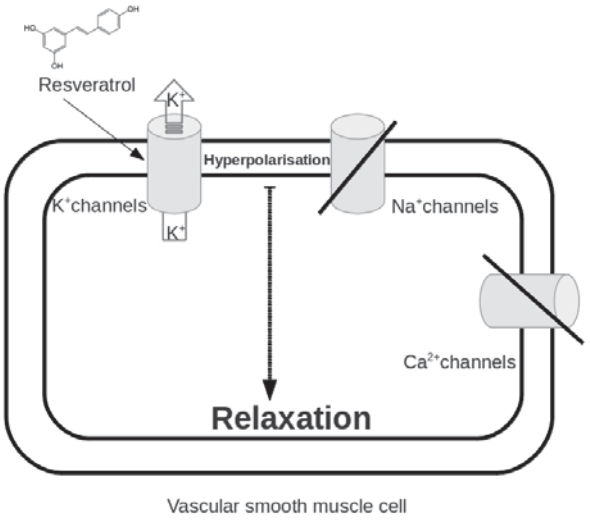


Fig. 2 – The role of potassium channels in vasorelaxation induced by resveratrol.

Resveratrol activates potassium (K^+) channels in vascular smooth muscle cells and induces hyperpolarisation. The change in voltage of membrane of smooth muscle cells leads to inactivation of sodium (Na^+) and calcium (Ca^{2+}) channels and induces vasorelaxation.

The effects of resveratrol on vascular inflammation

It is well-known that resveratrol has anti-inflammatory effect. The major molecular target for the anti-inflammatory effects of resveratrol in the vasculature is nuclear factor kappa-beta (NF-κß). Resveratrol inhibits NF-κß and there are several mechanisms which are involved in this inhibition. The inhibitory effect of resveratrol on NF-κß may be mediated by SIRT1 [37]. Also, resveratrol inhibits reactive oxygen species (ROS)-mediated NF-κß activation by reducing H_2O_2 levels. Resveratrol inhibits activation of Iκß kinases (IKK). IKK kinases are upstream kinases known to activate NF-κß. Those results were obtained in experimental study in skin tumor models [38]. Also, the transcription of NF-κß is blocked by resveratrol [38, 39].

Antiplatelet effects of resveratrol

Resveratrol has antiplatelet effects. This effect of resveratrol has been shown on isolated platelets from healthy subjects [40]. There is experimental study which described that resveratrol has prophylactic effects on portal vein thrombosis in the rat. According to this study, foods containing resveratrol can be advised to minimize portal vein thrombosis, at least among patients undergoing liver transplantation and displaying certain cardiovascular disease risk factors [41]. The mechanism of antiplatelet effect of resveratrol is not completely clear. Resveratrol enhances endothelial NO production, NO could diffuse into platelets and inhibits platelet aggregation by activation of guanylyl cyclase and production of cyclic guanosine monophosphate (cGMP). It was described in the isolated human platelets. The level of cGMP was increased in endothelium-independent manner [40]. There are assumptions that resveratrol enhanced platelet NO production and improved NO bioactivity due to the reduction of oxidative stress. Also, resveratrol is a potent inhibitor of cyclooxygenase 1 (COX-1). The inhibition of COX-1 is irreversible and non-competitive [42]. On the other hand, Kundu et al. [38] 2006 described that resveratrol inhibited both COX-1 and COX-2. There are some evidences that resveratrol inhibited thromboxane synthesis by inhibition of a pathway involving p38 mitogen-activated protein (MAP) kinase [19].

Resveratrol and atherosclerosis

In an experimental study, which included rabbits on the hypercholesterolemic diet, resveratrol had significant anti-atherogenetic effects [43]. The effect of resveratrol supplements, with regard to the modulation of lipid profiles, cholesterol synthesis and anti-atherogenesis, were examined in apo E-deficient (apoE(-/-)) mice fed a normal diet. The concentration of total cholesterol (total C) and low-density lipoprotein cholesterol (LDL-C) in plasma was significantly lower in the resveratrol-supplemented groups compared to the control group of mice [44]. The effect of resveratrol on intimal hyperplasia after endothelial denudation was examined in experimental rabbits. The results of this examination suggest that this polyphenol might have clinical potential in prevention and treatment of restenosis after angioplasty [45]. Also, this compound may inhibit lipid peroxidation by scavenging free radicals. The mechanism which is, also, involved in anti-atherogenetic effect of resveratrol is inhibition of vascular inflammation (described above). Resveratrol inhibits proliferation and migration of the vascular smooth muscle cell. There is evidence that resveratrol blocks oxidized LDL-induced proliferation of smooth muscle cell. Actually, resveratrol inhibits the mammalian target of rapamycin (mTOR) mitogenic signaling pathway [46]. According to this, it is obvious that all the described mechanisms might be involved in an anti-atherogenetic effect of resveratrol.

Resveratrol and diabetes

In animal studies, resveratrol decreases blood glucose and protects pancreatic ß cells from oxidative damage [47]. It binds to sulfonylurea receptor and block pancreatic adenosine-5'-triphosphate (ATP)-sensitive K^+ channels. Also, resveratrol displaced binding of glibenclamide, the drug which blocks ATP-sensitive K^+ channels in ß cells [48]. Resveratrol stimulated secretion of insulin in ß cell insulinoma lines [49]. In the presence of resveratrol, the amplifying pathway of insulin secretion, independent of the closure of ATP-sensitive K^+ channels in ß cells, was reported [50].

In development of type 2 diabetes, the most critical factor is insulin resistance. It is well known that SIRT1 has involved in the processes of glucose metabolism and insulin secretion. Increased expression of SIRT1 improves insulin sensitivity. Resveratrol is potent activator of SIRT1. Also, it

attenuates high fat diet-induced insulin resistance *in vivo*, in dose of 2.5 mg/kg/day [51].

The effect of resveratrol on energy metabolism and metabolic profile was investigated in randomized double-blind, crossover study which included 11 healthy, obese men treated with placebo or with resveratrol 150 mg/day for 30 days. The conclusion of this study was that resveratrol induced metabolic changes in the obese humans, mimicking the effects of calorie restriction. Resveratrol decreases intrahepatic lipid content, circulating glucose, triglycerides and inflammation markers [52].

Resveratrol and oxidative stress

The direct antioxidant effect of resveratrol is not prominent. Well established antioxidants, such as ascorbate and cysteine are more potent than resveratrol [53]. Resveratrol has been shown to be a scavenger of hydroxyl, superoxide, metal-induced radicals and H_2O_2. In cardiovascular tissues, resveratrol induces antioxidant enzymes. The molecular mechanisms of induction of antioxidant enzymes by resveratrol are not completely understood. Studies have demonstrated that SIRT1 and the nuclear factor E2-related factor-2 (Nrf2) play crucial roles in this process. Resveratrol induced SOD2 upregulation in cultured human coronary arterial endothelial cells. Such upregulation can be blocked by small interfering RNA (siRNA)-mediated knockdown of SIRT1. An overexpression of SIRT1 leads to SOD2 upregulation. Nrf2 is transcription factor involved in the regulation of a number of ROS detoxifying enzymes. In Nrf2-dependent manner, in cultured endothelial cell, resveratrol induced NAD(P)H: quinine oxidoreductases (NQO1), heme oxygenase-1 (HO-1) and c-glutamylcysteine synthetase (GCLC). The listed enzymes are rate-limited for glutathione synthesis. Also, resveratrol inhibits ROS production [54]. Treatment with resveratrol reduces the expression of NOS in the heart of hypercholesteroemic mouse, as well as mono-nitrogen oxides in the aorta of trauma hemorrhagic rats [55, 56]. The activity of the NADPH oxidase enzyme complex is reduced by resveratrol [57].

Resveratrol and heart

According to numerous animal studies, proposed anti-ischemic mechanisms of resveratrol include: coronary vasodilatation, inhibition of atheroma formation, metabolic protection and less ischemic-reperfusion injury. In addition, resveratrol (5 μM/L) inhibited growth of cardiac fibroblasts stimulated by angiotensin II, epidermal growth factor and transforming growth factor β, which are essential in cardiac fibrosis and heart failure [12]. Zheng et al. [13] demonstrated antiarrhythmic effect of resveratrol. In the papillary muscles of guinea pig heart, resveratrol shortened duration of action potential and decreased velocity of phase 0 depolarization. Also, it inhibited delayed after depolarization and triggered activity. These effects were due to a decrease of calcium influx and intracellular calcium concentration.

Conclusion

Cardioprotection includes all the described effects of resveratrol. The most important evidence for cardioprotection rendered by resveratrol comes from *in vivo* studies carried out on animal models of chronic heart disease that resulted in heart dysfunction. Those include hypertension, obesity, metabolic syndrome, type I diabetes, type II diabetes, viral cardiomiopathy, toxin cardiomiopathy and aging.

According to the presented results, the cardioprotective effect of resveratrol is obvious. It is complex and not well defined. It is necessary to emphasize that all positive effects of resveratrol were demonstrated mainly in *in vitro* studies and in studies with experimental animals, while quality clinical studies are lacking.

Further clinical studies are necessary in order to define pharmacokinetics, efficacy, adequate therapeutic doses, tolerability and possible interactions of resveratrol with other drugs.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No TR 31020).

R E F E R E N C E S

1. *Arichi H, Kimura Y, Okuda H, Baba K, Kozawa M, Arichi S*. Effects of stilbene components of the roots of Polygonum cuspidatum Sieb. et Zucc. on lipid metabolism. Chem Pharm Bull (Tokyo) 1982; 30(5): 1766−70.
2. *Vastano BC, Chen Y, Zhu N, Ho CT, Zhou Z, Rosen RT*. Isolation and identification of stilbenes in two varieties of Polygonum cuspidatum. J Agric Food Chem 2000; 48(2): 253−6.
3. *Dudley J, Das S, Mukherjee S, Das DK*. Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose. J Nutr Biochem 2009; 20(6): 443-452.
4. *Mukherjee S, Dudley JI, Das DK*. Dose-dependency of resveratrol in providing health benefits. Dose Response 2010; 8(4): 478–500.
5. *Ibern-Gómez M, Roig-Pérez S, Lamuela-Raventós RM, de la Torre-Boronat MC*. Resveratrol and piceid levels in natural and blended peanut butters. J Agric Food Chem 2000; 48(12): 6352−4.
6. *Sobolev VS, Cole RJ*. Trans-resveratrol content in commercial peanuts and peanut products. J Agric Food Chem 1999; 47(4): 1435−9.
7. *Romero-Pérez AI, Lamuela-Raventós RM, Andrés-Lacueva C, de La Torre-Boronat MC*. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. J Agric Food Chem 2001; 49(1): 210−5.
8. *Soleas GJ, Goldberg DM, Diamandis EP, Karumanchiri A, Yan J, Ng E*. A derivatized as chromatographic mass spectrometric method for the analysis of both isomers of resveratrol in juice and wine. Am J Enol Vitic 1995; 46(3): 346–52.
9. *Sanders TH, McMichael RW Jr, Hendrix KW*. Occurrence of resveratrol in edible peanuts. J Agric Food Chem 2000; 48(4): 1243−6.

10. *Tokuşoglu O, Unal MK, Yemiş F.* Determination of the phytoalexin resveratrol (3,5,4'-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode array (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). J Agric Food Chem 2005; 53(12): 5003−9.
11. *Cvejic JM, Djekic SV, Petrovic AV, Atanackovic MT, Jovic SM, Brceski ID, et al.* Determination of trans- and cis-resveratrol in Serbian commercial wines. J Chromatogr Sci 2010; 48(3): 229−34.
12. *Opie LH, Leceur S.* The red wine hypothesis: from concept to protective signalling molecules. Eur Heart J 2007; 28(14): 1683−93.
13. *Zheng L, Zhang LP, Ma HJ, Wang C, Ming L, Wang QS.* Resveraatrol reduces free calcium concentration in rat ventricular myocytes. A Physiol Sin 2005; 57(5): 599−604.
14. *Soleas GJ, Angelini M, Grass L, Diamandis EP, Goldberg DM.* Absorption of trans-resveratrol in rats. Methods Enzymol 2001; 335: 145−54.
15. *Atanacković M, Posa M, Heinle H, Gojković-Bukarica L, Cvejić J.* Solubilization of resveratrol in micellar solutions of different bile acids. Colloids Surf B Biointerfaces 2009; 72(1): 148−54.
16. *Walle T.* Bioavailability of resveratrol. Ann N Y Acad Sci 2011; 1215: 9−15.
17. *Renaud S, De Lorgeril M.* Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992; 339(8808): 1523−6.
18. *Klinge CM, Blankenship KA, Risinger KE, Bhatnagar S, Noisin EL, Sumanasekera WK,* et al. Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells. J Biol Chem 2005; 280(9): 7460−8.
19. *Shen MY, Hsiao G, Liu CL, Fong TH, Lin KH, Chou DS,* et al. Inhibitory mechanisms of resveratrol in platelet activation: pivotal roles of p38 MAPK and NO/cyclic GMP. Br J Haematol 2007; 139(3): 475−85.
20. *Baur JA, Sinclair DA.* Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006; 5(6): 493−506.
21. *Thirunavukkarasu M, Penumathsa SV, Koneru S, Juhasz B, Zhan L, Otani H,* et al. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. Free Radic Biol Med 2007; 43(5): 720−9.
22. *Xanthoudakis S, Curran T.* Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. Embo J 1992; 11(2): 653−65.
23. *Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L,* et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 2005; 310(5746): 314−7.
24. *Caddeo C, Teskac K, Sinico C, Kristl J.* Effect of resveratrol incorporated in liposomes on proliferation and UV-B protection of cells. Int J Pharm 2008; 363(1−2): 183−91.
25. *Gojkovic-Bukarica L, Novakovic A, Kanjuh V, Bumbasirevic M, Lesic A, Heinle H.* A role of ion channels in the endothelium-independent relaxation of rat mesenteric artery induced by resveratrol. J Pharmacol Sci 2008; 108(1): 124−30.
26. *Novakovic A, Gojkovic-Bukarica L, Peric M, Nezic D, Djukanovic B, et.* The mechanism of endothelium-independent relaxation induced by the wine polyphenol resveratrol in human internal mammary artery. J Pharmacol Sci 2006; 101(1): 85−90.
27. *Novakovic A, Gojkovic-Bukarica L, Kanjuh V, Heinle H.* Potassium channels-mediated vasorelaxation of rat aorta induced by resveratrol. Basic Clin Pharmacol Toxicol 2006; 99(5): 360−4.
28. *Naderali EK, Doyle PJ, Williams G.* Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea pigs. Clin Sci (Lond) 2000; 98(5): 537–43.
29. *Jager U, Nguyen-Duong H.* Relaxant effect of trans-resveratrol on isolated porcine coronary arteries. Arzneimittelforschung 1999; 49(3): 207–11.
30. *Nagaoka T, Hein TW, Yoshida A, Kuo L.* Resveratrol, a component of red wine, elicits dilation of isolated porcine retinal arterioles: role of nitric oxide and potassium channels. Invest Ophthalmol Vis Sci 2007; 48(9): 4232−9.
31. *Rakici O, Kiziltepe U, Coskun B, Aslamaci S, Akar F.* Effects of resveratrol on vascular tone and endothelial function of human saphenous vein and internal mammary artery. Int J Cardiol 2005; 105(2): 209−15.
32. *Silan C.* The effects of chronic resveratrol treatment on vascular responsiveness of streptozotocin-induced diabetic rats. Biol Pharm Bull 2008; 31(5): 897–902.
33. *Li H, Forstermann U.* Resveratrol: a multifunctional compound improving endothelial function. Editorial to: «Resveratrol supplementation gender independently improves endothelial reactivity and suppresses superoxide production in healthy rats» by S. Soylemez et al. Cardiovasc Drugs Ther 2009; 23(6): 425−9.
34. *Spanier G, Xu H, Xia N, Tobias S, Deng S, Wojnowski L, et al.* Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). J Physiol Pharmacol 2009; 60(Suppl 4): 111−6.
35. *Wong RH, Howe PR, Buckley JD, Coates AM, Kunz I, Berry NM.* Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutr Metab Cardiovasc Dis 2010; 21(11): 851−6.
36. *Campos-Toimil M, Elíes J, Alvarez E, Verde I, Orallo F.* Effects of trans- and cis-resveratrol on Ca^{2+} handling in A7r5 vascular myocytes. Eur J Pharmacol 2007; 577(1−3): 91−9.
37. *Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA,* et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004; 23(12): 2369–80.
38. *Kundu JK, Shin YK, Kim SH, Surh YJ.* Resveratrol inhibits phorbol esterinduced expression of COX-2 and activation of NF-kappaB in mouse skin by blocking IkappaB kinase activity. Carcinogenesis 2006; 27(7): 1465–74.
39. *Manna SK, Mukhopadhyay A, Aggarwal BB.* Resveratrol suppresses TNFinduced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol 2000; 164(12): 6509–19.
40. *Wang Z, Huang Y, Zou J, Cao K, Xu Y, Wu JM.* Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro. Int J Mol Med 2002; 9(1): 77–9.
41. *Kirimlioglu V, Sozen H, Turkoglu S, Haberal M.* Protective effect of resveratrol, a red wine constituent polyphenol, on rats subjected to portal vein thrombosis. Transplant Proc 2008; 40(1): 290−2.
42. *Szewczuk LM, Forti L, Stivala LA, Penning TM.* Resveratrol is a peroxidasemediated inactivator of COX-1 but not COX-2: a mechanistic approach to the design of COX-1 selective agents. J Biol Chem 2004; 279(21): 22727−37.
43. *Wang Z, Zou J, Cao K, Hsieh TC, Huang Y, Wu JM.* Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. Int J Mol Med 2005; 16(4): 533–40.
44. *Do GM, Kwon EY, Kim HJ, Jeon SM, Ha TY, Park T,* et al. Long-term effects of resveratrol supplementation on suppression of atherogenic lesion formation and cholesterol synthesis in apo E-deficient mice. Biochem Biophys Res Commun 2008; 374(1): 55−9.
45. *Zou J, Huang Y, Cao K, Yang G, Yin H, Len J,* et al. Effect of resveratrol on intimal hyperplasia after endothelial denudation in an experimental rabbit model. Life Sci 2000; 68(2): 153−63.
46. *Brito PM, Devillard R, Nègre-Salvayre A, Almeida LM, Dinis TC, Salvayre R,* et al. Resveratrol inhibits the mTOR mitogenic sig-

naling evoked by oxidized LDL in smooth muscle cells. Atherosclerosis 2009; 205(1): 126−34.

47. *Zhang Y, Jayaprakasam B, Seeram NP, Olson LK, DeWitt D, Nair MG*. Insulin secretion and cyclooxygenase enzyme inhibition by cabernet sauvignon grape skin compounds. J Agric Food Chem 2004; 52(2): 228–33.
48. *Hambrock A, de Oliveira Franz CB, Hiller S, Grenz A, Ackermann S, Schulze DU,* et al. Resveratrol binds to the sulfonylurea receptor (SUR) and induces apoptosis in a SUR subtype-specific manner. J Biol Chem 2007; 282: 3347–56.
49. *Chen WP, Chi TC, Chuang LM, Su MJ*. Resveratrol enhances insulin secretion by blocking KATP and KV channels of beta cells. Eur J Pharmacol 2007; 568(1−3): 269–77.
50. *Szkudelski T*. Resveratrol-induced inhibition of insulin secretion from rat pancreatic islets: evidence for pivotal role of metabolic disturbances. Am J Physiol Endocrinol Metab 2007; 293(4): E901–7.
51. *Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q*. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab 2007; 6(4): 307−19.
52. *Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH,* et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 2011; 14(5): 612−22.
53. *Bradamante S, Barenghi L, Villa A*. Cardiovascular protective effects of resveratrol. Cardiovasc Drug Rev 2004; 22(5): 169–88.
54. *Li H, Xia N, Förstermann U*. Cardiovascular effects and molecular targets of resveratrol. Nitric Oxide 2012; 26(2): 102–10.
55. *Yu HP, Hwang TL, Hwang TL, Yen CH, Lau YT*. Resveratrol prevents endothelial dysfunction and aortic superoxide production after trauma hemorrhage through estrogen receptor-dependent hemeoxygenase-1 pathway. Crit Care Med 2010; 38(4): 1147–54.
56. *Xia N, Daiber A, Habermeier A, Closs EI, Thum T, Spanier G,* et al. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. J Pharmacol Exp Ther 2010; 335(1): 149−54.
57. *Hshu YC, Wang JS, Chen JK*. Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. J Appl Physiol 2007; 102(4): 1520−7.

Received on June 13, 2012.
Revised on July 15, 2012.
Accepted on August 2, 2012.

CASE REPORTS

UDC: 617.7/006.44
DOI: 10.2298/VSP1312151J

Two cases of uveitis masquerade syndrome caused by bilateral intraocular large B-cell lymphoma

Dva bolesnika sa maskiranim sindromom uveitisa nastalim kao posledica obostranog intraokularnog limfoma velikih B-ćelija

Svetlana Jovanović*†, Zorica Jovanović†, Jelena Paović‡§, Vesna Stanković Čeperković†, Snežana Pešić†, Vujica Marković‡§

***Clinic of Ophthalmology, Clinical Center "Kragujevac", Kragujevac, Serbia; †Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia; ‡Clinic of Ophthalmology, Clinical Center of Serbia, Belgrade, Serbia; §Faculty of Medicine, University of Belgrade, Serbia**

Abstract

Introduction. Sometimes it is not easy to clinically recognize subtle differences between intraocular lymphoma and non-infectious uveitis. The most common lymphoma subtype involving the eye is B-cell lymphoma. **Case report**. We presented two patients aged 59 and 58 years with infiltration of the subretinal space with a large B-cell non-Hodgkin intraocular lymphoma. The patients originally had clinically masked syndrome in the form of intermediate uveitis. As it was a corticosteroid-resistant uveitis, we focused on the possible diagnosis of neoplastic causes of this syndrome. During hospitalization, the neurological symptoms emerged and multiple subretinal changes accompanied by yellowish white patches of retinal pigment epithelium with signs of vitritis, which made us suspect the intraocular lymphoma. Endocranial magnetic resonance imaging established tumorous infiltration in the region of the left hemisphere of the cerebellum. The histopathological finding confirmed the diagnosis of large B-cell non-Hodgkin lymphoma of risk moderate degree, immunoblast – centroblast cytological type. The other patient had clinical chronic uveitis accompanied by yellowish shaped white echographic changes of the retina and localized changes in the level of the subretina. The diagnosis of lymphoma was made by brain biopsy. **Conclusion**. Uveitis masquerade syndrome should be considered in all patients over 40 years with idiopathic steroid-resistant uveitis. Treatment begun on time can affect the course and improve the prognosis of uveitis masquerade syndrome (UMS) and systemic disease.

Key words:
eye neoplasms; lymphoma, non-hodgkin; uveitis; diagnosis, differential.

Apstrakt

Uvod. Ponekad je teško ustanoviti suptilnu razlika između intraokularnog limfoma i neinfektivnog uveitisa. Najčešći podtip intraokularnog limfoma je B-ćelijski limfom. **Prikaz bolesnika**. Prikazali smo dva bolesnika, starosti 59 i 58 godina, sa infiltracijom subretinalnog prostora velikim B-ćelijama non-Hodgkin itraokularnog limfoma. Prvi bolesnik prvobitno je imao kliničku sliku maskiranog sindoma u vidu intermedijalnog uveitisa. Kako se radilo o kortikosteroid-rezistentnom uveitisu, usredsredili smo se na moguću dijagnozu neoplastičnog uzroka ovog maskiranog sindroma. U toku hospitalizacije na neurologiji pojavili su se subretinalni eksudati praćeni žućkastobeličastim promenama retinalnog pigmentnog epitela i znacima vitritisa koji su nas naveli na sumnju na intraokularni limfom. Magnetna rezonanca (MR) endokranijuma potvdila je infiltraciju leve hemisfere cerebeluma. Patohistološki nalaz operisanog tumora cerebeluma potvrdio je dijagnozu non-Hodgkin limfoma velikih B-ćelija, umerenog stepena rizika, imunoblast-centroblastom citološkog tipa. Drugi bolesnika imao je kliničku sliku hroničnog zadnjeg uveitisa sa žućkasto beličastim promenama retine i ehografski lokalizovanim promenama u nivou subretine. Dijagnoza limfoma postavljena je biopsijom mozga. **Zaključak**. Uveitis maskirani sindom (UMS) treba razmotriti kod svih bolesnika starijih od 40 godina sa idiopatskim kortikosteroid-rezistentnim uveitisom. Lečenje započeto na vreme može uticati na tok i poboljšati prognozu UMS i sistemske bolesti.

Ključne reči:
oko, neoplazme; limfom, nehodžkinov; uveitis; dijagnoza, diferencijalna.

Correspondence to: Svetlana Jovanović, Department of Ophthalmology, Clinical Center "Kragujevac", Grada Sirena 30, 34 000 Kragujevac, Serbia. Phone: +381 63 683 054. E-mail: micaj034@yahoo.com , drsvetlanajovanovic@yahoo.com

Introduction

Uveitis masquerade syndromes (UMSs) are a group of non-inflammatory ocular diseases of benign or malign origin [1, 2]. A neoplastic lesion causes intraocular cellular infiltration and mimics intraocular inflammation, simulating immune mediated uveitis, poorly or not at all responsive to corticosteroid treatmant. Because UMSs are not only sight-threatening but in case of malign UMSs also a life-threatening disease, prompt and correct diagnosis and treatment is very important [3].

The World Health Organization (WHO) / Revised European-American Classification of Lymphoid Neoplasms (REAL) immunophenotypic classification identifies 3 types of lymphomas: B-cell neoplasms, T-cell and natural killer (NK) cell neoplasms and Hodgkin's disease [4].

Intraocular lymphoma can be further classified either as primary B-cell lymphoma of the retina and central nervous system (CNS) or as extranodal lymphoma of the uvea, or as a secondary B-cell lymphoma that represents uveal manifestation of systemic lymphoma. The most common lymphoma subtype involving the eye is B-cell lymphoma [4]. Suspicion is needed if symptoms are uniteral or occurs in very young children or in the elderly.

Primary intraocular lymphoma (PIOL) is, in fact, a subtype of primary central nervous system lymphoma (PCNSL) and non-Hodgkin's lymphoma (NHL). Neuraxis consists of not only the brain and spinal cord, but also the neurosensory retina. PCNSL is most commonly a B-cell tumor, though T-cell PCNSL has been described [4]. As an NHL B-cell disease, PCNLS is most frequently a subtype of diffuse large B-cell lymphoma (DLBCL).

There are 3 major subtypes of DLBCL: activated B-cell DLBCL (ABC DLBCL), germinal center B-cell (GCB DLBCL), and primary mediastinal (thymic large) B-cell DLBCL (PMB DLBCL) also known as type 3, based on gene signature profiling [5].

Ocular disease is bilateral in 50% of patients with PIOL [6]. Differential diagnosis include various diseases, such as sarcoidasis, tuberculosis, syphilis, toxophamosis, toxocardiasis, idiopathic vasculitis and scleritis, primary ocular – CNS non Hodgkins lymphoma, large B cell lymphoma, etc.

This small, retrospective observation case study reports the clinical presentation and pathophysiologic correlation in 2 patients over 50 years of age with masquerade syndrome. Informed consent was obtained from each patient.

Comprehensive clinical ophthalmic examination using ultrasound and fluorescein angiography diagnostics was performed in both patients, who were further evaluated with computed topography (CT) and magnetic resonance imaging (MRI). All imaging modalities were conducted according to the standard protocol [7]. The diagnosis of intraocular lymphoma was based on the histopathology pattern on biopsy spacemen [8].

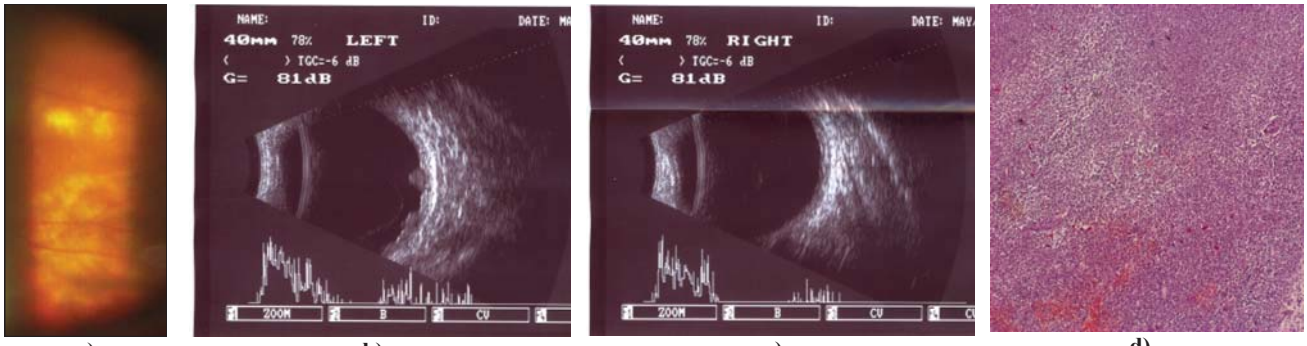
Case report 1

The first patient, a 59-year-old man, presented with an 11-month history of intraocular inflammation like uveitis, anterior and posterior. He had no ophthalmological disease in the past. The first ophthalmological manifestations were in the form of unilateral anterior and then posterior uveitis followed changes developed later in the same sense and on the other eye. The front uveitis was a small whitish precipitate of the endothel without plastic reactions in the sense of creating synechia. Posterior uveitis was in the form of vitritis with creamy white lesions in the level of the retina and retinal pigment epithelium (RPE) lesion boundary (Figure 1a).

The findings of ultrasound a month after showed lifting of the retinas in both eyes with masses in the subretinal space (Figures 1b and c).

Visual acuity and field of vision were changed. Initially the patient only slightly reacted to the corticosteroid therapy. After a year from the first appearance of symptoms of uveitis the patient developed neurological manifestations of the disease but still the CT finding was negative.

The third attempt with MRI detected tumor in the left hemisphere of the cerebellum. Pathohistological findings showed a diffuse large B-cell non-Hodgkin lymphoma of a moderate degree risk, immunoblast-centroblast cytological type. A diffuse large cell tumor consisted of large oval or irregular cell nuclei, with a large nucleolus and a lower number of smaller, medium or heavy amphophil basophil cytoplasm, with a rare presence of apoptosis, with a tangible body macrophage histiocytoma and numerous mitoses (Figure 1d).



a) b) c) d)

Fig. 1 – A patient with primary intraocular lymphoma showing creamy white lesions at the level of the retina and retinal pigment epithelium lesion boundary.

a) Fotofundus; b, c) Ultrasound – lifting of the retinas in both eyes with masses in the subretinal space; d) Non-Hodgkin lymphoma in the brain – B-cell, large cell diffusum (immunoblast-centroblast cytological type)

He was afebrile and his routine blood test was normal except low total red blood cell count (RBC), hemoglobin (Hgb), mean platelet volume (MPV) and relative volume of thrombocytes (Pct) were on little level. The patient did not allow biopsy of the retina.

Localization of the tumor was in the brain and subretinal space, actually in the immune privileged sites.

Case report 2

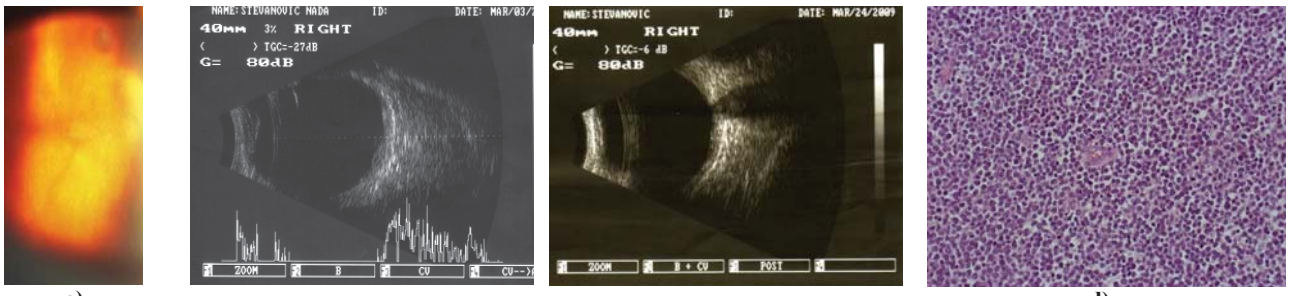
The second patient was a 58-year-old woman with anterior and posterior uveitis for several months. She showed no satisfactory respond to the treatment with corticosteroids. In fact, the therapy with corticosteroids had led to an incomplete reabsorption flare and precipitate in the front segment, with signs of vitritis in the back. The last segment was visible and the ultrasound findings showed an improvement. The posterior uveitis was manifested in the form of creamy, yellow white to orange subretinal pigment epithel (RPE) infiltrates, which also disappeared as a result of the treatment with corticosteroids, but the last segment as a whole was changed (Figure 2a).

Ultrasound suggested that intraocular lymphoma still responded to corticosteroid therapy. The fluorescein angiography in the intraocular lymphoma had a characteristic appearance. RPE disturbances included granularity, mottling, and late staining patterns. Fluorescence blockage at the level of the RPE, due to tumor infiltration could correspond to the deep retinal or subretinal creamy colored lesions noted on fundus photography. Visual acuity and visual field testing, were both reduced significantly. Intraocular (IOP) was 14 mm Hg. A routine blood test was normal except for Hgb. MR images demonstrated enhancement in the left temporal lobe. The diagnosis of lymphoma was made by brain biopsy. Histopathological finding was diffuse large B-cell non-Hodgkin lymphoma (Figures 2 b, c and d).

ophthalmologist is particularly important. Imaging of the central nervous system should be included. Imaging modalities are: full field fundus photography, ultrasound, fluorescein angiography, indocyanine green angiography, ocular coherence tomography, brain imaging. Systemic organs imaging is generally not necessary in the cases of suspected PIOL or PCNSL, and this practice is not highly recommended [11]. When there is a reason to suspect systemic lymphoma added to the standard tests are a complete blood cell count, erythrocyte sedimentation rate, and bone marrow evaluation [12]. Vitreous biopsy is a useful tool to diagnose PIOL. If it is implemented prior to steroid therapy, it might suppress the number of vitreous cells, including lymphoma cells, which may result in a negative vitreous cytology [13]. The lymphoma cells of POIL and PCNSL are very fragile, and if systemic corticosteroids are used to treat a presumed "uveitis", the lymphoma cells may be even more fragile [14]. Conventional ocular and brain examination includes cytological and histological examination (macroscopic and microscopic). Molecular pathology involves gene rearrangements, translocations, molecular signals, infectious deoxyribonucleic acid (DNA) [15]. Immunohistochemistry shows the same picture as that for PIOL. As the majority of PCNSL cells are B-cells, they stain for CD19, CD20, and surface immunoglobulin [16, 17].

Conclusion

Sometimes, uveitis is the only initial manifestation of an occult systemic problem in patients older than 40 years. UMS should be considered in all patients with idiopathic corticosteroid resistant chronic uveitis. Primary intraocular lymphoma should be considered in all patients aged 40 and older with ultrasonographic findings of subretinal lesions and vitreous cells. Malignancies and other diseases should be considered, with implementation of diagnosis biopsies of vi-



a) b) c) d)

Fig. 2 – A patient with primary intraocular lymphoma showing creamy, yellow-white to orange sub-retinal pigment epithelium infiltrates

a) Fotofundus; b) Ultrasound – lifting of the retinas in both eyes with masses in the subretinal space before the therapy c) after the therapy; d) Non-Hodgkin lymphoma in the brain (B-cell, large cell diffusum).

Discussion

Primary intraocular lymphoma is the most common manifestation of masked uveitis syndromes [9]. It is typically presented as posterior uveitis with cellular exudation in the vitreous fluid [10]. Imaging of the eye is the first step in evaluating the diagnosis with suspicion of PIOL. The role of an

trous fluid and brain. Timely treatment may improve the prognosis of UMS. Direct treatment of malignancy or underlying condition may be required to control uveitis.

Acknowledgements

This work was supported by the Grant No. 500-01-00035, the Ministry of Health of the Republic of Serbia.

REFERENCES

1. *Chan CC, Wallace DJ*. Intraocular Lymphoma: Update on Diagnosis and Management. Cancer Control 2004; 11(5): 285−95.
2. *Kubicka-Trzaska A, Romanowska-Dixon B*. Malignant uveitis masquerade syndromes. Klin Oczna 2008; 110(4−6): 199−202.
3. *Choi JY, Kafkala C, Foster CS*. Primary intraocular lymphoma: A review. Semin Ophthalmol 2006; 21(3): 125−33.
4. *Parikh AH, Khan SH, Wright JD Jr, Oh KT*. Systemic non-Hodgkin's lymphoma simulating primary intraocular lymphoma. Am J Ophtalmol 2005; 139(3): 573−4.
5. *Choi JS, Nam DH, Ko YH*. Primary central nervous system lymphoma in Korea: comparison of B- and T- cell lymphomas. Am J Surg Pathol 2003; 27(7): 919−28.
6. *Wright G, Tan B, Rosenwald A, Hurt EH*. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B-cell lymphoma. Proc Natl Acad Sci USA 2003; 100(17): 9991−6.
7. *Hoffman PM, McKelvie P, Hall AJ, Stawell RJ, Santamaria JD*. Intraocular lymphoma: a series of 14 patients with clinicopathological features and treatment outcomes. Eye (Lond) 2003; 17(4): 513−21.
8. *Pileri SA, Agostinelli C, Sabattini E, Bacci F, Sagramoso C, Pileri A Jr*, et al. Lymphoma classification: the quiet after the storm. Semin Diagn Pathol 2011; 28(2): 113−23.
9. *Cogliatti SB, Schimid U*. Who is Who and what is REAL? Swiss Med Wkly 2002; 132(43−44): 607−17.
10. *Rothova A, Ooijman F, Kerkhoff F, Van Der Lelij A, Lokhorst HM*. Uveitis masquerade syndromes. Ophthalmology 2001; 108(2): 386−99.
11. *Nussenblatt RB, Chan CC, Wilson WH, Hochman J, Gottesman M;* CNS and Ocular Lymphoma Workshop Group. International Central Nervous System and Ocular Lymphoma Workshop: recommendations for the future. Ocul Immunol Inflamm 2006; 14(3): 139−44.
12. *Leavy-Clarke GA, Chan CC, Nussenblatt RB*. Diagnosis and management of primary intraocular lymphoma. Hematol Oncol Clin North Am 2005; 19(4):739−49.
13. *Buggage RR, Chan CC, Nussenblatt RB*.Ocular manifestation of centralnervous system lymphoma. Curr Opin Oncol 2001; 13(3): 137−42.
14. *Kim E, Kim C, Lee J, Cho Y*. A case of primary intraocular lymphoma treated by intravitreal methotrexate. Korean J Ophthalmol 2009; 23(3): 210−4.
15. *Wallace DJ, Shen D, Read GF, Miyanaga M, Mochizuki M, Sen HN,* et al. Detection of the bcl-2 t(14;18) translocation and proto-oncogene expression in primary intraocular lymphoma. Invest Ophthalmol Vis Sci 2006; 47(7): 2750−6.
16. *Bashir R, McManus B, Cuningham C, Weisburger D, Hoshberg F*. Detection of Eber-1 RNA in primary brain lymphomas in immunocompetent and immunocompromised patients. J Neurooncol 1994; 20(1): 47−53.
17. *Schlegel U*. Primary CNS Lymphoma Ther Adv Neurol Disord 2009; 2(2): 93−104.

Received on September 12, 2011.
Accepted on June 11, 2012.

CASE REPORT

UDC: 616.36-006
DOI: 10.2298/VSP1312155J

Stromal reaction and prognosis in acinic cell carcinoma of the salivary gland

Stromalna reakcija i prognoza acinoćelijskog karcinoma pljuvačne žlezde

Ljubinka Janković Veličković*, Irena Dimov†, Dragan Petrović‡, Slavica Stojnev*, Stefan Dačić‡, Stefan Veličković‡, Vladisav Stefanović§

***Institute of Pathology, †Department of Immunology, ‡Clinic of Stomatology, §Institute of Nephrology, Faculty of Medicine, University of Niš, Niš, Serbia**

Abstract

Introduction. Primary acinic cell carcinoma (ACC) is an uncommon malignant neoplasm of the salivary gland (SG), which usually presents as slow growing tumor. **Case report.** We reported a 69-year-old woman with tumor in the right parotid gland with a 5-year progress. Biopsy sections revealed a hybrid form of ACC with a low- and high-grade component and prominent lymphoid tissue in tumor stroma. Immunohistochemistry was performed to define the molecular profile of this unusual ACC, with special interest for stromal influence on to the proliferative activity of ACC with dedifferentiation. We detected that the level and the type of stromal lymphoid reaction (particularly $CD8^+/CD4^+$ ratio) had a significant influence on to Ki-67 index in the high-grade component of ACC, as well as the involvement of the CXCR4 signaling axis in the stromal reaction influence. **Conclusion.** We suggest that tumor stroma may be a source of potential new tumor biomarkers which can determine the aggressivity of this tumor.

Key words:
parotid neoplasms; carcinoma, acinar cell; ki-67 antigen; prognosis.

Apstrakt

Uvod. Primarni acinoćelijski karcinom (ACC) je redak maligni tumor pljuvačnih žlezda koji obično ima sporu progresiju. **Prikaz bolesnika.** U radu je prikazana bolesnica, stara 69 godina, sa tumorom u desnoj parotidnoj žlezdi. Na presecima tkiva otkrivena je hibridna forma ACC, sa komponentom niskog i visokog gradusa, i izraženim limfnim tkivom u stromi tumora. Tumor je pokazivao 5-godišnju progresiju. Imunohistohemijska metoda korišćena je kako bi se definisao molekularni profil ove retke forme ACC, kao i uticaj strome na proliferativnu aktivnost ACC sa njegovom dediferencijacijom. Pokazali smo da nivo i tip stromalne limfoidne reakcije (pogotovo odnos $CD8^+/CD4^+$) ima značajan uticaj na Ki-67 indeks u komponenti visokog gradusa ACC, kao i uticaj CXCR4 signalnog puta. **Zaključak.** Tumorska stroma može biti izvor potencijalno novih tumorskih biomarkera, kada imuni odgovor može uticati na agresivnost ovog tumora.

Ključne reči:
parotidne žlezde, neoplazme; karcinom acinusnig ćelija; antigen, ki-67; prognoza.

Introduction

Although acinic cell carcinomas (ACCs) represent only 2–6% of salivary gland tumors, they are the third most common epithelial malignancy after mucoepidermoid carcinoma, thus being about 10% of all malignant salivary gland tumors [1]. Well-differentiated ACCs present as well circumscribed encapsulated tumors with a solid or microcystic pattern in which tumor cells are surrounded and intermingled with prominent lymphoid response. Dedifferentiated ACC presents itself with areas of low-grade ACC and areas of dedifferentiated high grade ACC, or undifferentiated carcinoma within the same tumor [2, 3]. Investigation of biological markers is very important and necessary for predicting prognosis of salivary malignancies and better understanding the pathogenesis of salivary cancer. Ki-67 was already proven as useful prognostic markers for survival in a few studies of patients with ACC and other salivary glands tumors [4, 5]. Tumor stroma may be a source of potential new tumor biomarkers, in which the immune response is of major importance. Here we investigated stromal influence onto the proliferative activity of ACC with dedifferentiation and evolution of this parotid gland tumor.

Case report

A 69-year-old woman presented with a superficial lobulated swelling in the lower half of the right parotid

Correspondence to: Vladisav Stefanović, Faculty of Medicine, Bul. Zorana Djindjića 81, 18 000 Niš, Serbia. E-mail: stefan@ni.ac.rs

gland. The tumor had been detected 5 years before with the diameter of 1 × 1 cm, clinically manifested as slowly enlarging, asymptomatic, painless tumor mass. Five years later surgical resection of the lower part of the parotid gland was performed, as well as extirpation of a lymph node along the front edge of the sternocleidomastoidal muscle.

Pathological and immunohystochemical findings

In a fragment of the parotid gland (45 × 40 mm), gross analysis showed a well- circumscribed tan-gray tumor mass of strong consistency (38 × 30 × 10 mm), and a focus of bleeding (12 mm). Microscopically, biopsy sections revealed feature of ACC where tumor cells had basophilic cytoplasm and acinar differentiation. The tumor showed predominantly a low-grade component, but in some parts of ACC, a high-grade component was detected. Abundant lymphoid tissue with germ cell follicles was present in tumor stroma (Figure 1a). Some parts of a high-grade component showed prominent stromal lymphoid reaction with intratumoral infiltration of lymphocytes. Regional lymph node showed reactive hyperplastic change. The tumor was in pathological stage 2.

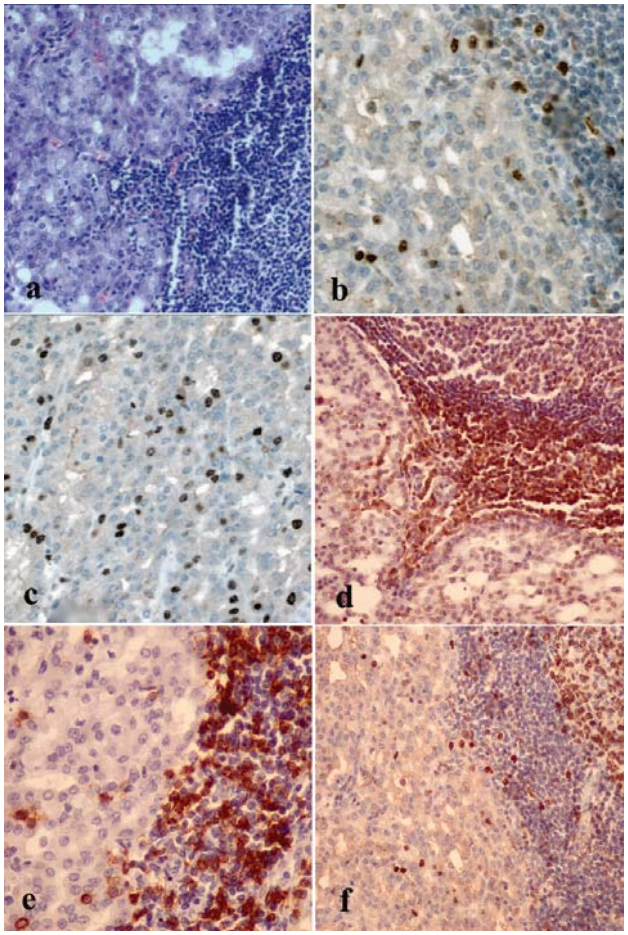


Fig. 1 – a) Acinic cell carcinomas (ACC) of the parotid gland with abundant stromal lymphoid tissue in high grade component (HE staining, × 40); b) Immunoreactivity in high grade ACC with stromal reaction and low Ki-67 index (× 40); c) prominent Ki-67 index in part of tumor with scanty stromal reaction (× 40); d) with infiltration of tumor with $CD8^+$ cell (× 40); e) and $CD4^+$ cells (× 40); f) CXCR4 positivity in both tumor cells and lymphocytes (× 40).

Broad spectrum of monoclonal antibodies was applied to define the molecular profile of low and high-grade components of ACC, and surrounding lymphoid stromal influence on to Ki-67 index. Immunohistochemical analysis was performed on the following 3 different tumor regions: low-grade, high-grade near prominent stromal reaction, and high-grade with a low stromal reaction. The tumor was analyzed using the mouse monoclonal antibody against p53, $p21^{WAF1/Cip1}$, HER-2, Ki-67, Bcl-2, Survivin, Bax, Fas, Caspase-3, CD4, CD8, CXCR4, GFAP, EMA, S-100, CEA, and CK (AE1/AE3). We defined indexes of Ki-67, p53, p21 and Survivin. On the literature data scoring system was performed to p21 [6]; Bcl-2, Bax, Fas, and Caspase 3 [7], and CXCR4 [8]. For testing the HER-2 (C-erbB2) status we used the HercepTest scoring system devised by DAKO. Characterization of the stromal reaction and a mononuclear cell infiltrate was determined based on the hot spot technique, which means that, in each studied tumor area, density was measured at the region of the highest tumor-infiltrating lymphocytes (TILs) density [9]. The TILs were further characterized by CD4 and CD8 markers expression and the percentage of CD4 and CD8 positive TILs was scored as: score 0, no immunoreactive cells; score +1, positivity in < 10% cells; score +2, positivity in 10–30% cells; and score +3, positivity in > 30% of cells. Pathology data regarding proliferative index Ki-67, p21, CD4 and CD8 positivity were analyzed by the analysis of variance (ANOVA), single-factor analysis and the $\chi 2$ test, and $p < 0.05$ was considered to be statistically significant.

Mitotic activity increased in the part of tumor with a high-grade component (Figure 1b, 1c), but it was significantly higher ($p < 0.05$) in high-grade AAC with scanty stroma. TILs showed the specific pattern of immunoreactivity. In high-grade tumor with strong lymphoid stromal reaction, $CD8^+$ cells infiltrating the tumor outnumbered the CD4+ cells (Figure 1d, 1e), and as a result, the $CD4^+/CD8^+$ ratio was below 0.5 (Figure 1d, 1e). In the low grade tumor and normal immune response this ratio is between 0.9 and 1.9. In the same area both TILs and tumor cells showed an increase in the density of CXCR4 positive cells (Figure 1f) and a decrease in the tumor cells mitotic activity (Figure 1b). A similar trend was seen in the case of stromal $CD4^+$ and $CD8^+$ cells.

Ki-67 index and quantification of $CD4^+$ cells and $CD8^+$ cells was shown in Figure 2. The molecular profile of investigated components in ACC is presented in Table 1.

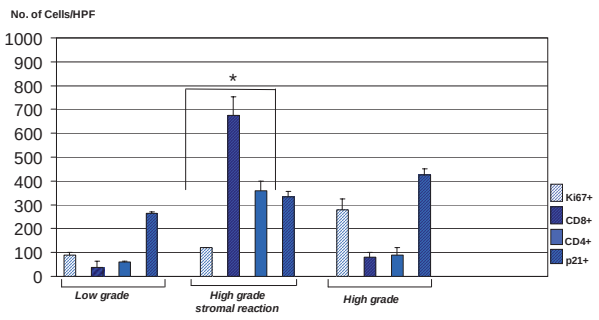


Fig. 2 – Lymphoid stromal reaction, Ki-67 and p21 expression in acinic cell carcinoma.
HPF – high power field.

Table 1
Expression of molecular markers in acinar cell carcinoma

Molecular markers	Low grade	High-grade stromal reaction	High grade
p53	–	–	–
p21%	–	Score 1	Score 2
HER-2	–	Score 2	Score 2
Ki-67	Score 1	Score 2	Score 1
Bcl-2	–	altered	altered
Survivin	–	–	–
Bax	normal	altered	altered
Fas	altered	altered	altered
Caspase-3	altered	–	–
CD4	Score 1	Score 3	Score 1
CD8	Score 1	Score 3	Score 1
CXCR4	+	+	–
GFAP	–	–	–
EMA	–	–	–
S-100	+	+	+
CEA	–	–	–
Cytokeratin	+	+	+

Discussion

Prognosis of salivary tumors depends mostly on the microscopic grade and the tumor type, as well as the stage of the disease and localization [1–3]. Investigation of proliferative activity in ACCs and other malignant salivary gland tumors, evaluating MIB-1 or Ki-67 index, shows that this is highly effective tool in patient follow-up and prognosis. Patients with MIB-1-negative ACCs had significantly better survival than patients with MIB-1-positive tumors. This is an independent prognostic factor for survival in patients with ACCs [4].

Furthermore, TILs showed the specific pattern of immunoreactivity regarding the tumor grade as well as the stroma/tumor ratio. In high-grade tumor with strong lymphoid stromal reaction, $CD8^+$ cells infiltrating the tumor were a dominant component of immune infiltrate. $CD4^+$ also revealed strong expression, although $CD8^+$ cytotoxic lymphocytes highly outnumbered $CD4^+$ TILs. This would suggest a later phase of the T-cell activation process [10–12] and may be a result of a relatively long period of tumor evolution.

In view of clinical and pathological data, it is speculated that the tumor foci lacking lymphoid stroma possibly represented a clone of high-grade malignancy arising within low-grade acinic cell carcinoma with lymphoid stroma. Several studies on various carcinomas have shown that a high $CD8^+/CD4^+$ T cell ratio is associated with favorable prognosis and *vice versa* [11, 13, 14]. The same area showed an increase in the density of CXCR4 positive cells in both TILs and tumor cells shown, which also correlated with low proliferation index. CXCR-4 is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12), a molecule endowed with a potent chemotactic activity for lymphocytes. The CXCR4 signaling pathway is a key regulator of many essential biological processes, such as cell motility, differentiation switch, apoptosis and lymphocyte homing [15, 16]. However, data regarding the role of CXCR4 is sometimes dubious. Until recently, SDF-1 and CXCR4 were believed to be a relatively "monogamous" ligand-receptor pair correlated to other chemokine receptors Recent evidence demonstrates ubiquitin (a well-known anti-inflammatory immune modulator and endogenous opponent of proinflammatory damage) may also be a natural ligand of CXCR4 [17, 18]. There is still no data on the role of this signaling axis in the ACC pathogenesis.

It is well-known that salivary gland cancer may resist programmed cell death with altered expression of both proapoptotic and antiapoptotic proteins [4, 5, 19], but this process can be regulated by expression of HER-1 and p21 20. So, a weak bcl-2 expression in SG tumors is associated with a high frequency of apoptosis, but strong Bax expression has no influence [19]. Some authors suggest that HER2/neu overexpression in cancer cells, in addition to stimulating tumor cell proliferation, acts as an antiapoptotic cell survival factor [20]. On the other hand, cancer cells lacking p21 are more sensitive to apoptosis, by arresting cell cycle progression or p21 could interact with and inhibit proapoptotic molecules, such as procaspase-3, caspase-8, and apoptosis signal-regulating kinase 1 [21].

This article describes a very unusual hybrid form of ACC with slow progression. In this case, a high-grade component of ACC showed deregulation of cell cycle, with high expression of HER-2, p21, and Bcl-2. Proapoptotic markers were effective in low grade ACC, but with dedifferentiation of ACC, tumor cells expressed Bax and Fas, however without Caspase-3 activity. Stromal lymphoid tissue had a significant influence on to Ki-67 index in a high-grade component of ACC, as well as involvement of the CXCR4 signaling axis in the stromal reaction influence.

Conclusion

Stromal lymphoid reaction (particularly $CD8^+/CD4^+$ ratio) has a significant influence onto Ki-67 index in high-grade ACC of the parotid gland, which can determine the aggressivity of this tumor, and therefore may be used as a novel prognostic biomarker.

Acknowledgements

This work was supported by the grant No. 175092 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

1. *Michail P, Karavokyros I, Pikoulis E, Arvelakis A, Charminis G, Michail O,* et al. Acinic cell carcinoma of the parotid gland in children a case report and literature review. West Indian Med J 2008; 57(1): 70−2.

2. *Skálová A, Sima R, Vanecek T, Muller S, Korabecna M, Nemcova J,* et al. Acinic cell carcinoma with high-grade transformation: a report of 9 cases with immunohistochemical study and analysis of TP53 and HER-2/neu genes. Am J Surg Pathol 2009; 33(8): 1137−45.
3. *Toshitaka N, Isamu S, Yasuo I, Akira A, Shigeru M., Kazuto Y,* et al. Hybrid carcinomas of the salivary glands: report of nine cases with a clinicopathologic, immunohistochemical, and p53 gene alteration analysis. Mod Pathol 2002; 15(7): 724–33.
4. *Hellquis HB, Sundelin K, Di Bacco A, Tytor M, Manzotti M, Viale G.* Tumour growth fraction and apoptosis in salivary gland acinic cell carcinomas. Prognostic implications of Ki-67 and bcl-2 expression and of in situ end labelling (TUNEL). J Pathol 1997; 181(3): 323−9.
5. *Yin HF, Okada N, Takagi M.* Apoptosis and apoptotic-related factors in mucoepidermoid carcinoma of the oral minor salivary glands. Pathol Int 2000; 50(8): 603−9.
6. *Tarakji B, Mohammad NZ.* Immunohistochemical expression of p21 in normal tissues of salivary gland, pleomorphic adenoma and carcinoma ex pleomorphic adenoma-(undifferentiated and adenocarcinoma types). Med Oral Patol Oral Cir Bucal 2010; 15(5): e697−703.
7. *Giannopoulou I, Nakopoulou L, Zervas A, Lazaris AC, Stravodimos C, Giannopoulos A*, et al. Immunohistochemical study of pro-apoptotic factors Bax, Fas and CPP32 in urinary bladder cancer: prognostic implications. Urol Res 2002; 30(5): 342−5.
8. *Burger M, Glodek A, Hartmann T, Schmitt-Gräff A, Silberstein LE, Fujii N,* et al. Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation, and adhesion to stromal cells. Oncogene 2003; 22(50): 8093–101.
9. *Tímár J, Forster-Horváth C, Lukits J, Döme B, Ladányi A, Remenár E,* et al. The effect of leukocyte interleukin injection (Multikine) treatment on the peritumoral and intratumoral subpopulation of mononuclear cells and on tumor epithelia: A possible new approach to augmenting sensitivity to radiation therapy and chemotherapy in oral cancer—A multicenter phase I/II clinical trial. Laryngoscope 2003; 113(12): 2206−17.
10. *Kumamoto Y, Mattei LM, Sellers S, Payne GW, Iwasaki A.* CD4+ T cells support cytotoxic T lymphocyte priming by controlling lymph node input. Proc Natl Acad Sci USA 2011; 108(21): 8749−54.
11. *Shedlock DJ, Shen H.* Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 2003; 300(5617): 337−9.
12. *Sun JC, Bevan MJ.* Defective CD8 T cell memory following acute infection without CD4 T cell help. Science 2003; 300(5617): 339−42.
13. *Piersma SJ, Jordanova ES, van Poelgeest MI, Kwappenberg KM, van der Hulst JM, Drijfhout JW*, et al. High number of intraepithelial CD8+ tumor-infiltrating lymphocytes is associated with the absence of lymph node metastases in patients with large early-stage cervical cancer. Cancer Res 2007; 67(1): 354−61.
14. *Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F,* et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci USA 2005; 102: 18538−43.
15. *Khan MZ, Brandimarti R, Musser BJ, Danielle Marie Resue BJ, Fatatis DM, Meucci O.* The chemokine receptor CXCR4 regulates cell-cycle proteins in neurons. J Neurovirol 2003; 9(3): 300–14.
16. *Chen AM, Granchi PJ, Garcia J, Bucci MK, Fu KK, Eisele DW.* Local-regional recurrence after surgery without postoperative irradiation for carcinomas of the major salivary glands: implications for adjuvant therapy. Int J Radiat Oncol Biol Phys 2007; 67(4): 982−7.
17. *Majetschak M.* Extracellular ubiquitin: immune modulator and endogenous opponent of damage-associated molecular pattern molecules. J Leukoc Biol 2010; 89(2): 205–219.
18. *Winsauer P, Walker E, Vande Stouwe C, Porretta C, Molina P.* Chronic Δ-9-tetrahydrocannabinol administration increases lymphocyte CXCR4 expression in rhesus macaques. J Neuroimmune Pharmacol 2011; 6(4): 540−5.
19. *Soini Y, Törmänen U, Pääkkö P.* Apoptosis is inversely related to bcl-2 but not to bax expression in salivary gland tumours. Histopathology 1998; 32(1): 28−34.
20. *Roh H, Pippin J, Drebin JA.* Down-regulation of HER2/neu expression induces apoptosis in human cancer cells that overexpress HER2/neu. Cancer Res 2000; 60(3): 560−5.
21. *Chiang CT, Way TD, Lin JK.* Sensitizing HER2-overexpressing cancer cells to luteolin-induced apoptosis through suppressing p21$^{WAF1/CIP1}$ expression with rapamycin. Mol Cancer Ther 2007; 6(7): 2127–38.

Received on December, 20 2011.
Revised on October 8, 2012.
Accepted on October 24, 2012.

CASE REPORT

UDC: 616.24-006-033.2 + 616.379-091.8-031
DOI: 10.2298/VSP1312159L

Multisystem Langerhans cell histiocytosis coexisting with metastasizing adenocarcinoma of the lung: A case report

Multisistemska histiocitoza Langerhansovih ćelija udružena sa metastazirajućim adenokarcinomom pluća

Aleksandra Lovrenski*†, Mirna Djurić†‡, Ištvan Klem*†, Živka Eri*†, Milana Panjković*†, Dragana Tegeltija*†, Djordje Považan†‡

***Center for Pathology, ‡Clinic for General Pulmology, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; †Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

Abstract

Introduction. Langerhans cell histiocytosis (LCH) is an uncommon disease of unknown etiology characterized by uncontrolled proliferation and infiltration of various organs by Langerhans cells. **Case report.** We presented a 54-year-old man, heavy smoker, with dyspnea, cough, hemoptysis, headache and ataxia, who died shortly after admission to our hospital. On the autopsy, tumor was found in the posterior segment of the right upper pulmonary lobe as well as a right-sided occipitoparietal lesion which penetrated into the right ventricle resulting in internal and external hematocephalus. Histologically and immunohistohemically, the diagnosis of primary lung adenocarcinoma with brain metastasis was made (tumor cells showed positivity for CK7 and TTF-1 which confirmed the diagnosis). In the lung parenchyma around the tumor, as well as in brain tissue around the metastatic adenocarcinoma histiocytic lesions were found. Light microscopic examination of the other organs also showed histiocytic lesions involving the pituitary gland, hypothalamus, spleen and mediastinal lymph nodes. Immunohistochemical studies revealed CD68, S-100 and CD1a immunoreactivity within the histiocytes upon which the diagnosis of Langerhans' cells histiocytosis was made. **Conclusion.** The multisystem form of LCH with extensive organ involvement was an incidental finding, while metastatic lung adenocarcinoma to the brain that led to hematocephalus was the cause of death.

Key words:
histiocytosis, langerhans-cell; diagnosis; lung neoplasms; adenocarcinoma; immunohistochemistry.

Apstrakt

Uvod. Histiocitoza Langerhansovih ćelija je retko oboljenje nepoznate etiologije koje se karakteriše nekontrolisanom proliferacijom i infiltracijom različitih organa Langerhansovim ćelijama. **Prikaz bolesnika.** Prikazan je bolesnik, star 54 godine, teški pušač, sa simptomima dispneje, kašlja, hemoptizija, glavobolje i ataksije koji je kratko nakon prijema u našu ustanovu egzitirao. Na obdukciji, nađen je tumor u posteriornom segmentu desnog gornjeg režnja, kao i tumorska masa lokalizovana desno okcipitoparijetalno koja je penetrirala u desnu komoru dovodeći do unutrašnjeg i spoljašnjeg hematocefalusa. Histološki i imunohistohemijski, postavljena je dijagnoza primarnog plućnog adenokarcinoma sa metastazom u mozak (tumorske ćelije su pokazale pozitivnost za CK7 i TTF-1, što je potvrdilo dijagnozu). U plućnom parenhimu oko tumora, kao i u moždanom parenhimu oko tumora nađene su histiocitne lezije. Histološka analiza isečaka uzetih iz hipofize, hipotalamusa, slezine i medijastinalnih limfnih čvorova otkrila je, takođe, prisustvo ovih lezija. Imunohistohemijski, ove lezije pokazale su pozitivnost na CD68, S-100 i CD1a, na osnovu čega je i postavljena dijagnoza histiocitoze Langerhansovih ćelija. **Zaključak.** Multisistemska forma histiocitoze Langerhansovih ćelija sa ekstenzivnim zahvatanjem organa je slučajan nalaz, dok je hematocefalus izazvan metastatskim adenokarcinomom bio uzrok smrti kod ovog bolesnika.

Ključne reči:
histiocitoza x; dijagnoza; pluća, neoplazme; adenokarcinom; imunohistohemija.

Correspondence to: Aleksandra Lovrenski, Center for Pathology, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia. E-mail: ajalovrenski@yahoo.com

Introduction

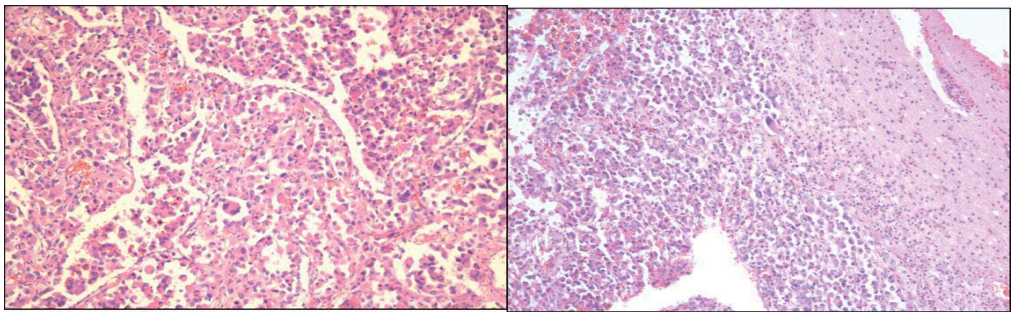
Langerhans cell histiocytosis (LCH) includes diseases previously designated as histiocytosis X, eosinophilic granuloma, Letterer-Siwe disease, Hand-Schuller-Christian syndrome and Langerhans cell granulomatosis. This is an uncommon disease of unknown etiology characterized by uncontrolled proliferation and infiltration of various organs by Langerhans cells, often organized into granulomas [1]. It occurs predominantly in children and young adults, but no age is an exemption. Practically any organ can be involved, but bone and skin are the sites of predilection. Patients are categorized as having single-system disease at a single or multiple sites, or as having multisystem disease. Single-system disease of LCH involves mainly bones or lungs and usually follows a benign course and can regress spontaneously. The clinical presentation of multisystem LCH, which carries a poor prognosis in a number of cases, is highly variable depending on the organs involved, mainly bones, skin, lungs, pituitary glands and less commonly liver, spleen, hematopoietic and central nervous system [1–3]. The association of single-system disease with malignant neoplasm is not so rare (particularly in association with malignant lymphoma), but association of multisystem LCH with a malignant neoplasm is rare and, generally, has been the subject of isolated case reports [4]. To our knowledge, this is the first case reporting on the association of multisystem LCH with metastasizing adenocarcinoma of the lung.

Case report

A 54-year-old man, heavy smoker (30 years/3 packs a day), was admitted to our hospital for further diagnostic approach to the radiologically detected change in the right lung. Four months before admission shortness of breath and cough with hemoptysis occured, and a month before admission the patient was refered to the neurologist because of walking instability, loss of strength in the left half of the body, and morning headaches. Computerized tomography (CT) scan of the endocranium showed right-sided occipitoparietal lesion, which primarily exhibited characteristics of secondary infiltration, and CT scan of the thorax showed inhomogeneous, extensive infiltration predominantly localized in the upper lobe. On the fourth day after admission hemoptysis occured, and bronchoscopic examination was performed, but histopathological findings did not clarify the etiology of the change. After two days a deterioration in general condition with intense headache developed, and despite all the applied therapeutic measures the patient passed away.

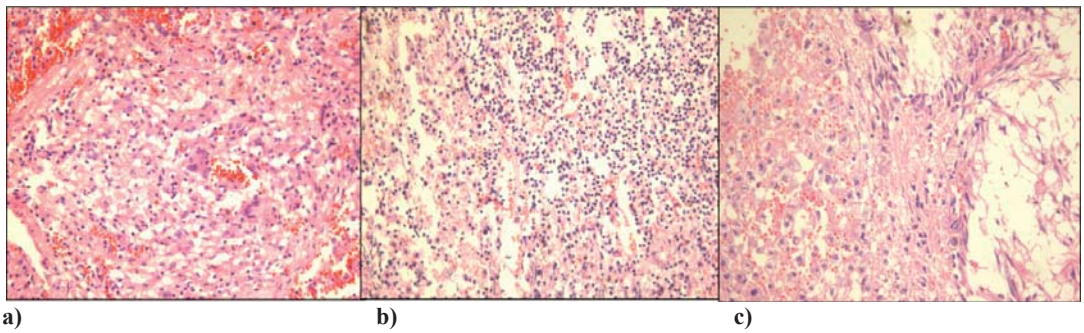
At autopsy, macroscopic examination of the lungs revealed an excavated tumor mass 1.8 cm in largest dimension in the posterior segment of the right upper lobe, which histopathologically corresponded to adenocarcinoma. In brain parenchyma, right-sided occipitoparietal necrotic, and hemorrhagic lesion 5.7 × 3,8 cm in largest diameter, which penetrated into the right ventricle resulting in internal and external hematocephalus, was observed. Histologically and immunohistohemically, the diagnosis of primary lung adenocarcinoma with brain metastasis was made (tumor cells showed positivity for CK7 and TTF-1 which confirmed the diagnosis) (Figures 1a and 1b).

Within the tumor, in the lung parenchyma around the tumor, as well as in the brain tissue around metastatic adenocarcinoma, histiocytic lesions were found (Figure 2a). Light microscopic examination of the other organs also showed histiocytic lesions involving the pituitary gland, spleen, hypothalamus, and mediastinal lymph nodes (Figures 2b and 2c).



a) b)

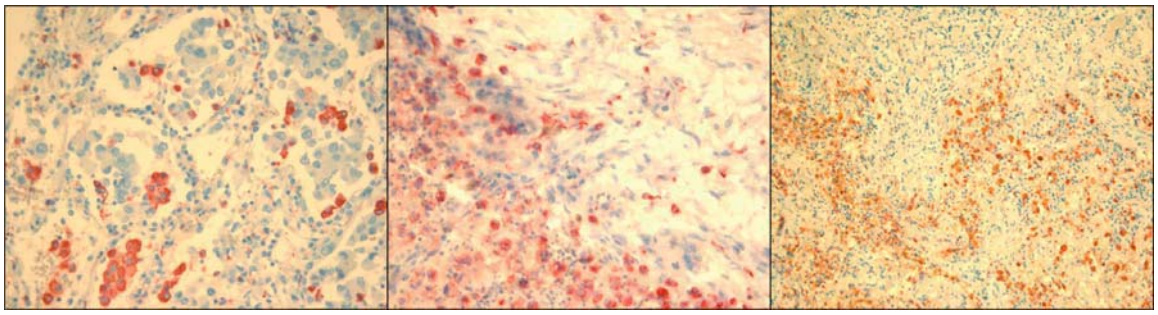
Fig. 1 – a) Adenocarcinoma of the lung (HE, × 10); b) Metastatic adenocarcinoma in the brain parenchyma (HE, × 5).



a) b) c)

Fig. 2 – a) Histiocytic lesion within the lung parenchyma around the tumor (HE, × 10); b) Histiocytic lesion in the mediastinal lymph node (HE, × 10); c) Histiocytic lesion in the hypothalamus (HE, × 20).

These histiocytic lesions were made of clusters and sheets of characteristically ovoid cells with abundant, lightly eosinophilic cytoplasm, grooved or contorted nuclei, fine chromatin, a thin nuclear membrane and inconspicuous nucleoli. Multinucleated giant cells were also present. Immunohistochemical studies revealed CD68 (histiocytic marker), cytoplasmic protein S-100 and glycoprotein CD1a (a marker of Langerhans' cells) immunoreactivity within the histiocytes upon which the diagnosis of LCH was made (Figures 3a, 3b and 3c).



a) **b)** **c)**

Fig. 3 – a) CD1a positivity of Langerhans cells within the lung tumor parenchyma (× 20); b) CD1a positivity of Langerhans' cells within the brain parenchyma around the tumor (× 20); c) CD1a positivity of Langerhans cells within the pituitary gland (× 10).

Discussion

Analysis of a large cohort of Mayo Clinic patients (314 patients between 1946 and 1996) with histologically proven LCH showed that 96 patients had LCH involving multiple systems, 114 had isolated osseous LCH, and remaining 104 had nonosseous single system LCH. Only 27 of 314 patients had coexisting neoplasms. Five patients had lung carcinoma (four adenocarcinoma and one small cell carcinoma), but all of them had pulmonary LCH without involvement of other organs with Langerhans' cells. Four patients had multisystem LCH (mostly affecting bones, skin, lymph nodes and pituitary gland) coexisting with breast adenocarcinoma, parathyroid adenoma, pancreatic cystadenoma and pontine mass [5].

A group of autors at the University of Minnesota reviewed their own charts as well as reported cases in the literature between 1960 and 1992. Of the 91 patients, 39 had LCH with malignant lymphoma (25 of these cases were Hodgkin disease), in 22 patients LCH was reported in association with leukemia, and in remaining 30 patients LCH was associated with a variety of solid tumors, including lung carcinoma in 12 patients (nine adenocarcinoma, two large cell carcinoma and one squamous cell carcinoma). In 11 cases of associated lung carcinomas and LCH, the diagnosis of LCH was confined pathologically to the lung [6]. In one case reported by Hammar et al. [7] LCH affected bone and lung and this is the only reported case of lung adenocarcinoma coexisting with LCH which was not limited to the lung. According to these two studies, simultaneous association of LCH with lung carcinoma suggest that pulmonary LCH represents a reaction to the tumor.

Conclusion

LCH remains a rare, enigmatic disease which, in most cases, is detected relatively late in its course and which should be included in the differential diagnosis of patients with multisystem disease. In this case, the coexistance of multisystem LCH with extensive organ involvement and metastastic lung adenocarcinoma (that led to hematocephalus and death) represents a coincidental finding, because only in case of a single-system disease one can speak about the reactive nature of LCH.

REFERENCES

1. *Makras P, Piaditis G, Kaltsas GA.* Systemic and endocrine manifestations of Langerhans' cell histiocytosis: current concepts in diagnosis and management. Expert Rev Endocrinol Metab 2007; 2(6): 773−83.
2. *Vassallo R, Ryu JH, Schroeder DR, Decker PA, Limper AH.* Clinical outcomes of pulmonary Langerhans'-cell histiocytosis in adults. N Engl J Med 2002; 346(7): 484−90.
3. *Wang CW, Colby TV.* Histiocytic lesions and proliferations in the lung. Semin Diagn Pathol 2007; 24(3): 162−82.
4. *Benharroch D, Guterman G, Levy I, Shaco-Levy R.* High content of Langerhans cells in malignant lymphoma-incidence and significance. Virchows Arch 2010; 457(1): 63−7.
5. *Howarth DM, Gilchrist GS, Mullan BP, Wiseman GA, Edmonson JH, Schomberg PJ.* Langerhans cell histiocytosis: diagnosis, natural history, management, and outcome. Cancer 1999; 85(10): 2278−90.
6. *Egeler RM, Neglia JP, Aricò M, Favara BE, Heitger A, Nesbit ME,* et al. The relation of Langerhans cell histiocytosis to acute leukemia, lymphomas, and other solid tumors. The LCH-Malignancy Study Group of the Histiocyte Society. Hematol Oncol Clin North Am 1998; 12(2): 369−78.
7. *Hammar SP, Bockus D, Remington F, Hallman KO, Winterbauer RH, Hill LD, et al.* Langerhans cells and serum precipitating antibodies against fungal antigens in bronchioloalveolar cell carcinoma: possible association with pulmonary eosinophilic granuloma. Ultrastruct Pathol 1980; 1(1): 19−37.

Received on May 31, 2012.
Accepted on June 26, 2012.

CASE REPORT

UDC: 616.14-056.7::611.94]:615.817
DOI: 10.2298/VSP1312162V

Successful implantation of a biventricular pacing and defibrillator device *via* a persistent left superior vena cava

Uspešna ugradnja resinhronizaciono-defibrilatorskog aparata preko perzistentne leve gornje šuplje vene

Mihailo Vukmirović*, Lazar Angelkov†, Filip Vukmirović‡, Irena Tomašević Vukmirović§

***Center of Cardiology, ‡Center of Patology, §Center of Radiology, Clinical Center of Montenegro, Podgorica, Montenegro; †Institute of Cardiovascular Disease "Dedinje", Belgrade, Serbia**

Abstract

Introduction. Persistent left superior vena cava is the most common thoracic venous abnormality which is usually asymptomatic, found incidentally during pacemaker implantation. The main problem is related to reaching the appropriate pacing site and ensuring stable lead placement. **Case report.** We reported a successful implantation of a biventricular pacing and defibrillator device (CRT-D) *via* a persistent left superior vena cava in a 55-year-old man with dilated cardiomyopathy and severe heart failure. A persistent left superior vena cava was detected during CRT-D implantation. We managed to position electrodes in the right ventricular outflow tract, a posterior branch of the coronary sinus and in the right atrium. **Conclusion.** Congenital anomalies of thoracic veins may complicate lead placement on the appropriate and stable position. The presented case demonstrates a successful biventricular pacing and defibrillator therapy device implantation in a patient with dilated cardiomyopathy and severe heart failure.

Key words:
vena cava superior; vascular malformations; cardiac pacing artificial; defibrilator implantable; treatment outcome.

Apstrakt

Uvod. Leva gornja šuplja vena je najčešća anomalija vena toraksa koja je uglavnom asimptomatska. Obično se verifikuje prilikom ugradnje pejsmejkera kada može jako otežati, a nekada i potpuno onemogućiti postavljanje elektroda. Problem koji nastaje zbog anomalije venskog sistema je u odgovarajućem pozicioniranju elektroda, odnosno u njihovoj stabilnosti. **Prikaz bolesnika.** Prikazali smo uspešnu implantaciju resinhronizacionog defibrilatorskog aparata (CRT-D) kod bolesnika starog 55 godina, sa dilatativnom kardiomiopatijom, odnosno teškom srčanom slabošću, kojem je u toku intervencije detektovana perzistentna leva gornja šuplja vena. Uprkos anomaliji venskog sistema toraksa, uspeli smo da pozicioniramo elektrodu u izlazni trakt desne komore, posteriornu granu koronarnog sinusa, odnosno u desnu pretkomoru. **Zaključak.** Urođene anomalije vena toraksa mogu jako da otežaju postavljanje elektroda na adekvatnu, odnosno stabilnu poziciju. Ipak, pokazali smo da se i kod ovakvih bolesnika može uspešno ugraditi resinhronizacioni defibrilator.

Ključne reči:
v. cave superior; krvni sudovi, malformacije; srce, veštačko usklađivanje ritma; defibrilator, implantabilni; lečenje, ishod.

Introduction

Persistent left superior vena cava (PLSVC) is the most common variation in the thoracic venous system. PLSVC is found in 0.3% to 0.5% of the population and in 5% to 10% of patients with other congenital heart defects (atrial septal defect, bicuspid aortic valve, coarctation of aorta, coronary sinus ostial atresia and cor triatriatum) [1–6]. Several subtypes of PLSVC can be distinguished. In 68% of cases an innominate vein bridges the two superior venae cavae [7]. In about 20% of patients, as well as in the presented case, the right superior vena cava (RSVC) is absent resulting in drainage of venous blood from the head and both arms through the left brachiocephalic vein, PLSVC and the coronary sinus into the right atrium [8]. This condition is typically asymptomatic, usually incidentally discovered during pacemaker implantation can complicate lead placement through the subclavian approach. We reported a case of a successful biventricular pacing and defibrillator device implantation (CRT-D) *via* a persistent left superior vena in

Correspondence to: Mihailo Vukmirović, Clinical Center of Montenegro, Center of Cardiology, Ljubljanska bb, 81000 Podgorica, Montenegro. Phone: +382 20 412 258. Mob. +382 69 304 400. E-mail: mihailovukmirovic@t-com.me

a patient with dilatative cardiomyopathy and severe heart failure.

Case report

A 55-year-old man with weakness, fatigue, dizziness, syncope and the history of idiopathic dilated cardiomyopathy underwent implantation of a biventricular pacemaker and defibrillator therapy device. ECG showed a wide left bundle branch block with the duration of 180 msec (Figure 1). The patient was categorized as the New York Heart Association (NYHA) functional class III.

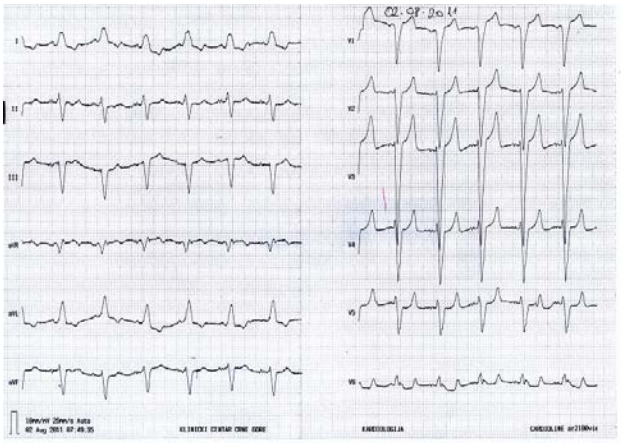


Fig. 1 – Electrocardiography (ECG) on admission showed a wide left bundle branch block with the duration of 180 msec.

An echocardiogram showed an enlarged left ventricular cavity (end-diastolic diameter 7.8 cm and end-systolic diameter 6.2 cm) with severe impaired left ventricular ejection fraction (LVEF) of 15%, wide coronary sinus and moderate to severe mitral regurgitation. Coronary angiography showed normal coronary arteries.

After cannulation of the left subclavian vein, the guidewire passed along the left side of the mediastinum when the left superior vena cava became evident. An active fixation ventricular defibrillator (ICD) lead was placed through a curved guiding stylet on the right ventricule outflow tract. The stimulation threshold was 1.5 V at 0.5 ms with the impedance of 530 Ω, high-voltage shock impedance was 47 Ω and R wave amplitude was 8.9 mV. A subselection catheter and the guidewire facilitate placement of unipolar left ventricular electrode in the posterior branch of the coronary sinus. Its stimulation threshold was 1.25 V at 0.5 ms, R wave amplitude was 12 mV and impedance was 300 Ω. A passive fixation right atrial lead was positioned using a standard atrial stylet in the septum of the right atrium. The stimulation threshold was 0.5 V at 0.5 ms with the impedance of 680 Ω and P wave amplitude of 4.7 mV (Figures 2 and 3).

There were no other complications in the course of the procedure.

After a 6-month follow-up, the patient fitted into NYHA functional class I, sensing and capture threshold remained stable and ventricular pacing was more than 95%. ECG showed narrowing of QRS to 120 msec (Figure 4). There was a mild mitral regurgitation, and LVEF was 40%, showing an excellent response to CRT-D.

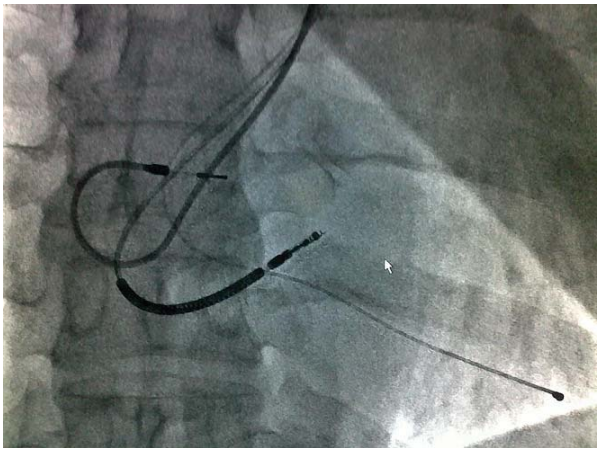


Fig. 2 – Antero-posterior (AP) view: an active fixation of ventricular defibrillator lead positioned in the outflow tract of the right ventricle; an unipolar left ventricular electrode positioned in the posterior branch of the coronary sinus; a passive fixation atrial lead positioned in the septum of the right atrium.

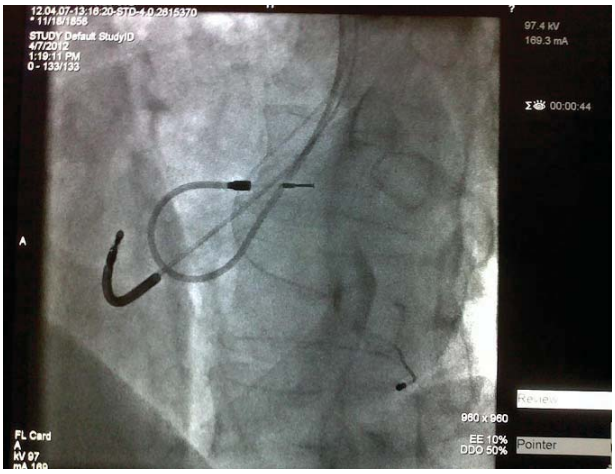


Fig. 3 – Left anterior oblique (LAO) 45° view: a right ventricular defibrillation lead, left ventricular unipolar lead and atrial lead insertion place.

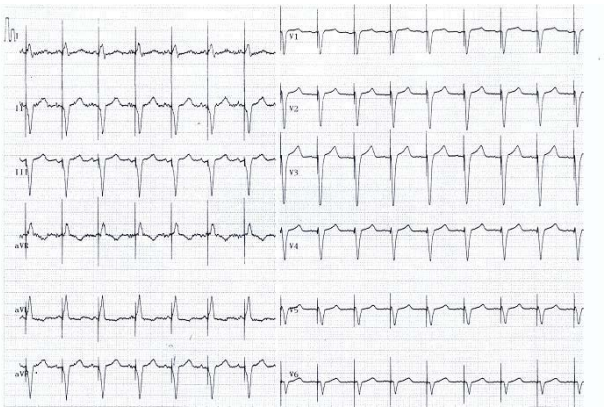


Fig. 4 – Electrocardiography (ECG) showing biventricular stimulation with narrowing of QRS to 120 msec.

Discussion

Although the left superior vena cava may complicate or completely disable biventricular pacemaker and defibrillator devices implantation, several cases of successful implantation have been described in the literature [7, 9, 10]. However, some difficulties may occur during this procedure. Right ventricular lead placement is the major problem. In patients with the innominate vein which connects the right and left superior vena cava, right ventricular lead implantation is usually achievable through this one using a conventional method [7]. Typically, the PLSVC drains directly into the right atrium through the greatly enlarged coronary sinus because of a significant increase in blood flow [7]. When an electrode is introduced into the right atrium, the tip of the right ventricular lead usually tends to deflect away from the tricuspid annulus. There are several methods to overcome this difficulty. Biffi et al. [11] used a manually formed U-shaped stylet, requiring considerable manoeuvring, forming a loop in the right atrium, using the right atrial free wall for support. Srimannarayana et al. [12] reported the use of atrial J-shaped stylet for ventricular lead placement [12]. Konstantino et al. [13] demonstrated ventricular lead placement into the right ventricular outflow tract using a coronary sinus delivery system. These techniques allow acute angulation of ventricular electrode to reach right ventricul through the tricuspidal valve, which is the critical juncture mostly requiring additional complex maneuvers. These maneuvers have not been completely described in the published literature so far [14, 15].

In the presented case of ICD lead implantation we used a manually formed U-shaped stylet with the rotation of electrode in clockwise direction toward the tricuspid valve annulus and after a sudden withdrawal of the stylet, the ventricular lead got into the right ventricul across the tricuspidal valve. The atrium lead was implanted using J-shaped stylet, but with contraclockwise rotation in the right atrium septum. It is advisable to apply both maneuvers in the direction of rotation opposite to the one done during the conventional biventricular pacemaker device lead implantation.

The left ventricular lead, if possible, is introduced through the PLSVC into the coronary sinus and implanted in the appropriate coronary vein. The difficulty in coronary sinus cannulation is still one of the reasons for failing biventricular pacing system in implantation. Balloon-occlusion retrograde angiography is not possible because of coronary sinus dilation. Coronary cannulation vein requires a lead to be manipulated through sharp angles. Sometimes, left coronary angiography is performed in order to evaluate possible position for left ventricular lead placement. However, this can lead to large contrast volume and prolonged angiographic time. In the case, we used the sub-selection catheter with a curve of 90° and a guidewire which facilitates unipolar left ventricular electrode placement in the posterior branch of the coronary sinus.

Conclusion

Despite venous abnormalities, biventricular pacing and defibrillator device implantation *via* a persistent left superior vena cava is feasible. According to our findings, these maneuvers have not been completely described in the published literature so far. We hope that these techniques may facilitate lead implantation *via* the left superior vena cava.

REFERENCES

1. *Fry AC, Warwicker P.* Bilateral superior vena cava. N Engl J Med 2007; 356: 18.
2. *Peltier J, Destrieux C, Desme J, Renard C, Remond A, Velut S.* The persistent left superior vena cava: anatomical study, pathogenesis and clinical considerations. Surg Radiol Anat 2006; 28(2): 206−10.
3. *Schreve-Steensma AM, Van der Valk PH, Ten Kate JB, Kofflard MJ.* Discovery of a persistent left superior vena cava during pacemaker implantation. Neth Heart J 2008; 16(7−8): 272–4.
4. *Tak T, Crouch E, Drake GB.* Persistent left superior vena cava: incidence, significance and clinical correlates. Int J Card 2002; 82(1): 91−3.
5. *Jović Z, Mijailović Z, Obradović S, Tavčiovski D, Matunović R, Rusović S.* Successful implantation of a permanent pacemaker through a persistent left superior vena cava by using a right subclavian approach. Vojnosanitetski pregled 2011; 68(9): 792−4.
6. *Reinhardt D, Surber R, Kuehnert H, Heinke M, Figulla HR.* Implantation of a re- synchronization device in a patient with persistent left superior vena cava-a case report. Indian Heart J 2010; 62(4): 344−5.
7. *Antonelli D, Freedberg M, Feldman A.* Implantation of a Resynchronization Implantable Cardioverter Defibrillator in a Patient with Persistent Left Superior Vena Cava. Indian Pacing Electrophysiol J 2007; 7(4): 246−8.
8. *Lappegard KT, Prytz JF, Haug B.* Pacemaker implantation in patients with persistent left superior vena cava. Heart Vessels 2004; 19(3): 153−4.
9. *Smyth YM, Barrett CD, Fahy GJ.* Biventricular pacemaker implant in a patient with persistent left sided superior vena cava. Heart 2005; 91(11): 1427.
10. *Gasparini M, Mantica M, Galimberti P, Coltorti F, Simonini S, Ceriotti C,* et al. Biventricular pacing via a persistent left superior vena cava: report of four cases. Pacing Clin Electrophysiol 2003; 26(1): 192−6.
11. *Biffi M, Boriani G, Frabetti L, Bronzetti G, Branzi A.* Left superior vena cava persistence in patients undergoing pacemaker or cardioverter-defibrillator implantation: a 10-year experience. Chest 2001; 120(1): 139−44.
12. *Srimannarayana J, Sekhlar Varma R, Satheesh S, Anilkumar R, Balachander J.* Transvenous permanent pacemaker implantation through persistent left superior vena cava. Indian Heart J 2004; 56(4): 346−8.
13. *Konstantino Y, Kusniec J, Shohat-Zabarski R, Battler A, Strasberg B.* Cardiac defibrillator implantation via persistent left superior vena cava facilitated by a coronary sinus delivery system. Europace 2009; 11(1): 119−20.
14. *Rogers D, Walker F, Chow F, Lambiase PD.* Biventricular Device Implantation in a Patient with Congenitally Corrected Transposition and Left-Sided Superior Vena Cava. Pacing Clin Electrophysiol 2008; 31(4): 499−502.
15. *Kapetanopoulos A, Peckham G, Kiernan F, Clyne C, Kluger J, Migeed MA.* Implantation of a biventricular pacing and defibrillator device via a persistent left superior vena cava. J Cardiovasc Med (Hagerstown) 2006; 7(6): 430−3.

Received on June 10, 2012.
Revised on August 14, 2012.
Accepted on September 28, 2012.

HISTORY OF MEDICINE

UDC: 575:61]:577.2(091)

The 60th anniversary of the discovery of DNA secondary structure

Otkriće sekundarne strukture molekula DNK – 60-godišnjica

Goran Brajušković, Dušanka Savić Pavićević, Stanka Romac

Faculty of Biology, University of Belgrade, Belgrade, Serbia

Key words:
DNA; molecular biology; genomics; history of medicine; nobel prize.

Ključne reči:
DNK; biologija, molekulska; genomika; istorija medicine; nobelova nagrada.

Introduction

The year 2013 is the year of great anniversaries in molecular biology. In 1953 Watson and Crick published the model of DNA structure in the scientific journal "Nature", indicating the model of its self-replication as well [1, 2]. This was the final proof that DNA molecule contains genetic information. With this discovery, exactly 60 years ago, molecular biology became distinct scientific discipline and today it is one of the most dynamic fields of science. There is no doubt that completion of one of the largest and the most expensive scientific project of all times, deciphering the sequence of the human genome, is one of the milestones that marked the beginning of the 21st century. The International Human Genome Sequencing Consortium published the results of their project in "Nature" in 2001 [3]. The very next day the results of the work done by a group of scientists employed by the company "Celera" appeared in "Science" [4]. The complete sequence of the human genome with the assessment of the gene number was published in 2003 [5]. At the time, the estimation of the number of the protein-coding genes was 30,000. Since then every year we witness a new revision of the number of human genes. According to the last data, the number of protein-coding genes is slightly more than 20,000 [6]. Starting in 2003, the Encyclopedia of DNA Elements (ENCODE) project set out to map which parts of human chromosomes are transcribed, how transcription is regulated and how the process is affected by the way DNA is packaged in the cell nucleus. The results of this project, published in 2012, showed that 80.4% of the human genome displays some functionality in at least one of 147 different cell types analysed [7].

While deciphering the human genome and other species' genomes, the new scientific branch, genomics, was established. The object of study in genomics is the whole genomes of species. It is the opinion of the scientific community that genomics of all species will bring about the most progress in the everlasting human struggle against incurable diseases and ageing [8]. The most important discoveries that enabled the advances in molecular biology and the beginning of the genomic era will be presented in this article.

Discovery of the secondary structure of DNA molecule

In spring 1951, during a zoology congress, the American zoologist James Dewey Watson (born 1928) met the New Zealander Maurice Hugh Frederick Wilkins (1916–2004) who showed him diffraction images of DNA molecule. For young Watson, this experience was initiation into the research of the chemical structure of nucleic acids and proteins. By the end of 1952 Watson also met a British physicist and biologist Francis Harry Compton Crick (1916–2004). The two started working together on the 3-dimensional model of a DNA molecule. In no more than couple of months, making molecular models based on characteristics of diffraction images of DNA, Watson and Crick established that a DNA molecule consisted of two antiparallel polynucleotide strands joined together by hydrogen bonds [9].

On April 25, 1953 in its 171st volume, the journal "Nature" published the article "Molecular structure of nucleic acids – A Structure for Deoxyribose Nucleic Acid" [1]. It was known at that time that DNA is the carrier of hereditary information. This was mainly due to the work of Avery et al. [10] who investigated the transformation of *Pneumococcus* type III bacteria and the horizontal gene transfer in this species. Therefore, it is not surprising that this publication was met by the utmost interest of the scientific community as soon as it ap-

Correspondence to: Goran Brajušković, Faculty of Biology, University of Belgrade, 11 000 Belgrade, Serbia. Phone: + 381 11 263 9 100. E-mail: brajuskovic@bio.bg.ac.rs

peared. Watson and Crick published their discovery of the DNA molecule structure based on the double stranded right-handed spiral model on only one page with 6 cited references. According to their original double helix model, purine and pyrimidine bases are facing each other on the inside of the molecule, stacked one on another (due to hydrophobic interactions) while phosphate groups are turned to the outside. The backbone of the molecule is formed by pentose sugar and phosphate groups (Figure 1). Since the diameter of the helix is the same along the length of the molecule, purine base in one strand is facing the pyrimidine base in another one. This principle designated as the complementary base pairing rule states that adenine (A) and thymine (T), respectively cytosine (C) and guanine (G) form hydrogen bonds and make base pairs with the same geometry [1].

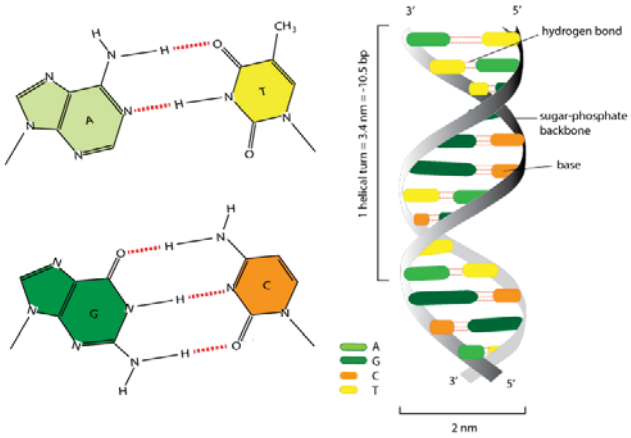


Fig. 1 – Complementary base pairs (left) and secondary structure of a DNA molecule (right)

Relying on the model of DNA secondary structure they proposed, Watson and Crick reflected on the possible way of the replication of DNA molecule. Therefore, the scientists emphasized in their article: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" [1].

At the time of this discovery, Watson was only 25 years old, and he won the Nobel Prize at the age of 34. Even today, in his late years, Watson is active and certainly is one of the greatest authorities in molecular biology. Last years have been marked by his controversial statements about the influence of genes on the human nature [11].

Discovery of the secondary structure of a DNA molecule still captures the interest of the scientific as well as general public. Crick wrote a letter to his son, sharing with him the news about the discovery. Sixty years later, in April 2013, this letter, known as Francis Crick's DNA letter sells at auction for a record $6 million. The letter was purchased by an anonymous buyer who made the bid over the phone. Half of the proceeds will go to Michael Crick and his wife. The other half will go to the Salk Institute for Biological Studies in California, where the elder Crick worked up until his death in 2004 at the age of 88 [12].

The importance of Chargaff's rules and diffraction photographs of DNA molecule in the discovery of the double helix

Combining of the bases according to the complementary base-pairing principle, suggested in Watson and Crick's model of double helix DNA, corresponded with Chargaff's findings about frequency of nucleotides in DNA molecule. As a result of his research, Erwin Chargaff (1905–2002) came to the conclusion that the content of purines in a DNA molecule equals the content of pyrimydines, and also, that the content of A equals that of T and content of G equals the content of C. In 1951, his results were mathematically formulated as $A/T = G/C = 1$ and $A + T = G + C$ and today these equations are known under the name the rules of Chargaff [13, 14]. Chargaff recalled the 3 lucky factors that contributed to his success: a new method described in 1944 by Consden, Gordon, and Martin that came to be known as paper chromatography – applied to the analysis of nucleic acid constituents, purines and pyrimidines; the availability for the first time of commercial ultraviolet spectrophotometers – which enabled the analytic procedure to be strictly quantitative – for purines and pyrimidines exhibit strong characteristic absorption spectra in ultraviolet; and what he felt to be most important, two excellent collaborators – Dr. Ernst Vischer and Mrs. Charlotte Green [15]. The scientific community of Serbia was introduced to Chargaff's work by the scientist himself who gave a lecture in the Serbian Academy of Sciences and Arts on June 22, 1970.

There is an anecdote about Chargaff who, while in London in 1952, had a meeting with Watson and Crick. They discussed the impact of his rules on the model of DNA structure. At this point Crick had forgotten the names of the bases, which did not impress Chargaff, who arrogantly considered that he was wasting his time talking to a couple of 'pitchmen' [16].

As already mentioned, radiography (X-ray) diffraction images of DNA were as important for the discovery of the secondary structure of this molecule as Chargaff's rules. Supportive of this opinion is the fact that the same volume of "Nature" in which Watson and Crick's article appeared also published two other articles confirming the suggested model with X-ray diffraction images of DNA molecule [17, 18]. The author of one of these two papers was Wilkins who later shared the Nobel Prize for Physiology or Medicine with Watson and Crick. The authors of the other were Rosalind Franklin (1920–1958) and Raymond Gosling (born 1926).

It is widely appreciated today that X-ray photographs made by Rosalind Franklin were of key importance for the discovery of the secondary structure of the DNA molecule [19]. In lecture notes dated November 1951, Franklin wrote the following: "The results suggest a helical structure (which must be very closely packed) containing 2, 3 or 4 co-axial nucleic acid chains *per* helical unit, and having the phosphate groups near the outside" [20]. However, because of her premature death at the age of 37, she could not be awarded the Nobel Prize since this prize cannot be posthumously awarded. In the speeches at the Nobel ceremony "Crick and Watson

notoriously and shamefully did not mention Rosalind, and Wilkins's tribute was slight, but who can say what might have happened if she had lived" [19]. Moreover, it is no secret presently that she did not know that they had seen either her X-ray photograph, showing unmistakable evidence of a helical structure, or her precise measurements of the unit cell (the smallest repeating unit) of the DNA crystal [21]. After she had done the work on diffraction images of DNA molecule and proteins, she left King's College and turned to the investigation of the tobacco mosaic virus structure. Rosalind Franklin published nearly 50 scientific papers. In the year she died her model of the tobacco mosaic virus was displayed at the international exhibition in Brussels, to be moved later to the new Laboratory of Molecular Biology in Cambridge [19]. Truth be told, during the last year of her life, she became a close friend of Crick and his wife Odile who she stayed with while receiving her treatments for ovarian cancer. After her death, Franklin has become a feminist icon – the Sylvia Plath of molecular biology – seen as a genius whose gifts were sacrificed to the greater glory of the male [21].

This is by no means the end of controversy regarding diffraction photographs of a DNA molecule. There can be no doubt Franklin's role was crucial: it was her skill in the technique known as X-ray crystallography that resulted in the famous Photograph 51. But it was Franklin's student, 22 year-old Raymond Gosling (Figure 2), who actually took the photo [17].

Fig. 2 – Raymond Gosling.

The anniversary itself was an opportunity for Gosling to recall the days he had spent in Rosalind Franklin's laboratory and of the Watson and Crick's discovery he designated as "Eureka moment" [22]. "Standing in the dark room outside this lead-lined room, and looking at the developer, and up through the developer tank swam this beautiful spotted photograph, you are familiar with them now I'm sure. It took 90-something hours to take the photograph, again, pot luck. But it really was the most wonderful thing. And I knew at the time that what I'd just done was to produce a crystalline state in these fibres, and if then the DNA was the gene material, I must be the first person ever to make genes crystallize" [23]. According to Gosling himself, the importance of Rudolf Signer (1903–1990), a Swiss biochemist should not be forgotten [23]**.** Rudolf Signer delivered a lecture in the Royal Society on his method for producing DNA of a superior quality. In Gosling's words: "Signer asked at the end of the lecture if anybody would like some of this material and he had a specimen tube full of this freeze dried material. Only two people put their hand up. I'm glad to say that Maurice (Wilkins) was awake enough to put his hand up!" [24]. Anyway, this scientific discovery, as any other, is a merit of many scientists whose work has been directed towards common goal, elucidation of the DNA secondary structure in this case.

The second half of the 20th century, a golden age of molecular biology

Shortly after the discovery of the secondary structure of DNA, the central dogma of molecular biology was postulated. It states that in the cell, the hereditary information is being transmitted in one direction from nucleic acids to proteins and never the other way around, and from DNA to DNA between generations. Quite a few scientists were involved in defining central dogma, and Crick was the most influential among them [25]. It is the opinion of many that defining this rule (in the year 1958) actually represents the beginning of the golden age of molecular biology.

Five years after the discovery of the DNA secondary structure, the American Matthew Meselson (born 1930) gave an experimental proof for the model of replication suggested by Watson and Crick [26]. During the mid 50s of the last century, American biochemist Arthur Kornberg (1918–2007) isolated the first enzyme involved in replication of DNA molecule. It was DNA polymerase I from *E. coli*. Using this newly discovered enzyme, Kornberg successfully synthesized DNA *in vitro* in the presence of deoxyribonucleoside triphosphate, molecules of DNA, magnesium ions and adenosine triphosphate. In addition, he discovered that this process always takes place in 5'→3' direction [27]. On the day Arthur Kornberg received the Nobel Prize, his wife, biochemist in the same scientific group, Sylvy Ruth Levy (birth: unknown-1986), gave a brief statement to the press: "I was robbed". The science is "family business" of Kornbergs. Apart from Kornberg and his wife, their two sons are also distinguished scientists. Roger David Kornberg (born 1947) is a professor of structural biology and one of the pioneers in the field of chromatin structure and function research. In 2006 he was awarded the Nobel Prize for his work on transcription in eukaryotic cells. His brother, Thomas Bill Kornberg (born 1948) was a member of research group which discovered enzymes DNA polymerase II and DNA polymerase III in 1970 [28, 29].

In the field of molecular biology, the beginning of the 60s of the last century was marked by the discovery of the role of messenger RNA (mRNA) in the flow of genetic information in cells and by introducing the notion of codon. In relation to this, Crick's adaptor hypothesis should be mentioned. In the year 1961, Crick and colleagues discovered that DNA "communicates in specific language" in which the words (codons) are always composed of three letters (each letter corresponds to one nucleobase of the DNA sequence). During these years, a great number of scientists were involved in the work on transcription and translation processes, but the discovery of mRNA is considered mostly the achievement of three of them: Matthew Meselson, Briton Sydney Brenner (born 1927) and French François Jacob (1920–2013) [30].

In 1963 the American Marshall Nirenberg (1927–2010) and Indian Har Gobind Khorana (1922–2011), based on the results of their independent research, explained the way in which nucleic acids (with 4 bases, that is 4 letters) determine the sequence of 20 amino acids in polypeptide chains using codons (three letter words). Nirenberg gave a correct estimate of the total number of codons – 64 ($4 \times 4 \times 4$). Through experiments started in 1961 in collaboration with the American Philip Leder (born 1934) he confirmed the hypothesis that nucleic acids determine the sequence of 20 amino acids using 64 codons [31, 32].

The year 1974 was marked by one of the most unexpected discoveries in the molecular biology which had a tremendous influence on its further development. The Americans Richard John Roberts (born 1943) and Phillip Allen Sharp (born 1944) discovered that eukaryotic genes consist of coding (exons) and non-coding (introns) sequences. After transcription, the processing of the primary transcript takes place, which includes splicing out introns and joining together of exons to form the strand of mRNA [33].

The first recombinant DNA molecule was created in the begging of 70s of the 20th century, which initiated the new era of recombinant DNA technology. American biochemist Paul Berg (born 1926) created *in vitro* the first hybrid circular DNA molecule using the sequences of viral (*SV40*) and bacterial (*E. coli)* DNA [34]. This was possible only due to the discovery of restriction endonucleases and DNA ligase. The first isolated restriction enzyme was *Hind* III from the bacteria *Haemophilus influenzae*. It was isolated in 1970 by three microbiologists – the American Hamilton Smith (born 1931), the Swiss Werner Arber (born 1929) and another American Daniel Nathans (1928–1999) [30].

During the mid-seventies of the last century first techniques for DNA sequencing were developed. The British Frederick Sanger (born 1918) and the American Walter Gilbert (born 1932) independently created original methods to determine the sequence of nucleotides in DNA molecule [35, 36]. By applying his own method, Sanger managed to sequence a stretch of the DNA from *E. coli* bacteriophage ØX174 whose genome is 5.375 base pairs (bp) long [37]. Frederick Sanger, double laureate of the Nobel Prize, is one of the most famous living scientists.

Entry into the genomic era

With the development and automation of methods for DNA sequencing it was possible to consider sequencing of the human genome and genomes of other species. The idea about deciphering the human genome originated in the US Department for Energy in 1985. This idea was met with the approval of the scientific community and as a result, in 1988, the National Center for Human Genome Research (NCHGR) was founded. The estimate of the number of genes in human genome revolved around 100,000 at the end of the 80s of the last century. Based on this estimate the plan was devised for scientists from NCHGR to sequence the entire DNA in human cells over period of 15 years spending the budget of 3 billion dollars. October 1990 is usually considered as the official launch date of the project. At the very start it was clear that it surpasses the scope of a national project. It became an international project as the scientific institutes from the European Union, China, Japan, Australia and other countries (18 in total) joined. The Human Genome Organization (HUGO) was founded first with the task to coordinate the work of this large number of institutions and later the International Human Genome Sequencing Consortium with the same purpose. Watson was the director of the consortium for a period. One of the founders and directors of the NCHRG was Craig Venter (born 1946). Due to the misunderstanding regarding the methodology of sequencing, Venter leaves NCHGR and founds his private institute – TIGR (the Institute for Genomic Research) [38]. This institute succeeds to sequence the first whole genome of an organism in less than six months. It was the genome of the bacterium *Haemophilus influenzae* 1.830.137 bp long and consisting of 1,749 genes [39]. After deciphering the first genome of a living organism, interest in deciphering the human genome was growing over years. The number of pharmaceutical companies and companies of other type that would readily make considerable investments in this project was also growing. In 1998 Venter starts the private company "Celera Genomics". After the sequence of the first prokaryotic genome had been published, one of the most exciting races in the history of science begun. Its participants were scientists from NCHGR and from "Celera", while its goal was to read out the complete sequence of the human genome [38].

As the work progressed towards the end, the rivalry was becoming more open. Arguments in favour of one or the other sequencing strategy were the topic of many scientific, but also social debates. The confrontations of the two teams with two different approaches and mutual challenges threatened the whole project. Under the circumstances the US president Bill Clinton was bound to intervene. He organized meeting of the leaders of both teams in the East Room of the White House on June 26, 2000. Presidential mediation led the scientists to bury the hatchet and shake hands [40]. The wish to successfully complete the project of deciphering the human genome overtook. The exchange of the results of readout of the human genome obtained by that moment was arranged between the teams. The result of described efforts

was a successful completion of one of the most expensive scientific projects of all times – the deciphering of the human genome.

Ending of a project of this sort is the culmination of the investigations that started with the discovery of the secondary structure of the DNA molecule. At the same time it is the starting point for the new scientific branch named genomics. The research in genomics extends over many disciplines mostly including: sequencing of the genomes, determining the number and the structure of the genes, investigations of the gene expression profiles and determining the structure and function of proteins. Comparative genomics has as its main topic investigations into similarities and differences between the genomes of different species. In parallel with genomics another novel scientific discipline develops – bioinformatics. Bioinformatics is defined as application of information technologies in biology. It refers primarily to the collection, processing and analysis of experimental results. Deciphering of the billions of base pairs of the hundreds of genomes of different species is currently underway. The work of such a large scale requires the creation of specific data bases. The final goal of this approach is to make results of the work of scientists investigating genomes of many organisms accessible *via* Internet to all interested researchers around the world. The future of genomics can be seen in the application of its findings in numerous sciences such as agronomy (agrogenomics), pharmacology (pharmacogenomics), and especially medicine (genomic medicine) [41].

Acknowledgments

The research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project no. 173016).

REFERENCES

1. *Watson JD, Crick FH*. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. Nature 1953; 171(4356): 737−8.
2. *Watson JD, Crick FH*. Genetical implications of the structure of deoxyribonucleic acid. Nature 1953; 171(4361): 964−7.
3. *Cheung VG, Nowak N, Jang W, Kirsch IR, Zhao S, Chen XN*, et al. Integration of cytogenetic landmarks into the draft sequence of the human genome. Nature 2001; 409(6822): 953−8.
4. *Venter JC, Adams MD, Myers EW, Li PW, Mural RJ*. The sequence of the human genome. Science 2001; 291(5507): 1304−51.
5. *International Human Genome Sequencing Consortium*. Finishing the euchromatic sequence of the human genome. Nature 2004; 431(7011): 931−45.
6. *Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F*, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 2012; 22(9): 1760−74.
7. *Qu H, Fang X*. A Brief Review on the Human Encyclopedia of DNA Elements (ENCODE) Project. Genomics Proteomics Bioinformatics 2013; 11(3): 135−41.
8. *Brajušković G*. Genomics. Vojnosanit Pregl 2006; 63(6): 604−10. (Serbian)
9. *Watson JD*. The Double Helix. A Personal Account of the Discovery of the Structure of DNA. New York: W. W. Norton and Company; 1980
10. *Avery OT, Macleod CM, McCarty M*. Studies of the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med 1944; 79(2): 137−58.
11. *Khamsi R*. James Watson retires amidst race controversy. Science in Society 2007. Available from: http://www.newscientist.com/article/dn12835-james-watson-retires-amidst-race-controversy.html [cited 2007 October 25].
12. *Boyle A*. Francis Crick's DNA letter to his son sells at auction for a record $6 million. Available from: muckrack.com/.../francis-cricks-dna-letter-to-his [cited 2013 April 10].
13. *Chargaff E, Vischer E*. Nucleoproteins, nucleic acids, and related substances. Annu Rev Biochem 1948; 17: 201−26.
14. *Magasanik B, Chargaff E*. Studies on the structure of ribonucleic acids. Biochim Biophys Acta 1951; 7(3): 396−412.
15. *Weintraub B*. Erwin Chargaff (1905–2002) and the Chargaff's Rules. Available from: http://www.chemistry.org.il/booklet/22/pdf/bob_weintraub.pdf
16. *Manchester KL*. Historical opinion: Erwin Chargaff and his 'rules' for the base composition of DNA: why did he fail to see the possibility of complementarity. Trends Biochem Sci 2008; 33(2): 65−70.
17. *Franklin RE, Gosling RG*. Molecular configuration in sodium thymonucleate. Nature 1953; 171(4356): 740−1.
18. *Wilkins MH, Stokes AR, Wilson HR*. Molecular structure of deoxypentose nucleic acids. Nature 1953; 171(4356): 738−40.
19. *Glynn J*. Rosalind Franklin: 50 years on. Notes Rec R Soc 2008; 62: 253−5.
20. *Braun G, Tierney D, Schmitzer H*. How Rosalind Franklin Discovered the Helical Structure of DNA: Experiments in Diffraction. The Physics Teacher 2011; 49(3): 140−3.
21. *Maddox B*. The double helix and the 'wronged heroine'. Nature 2003; 421(6921): 407−8.
22. *Doolittle FW, Fraser P, Gerstein MB, Graveley BR, Henikoff S, Huttenhower C*, et al. Sixty years of genome biology. Genome Biol 2013; 14(4): 113.
23. *Attar N*. Raymond Gosling: the man who crystallized genes. Genome Biol 2013; 14(1): 402.
24. *Balaram P*. The Double Helix: Sixty Years On. Curr Sci 2013; 104(9): 1127−8.
25. *Crick FH*. On protein synthesis. Symp Soc Exp Biol 1958; 12: 138−63.
26. *Meselson M, Stahl FW*. The replication of DNA in Escherichia coli. Proc Natl Acad Sci U S A 1958; 44(7): 671−82.
27. *Lehman IR*. Historical perspective: Arthur Kornberg, a giant of 20th century biochemistry. Trends Biochem Sci 2008; 3(6): 291−6.
28. *Kornberg T, Gefter ML*. Purification and DNA synthesis in cell-free extracts: properties of DNA polymerase II. Proc Natl Acad Sci U S A 1971; 68(4): 761−4.
29. *Kornberg T, Gefter ML*. Deoxyribonucleic acid synthesis in cell-free extracts. IV. Purification and catalytic properties of deoxyribonucleic acid polymerase III. J Biol Chem 1972; 247(17): 5369−75.

30. *Brajušković G.* History of Molecular Biology. In: *Brajušković G*, editor. Molecular Biology 2. Belgrade: Savremena administracija; 2012. p. 237−60. (Serbian)
31. *Leder P, Clark BF, Sly WS, Pestka S, Nirenberg MW.* Cell-Free Peptide Synthesis Dependent upon Synthetic Oligodeoxynucleotides. Proc Natl Acad Sci U S A 1963; 50(6): 1135−43.
32. *Falaschi A, Adler J, Khorana HG.* Chemically synthesized deoxypolynucleotides as templates for ribonucleic acid polymerase. J Biol Chem 1963; 238: 3080−5.
33. *Sharp PA.* The discovery of split genes and RNA splicing. Trends Biochem Sci 2005; 30(6): 279−81.
34. *Singer M, Berg P.* Recombinant DNA: NIH Guidelines. Science 1976; 193(4249): 186−8.
35. *Sanger F, Coulson AR.* A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 1975; 94(3): 441−8.
36. *Maxam AM, Gilbert W.* A new method for sequencing DNA. Proc Natl Acad Sci U S A 1977; 74(2): 560−4.
37. *Sanger F, Nicklen S, Coulson AR.* DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977; 74(12): 5463−7.
38. Genome timeline. What a long, strange trip it's been. Nature 2001; 409(6822): 756−7.
39. *Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR*, et al. Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science 1995; 269(5223): 496−512.
40. *Weaver RF.* Molecular biology. 2nd ed. Boston: McGraw-Hill; 2002.
41. *Brajušković G.* Genomics. In: *Brajušković G,* editor. Molecular biology 2. Belgrade: Savremena administracija; 2012. p. 203−36. (Serbian)

Received on August 27, 2013.
Accepted on September 2, 2013.

LETTERS TO THE EDITOR / PISMO UREDNIKU

UDC: 616.8-009.836(045)(048.8)

Two original articles in the field of sleep medicine published in 1932 and 1934 by the authors from the Faculty of Medicine in Belgrade (on the occasion of the 90th anniversary of neuropsychiatric service in Serbia)

Prikaz dva originalna rada iz oblasti spavanja nastala na Medicinskom fakultetu u Beogradu 1932. i 1934. godine (povodom proslave 90. godišnjice osnivanja neuropshijatrijske službe u Srbiji)

To the Editor:

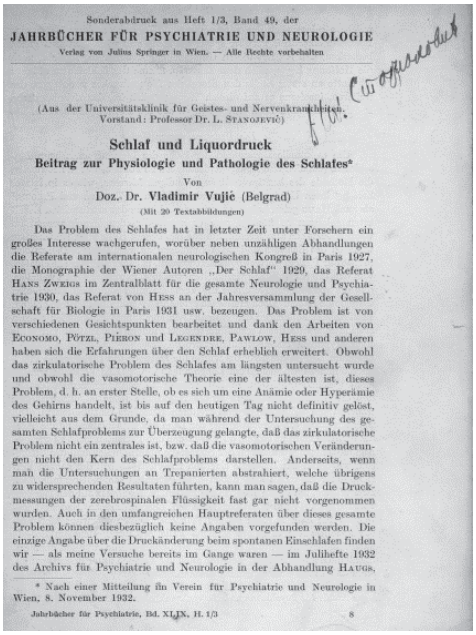
Articles originating from the first part of the 20th centry in the field of neurology and psychiatry by Serbian authors preserved in original are rare. We present two articles written by two Professors from the Faculty of Medicine in Belgrade, Serbia, (Prof. Dr. Vladimir F. Vujić, 1932, and Prof. Dr. Dimitrije T. Dimitrijević, 1934) dealing with the problems in sleep, a topic very popular in the first half of the past century.

Although their results are now a part of history, the idea and methodology are still attractive. The article by Prof. Vujić presents original research dealing with the measurement of pressure cerebrospinal liquor during sleep in patients with different diagnoses with the idea that a single disease may have a characteristic graph of a change in liquor pressure. The paper by Prof. Dimitrijević reports a patient with narcolepsy indicating medical attitudes on narcolepsy in Serbia prior to the Second World War.

There is only a small number of preserved original articles in the field of neurology and psychiatry, printed before the Second World War by the Serbian medical doctors. We present two of the articles. The first was written in 1932 by Prof. Dr. Vladimir Vujić from the University Clinic for Mental and Nervous Diseases in Belgrade, titled "Sleep and the liquor pressure. A contribution to the physiology and pathology of sleep" [1]. The second article was written in 1934 by Dr. Dimitrije T. Dimitrijević from Belgrade, titled "Contribution to the knowledge about traumatic narcolepsy" [2]. Both articles may be read or reprints taken from the History Section of the Serbian Somnologic Society web site [3].

The author of the first article, Prof. Dr. Vladimir F. Vujić, studied medicine in Paris and Prague. He finished the specialization in neuropsychiatry in 1925 in Vienna as a student of Prof. Dr. Julius Wagner Ritter von Jauregg, a Nobel Prize winner in Physiology or Medicine in 1927. At the end of the Second World War in 1945 as the first director of the Neuropsychiatric Clinic, Prof. Dr. Laza Stanojević retired, he became the Director and was immediately elected Vice Dean of the Medical Faculty of Medicine. He also became a Full Professor and head of the cathedras for neurology, psychiatry and medical psychology (from 1945 to 1953).

Professor Dr. Vladimir F. Vujić published in 1932 in Vienna the article in German Language titled "*Schlaf und Liquordruck, Beitrag zur Physiologie und Pathologie des Schlafes*" (Figure 1) (the publisher was famous 'Verlag von Julius Springer aus Wien'). The study was performed at the University Clinic for Mental and Nervous Diseases, the director at that time being Prof. Dr. Laza Stanojević (*'Universitätsklinik für Geistes – und Nervenkrankheiten; vorstand Professor Dr. L. Stanojević, Belgrade, Serbien'*). It was printed as a special issue on 16 pages, as a separate from the first of the three volumes of the 49. of the Yearbook for Psychiatry and Neurology (*Sonderabdruck aus Heft 1/3, Band 49 der Jahrbücher für Psychiatrie und Neurologie*) [1]. The text contains 20 tables with comments. Unfortunately, the pages with the literature used by Prof. Vujić in this article are not preserved. Prof. Vujić delivered oral presentation of the same title in Vienna, on November 8, 1932.



Sonderabdruck aus Heft 1/3, Band 49, der

JAHRBÜCHER FÜR PSYCHIATRIE UND NEUROLOGIE

Verlag von Julius Springer in Wien. — Alle Rechte vorbehalten

(Aus der Universitätsklinik für Geistes- und Nervenkrankheiten. Vorstand: Professor Dr. L. Stanojević)

Schlaf und Liquordruck

Beitrag zur Physiologie und Pathologie des Schlafes*

Von

Doz. Dr. **Vladimir Vujić** (Belgrad)

(Mit 20 Textabbildungen)

Das Problem des Schlafes hat in letzter Zeit unter Forschern ein großes Interesse wachgerufen, worüber neben unzähligen Abhandlungen die Referate am internationalen neurologischen Kongreß in Paris 1927, die Monographie der Wiener Autoren „Der Schlaf" 1929, das Referat Hans Zwaros im Zentralblatt für die gesamte Neurologie und Psychiatrie 1930, das Referat von Hess an der Jahresversammlung der Gesellschaft für Biologie in Paris 1931 usw. bezeugen. Das Problem ist von verschiedenen Gesichtspunkten bearbeitet und dank den Arbeiten von Economo, Pötzl, Pieron und Legendre, Pawlow, Hess und anderen haben sich die Erfahrungen über den Schlaf erheblich erweitert. Obwohl das zirkulatorische Problem des Schlafes am längsten untersucht wurde und obwohl die vasomotorische Theorie eine der ältesten ist, dieses Problem, d. h. an erster Stelle, ob es sich um eine Anämie oder Hyperämie des Gehirns handelt, ist bis auf den heutigen Tag nicht definitiv gelöst, vielleicht aus dem Grunde, da man während der Untersuchung des gesamten Schlafproblems zur Überzeugung gelangte, daß das zirkulatorische Problem nicht ein zentrales ist, bzw. daß die vasomotorischen Veränderungen nicht den Kern des Schlafproblems darstellen. Anderseits, wenn man die Untersuchungen an Trepanierten abstrahiert, welche übrigens zu widersprechenden Resultaten führten, kann man sagen, daß die Druckmessungen der zerebrospinalen Flüssigkeit fast gar nicht vorgenommen wurden. Auch in den umfangreichen Hauptreferaten über dieses gesamte Problem können diesbezüglich keine Angaben vorgefunden werden. Die einzige Angabe über die Druckänderung beim spontanen Einschlafen finden wir — als meine Versuche bereits im Gange waren — im Julihefte 1932 des Archivs für Psychiatrie und Neurologie in der Abhandlung Haugs,

* Nach einer Mitteilung fin Verein für Psychiatrie und Neurologie in Wien, 8. November 1932.

Jahrbücher für Psychiatrie, Bd. XLIX, H. 1/3 8

Fig. 1 – Title page of Prof. Vladimir Vujić's article published in 1932.

Correspondence to: Slavko Janković, Clinic for Neurology, Clinical Center of Serbia, Dr Subotica Street 6, 11 000 Belgrade, Serbia. Phone: +381 63 827 5748; +381 11 7471 661. E-mail: slavko@sezampro.rs

The introduction of his work on the pressure of cerebrospinal liquor Prof. Vujić dedicated to at that time contemporary meetings (reports at the International Neurologic Congress in Paris in 1927) and the literature on the problem of sleep (monograph "Sleep" of the Viennese authors from 1929; report of Hans Zweig in the "*Zentralblatt für Neurologie und Psychiatrie*" from 1931; report of Hess on the Yearly Meeting of the Society for Biology in Paris in 1931). As the most competent contemporaries in the field of sleep he quoted von Ekonomo, Plötzl, Piéron and Legendre, Pavlov and Hess "whose experience highly widened the knowledge on sleep". Starting from these points as the basis for his work Prof. Vujić quoted the oldest theories of sleep as 'circulatory' and 'vasomotor'; they deal with oligemia and hyperemia, respectively, of the brain during sleep. However, he cautiously noted that "none of these theories are proved".

Very cautiously Prof. Vujić also states that "with this exploration he does not expect to come closer to the central problem of sleep", however he wants to eventually clarify circulatory nature and some other questions on sleep. In methodology Prof. Vujić states that the measurement of the pressure of liquor was performed during wakefulness, falling asleep, sleep and again in wakefulness, with patients in lying position and administration of hypnotics. Professor Vujić, only four years after Hans Berger's formal description of human electroencephalography (EEG) [4] clearly states that he did not want to disturb the "process of falling asleep and the sleep depth", implying that he considers sleep to be an active process with a certain profile of development throughout the night; it should be stressed that at that time the dual nature non-repid eye movement (NREM) and rapid eye movement (REM) as well as the depth (i.e., sleep stages) of sleep were not known and were described 21 (by Aserinsky and Kleitman in 1953) [5] and 36 years (by Rechtschaffen and Kales in 1968) [6] later. Due to care for patients, attempted determination of the minimal liquor pressure with the Barany method *via* hyperventilation was abandoned for three reasons. First, the possibility that patients with epilepsy may provoke an epileptic attack. Second, the pressure of the liquor at the end of hyperventilation increases steeply. Third, demented patients could not perform that task. For induction of sleep he used paraldehide and ampoules of Somnifen-Roche, while "unfavorable" scopolamine in the form of Pantopon-Scopolamon Roche was rarely used. To determine liquor pressure he used a Claude's manometer, including 105 patients, all males except for 3 females. The diagnoses were: paralysis progressiva (33 patients), schizophrenia (13 patients), (erratic) imbecility (with epileptic attacks) (4 patients), encephalitis chronica (16 patients), catatonia chronica, hydrocephalus (1 patient), epilepsy (29 patients), chorea (1 patient), mild alcoholism, morbus Parkinsoni (1 patient), catatonia, hysteria (3 patients). All measurements were performed in the afternoons from 19 to 24 hours, with the help of Dr. Ilija Grujičić, Assis. Prof. at the Physiology Unit. A part of the article of Prof. Vujić is missing and is preserved only for the patients with progressive paralysis in whom, after falling asleep, an increase of 5 cm in liquor pressure was observed.

The idea to measure the pressure of liquor was original for that time. As Prof. Vujić states, "if we throw away the research with trephination", there is only one article dealing with the same problem, published in 1932 in the July issue of the *Archiv für Psychiatrie und Neurologie*. Professor Vujić says that "he tried to find a specific curve of the liquor pressure change in wakefulness, falling asleep and sleep that could be characteristic for each particular disease".

It is impressive that Prof. Vujić performed a scientific work at the time when the field of sleep was very up-to-date. This work was certainly pioneering since the book of Kleitman [7] and his seminal work with Aserinsky and Kleitman [5] were to appear only after a few decades. The idea to determine a specific graph of liquor pressure in a particular disease during wakefulness and sleep is original. Although today the idea may look simple, it is original and was certainly good to pursue. In respect to our short review the original article of Prof. Vujić appeared 81 years ago.

A close collaborator of Prof. Dr. Laza Stanojević, later Prof. of Neuropsychiatry at the Faculty of Medicine in Sarajevo, Dr. Dimitrije T. Dimitrijević published a number of studies. His "Neuroses with thalamopathic events" [8] appeared in 1953 on 98 pages; "Schisasthenia: psychopathologic-clinical study" [9] was published in 1954 on 52 pages; "Hysteria as a neurodynamic problem" [10] was published in 1956 on 94 pages.

The article on a case with traumatic narcolepsy, fully preserved to our days, was published in Serbian Language in 1934, titled "Contribution to the knowledge about traumatic narcolepsy" [2]. It appeared as a special print in Belgrade, in the third volume of "Medical Review", in March 1934 (Figure 2) (in Serbian and German, printed at that time by the

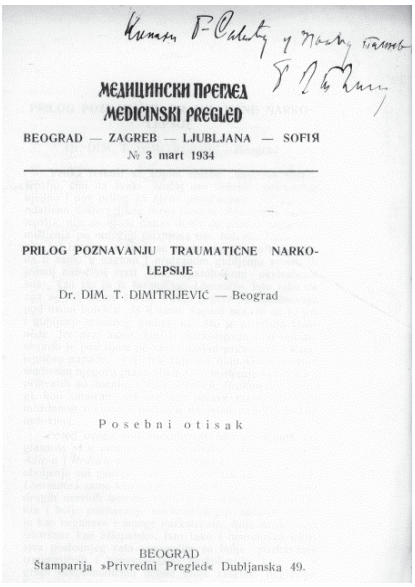

МЕДИЦИНСКИ ПРЕГЛЕД
MEDICINSKI PREGLED
BEOGRAD — ZAGREB — LJUBLJANA — SOFIЯ
№ 3 mart 1934
PRILOG POZNAVANJU TRAUMATIČNE NARKOLEPSIJE
Dr. DIM. T. DIMITRIJEVIĆ — Beograd
Posebni otisak
BEOGRAD
Štamparija »Privredni Pregled« Dubljanska 49.

Fig. 2 – Title page of Prof. Dimitrije T. Davidović's article published in 1934.

publishers from Belgrade-Zagreb-Ljubljana). The original article was published in the printing shop "Economic Review" (*Privredni pregled*) located in 49 Dubljanska Street, which was close to the then "General State Hospital". The

article bears a dedication to Dr. Savić "as a token of care". The article was printed on 18 pages with an abstract in German Language with 20 references most of which refer to the works of great contemporaries as were Lhermitte in 1927, Emil Redlich, C. Rosenthal, William Richard Gowers in German from 1921 and IA Barre from 1928.

From this point of time, the article on narcolepsy by Prof. Dimitrijević was published 79 years ago.

Professor Dimitrijević starts the article by listing the principal facts unknown about narcolepsy: is narcolepsy only a sleep disturbance or it encompass the loss of muscle tone (cataplectic attacks), as initialy described by Jean Baptiste Edouard Gélineau [11]. The etiology of narcolepsy at the time was unclear, ranging from the view that it is a neurosis to the view that it is a disease *sui generis*. Traumatic narcolepsy is described as immediate, born immediately after the injury, or as tardive "at a time distance from traumatic causes whose etiological relationship with the disease is sometimes difficult to prove". He describes a case of a 51-year-old man who was in a military exercise in Carinthia (Koruška) in 1919 who as a consequence of explosion of shells lost consciousness for several minutes. Six years after the injury excessive daytime sleepiness started at least four times a day (with no loss of muscle tone), which he tried to cure with ephedrine (which the patient, actually, did not take). Sleep attacks spontaneously thinned and disappeared. The author rejects the possibility of "idiopathic narcolepsy ... as it occurs in other circumstances and in much younger people. Commotion which the patient survived remains the only option for an explanation of narcolepsy". He also adds that "if epilepsies are given the opportunity to occur several years after the injury, why it would not be the same case with narcolepsy, as one part of narcolepsy undoubtedly belongs to the field of epilepsy". Professor Dimitrijević came to a conclusion that vasospasm mechanism responsible for epilepsy must be, as well, responsible for the development of narcolepsy. As a "hypnic center" he names the subcortical regions of the III ventricle in which violations of sympathetic centers lead to a "vagal hypertonia" that dominates during sleep. The paper concludes with the abstracts in German ("*Beitrag zur Kenntniss der traumatischen Narkolepsie*") and the references of 20 contemporary units.

The paper by Prof. Dimitrijevic on posttraumatic narcolepsy has two characteristics. It shows a good knowledge of the disease, as it was written at the time when a definitive stand on the disease only emerged: Löewenfeld [12] only in 1902 introduced the term "cataplexy" for a sudden muscle weakness caused by emotions, and in 1930, only 4 years before the work of Prof. Dimitrijevic, ephedrine (without any clinical effect) and amphetamine were introduced in therapy. Professor Dimitrijevic was familiar with the recent work of von Economo's [13] (which was already published in English), as quoted by, and accepts a new position on the localization of injury in narcolepsy. On the other hand, the article suggests a high competence of the author, but indirectly, also of the entire generation of his colleagues.

After the Second World War the registering of sleep on experimental animals was performed by academic Prof. Veselinka Šusić who in 1970 had her Ph.D. thesis in the field of sleep [14], and in 1977 published the first book in Serbia on sleep, entitled "Vigilance, sleep and dreaming" [15]. First registration of polysomnography (PSG) was performed by Prof. Dragoslav Ercegovac and Prof. Žarko Martinović who registered sleep in the afternoons from 1972 to 1974 [16], and a first whole-night polysomnography in 1978 and 1979 [17].

Papers by Professors Vladimir F. Vujić and Dimitrije T. Dimitrijević provide a clear picture of the achievements of Serbian neurology in the period between the two wars. The first paper is original in the study of physiological changes in the cerebrospinal fluid during wakefulness and sleep and the other provides an excellent insight into the understanding of the pathology and etiology of narcolepsy at the time that the disease has taken on, which became the basis for further research and development of the modern theory of narcolepsy. Both studies were performed at the time when Europe and America just released the first series of patients with narcolepsy (Addie 1926) [18], (Wilson, 1928) [19], (Daniels, 1934) [20], i.e., at the time when the first textbook on sleep appeared (Kleitman, 1939) [7].

Slavko Janković*, Dragoslav Sokić*, Nikola Vojvodić*, Aleksandar Ristić*, Maša Kovačević*, Vladimir Kostić*†

*Clinic for Neurology, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Belgrade, Serbia

REFERENCES

1. *Vujic V.* „Schlaf und Liquordruck. Beitrag zur Physiologie und Pathologie des Schlafes". (Universitätsklinik für Geistes- und Nervenkrankheiten; vorstand Professor Dr L. Stanojević, Belgrad, Serbien). Sonderabdruck aus Heft 1/3, Band 49 der Jahrbücher für Psychiatrie und Neurologie, Verlag von Julius Springer aus Wien, 1932, p: 113-128.
2. *Dimitrijević DT.* Contribution to the knowledge about traumatic narcolepsy. Belgrade: Medicinski pregled; 1934. (Serbian)
3. Serbian Sleep Society. Available from: http://www.sss.rs (Serbian)
4. *Berger H.* Über das Elektrenkephalogramm des Menschen. Archiv für Psychiatrie 1929; 87(1): 527–70.
5. *Aserinsky E, Kleitman N.* Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 1953; 118(3062): 273−4.
6. *Rechtschaffen A, Kales A.* A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, Md., U. S: National Institute of Neurological Diseases and Blindness, Neurological Information Network; 1968.
7. *Kleitman N.* Sleep and wakefulness. Chicago: Chicago University Press; 1939.
8. *Dimitrijević DT.* Neuroses with thalamopathic events. Sarajevo: Državna štamparija; 1953. (Serbian)
9. *Dimitrijević DT.* Schisasthenia: psychopathologic-clinical study. Sarajevo: Državna štamparija; 1954. (Serbian)

10. *Dimitrijević DT*. Hysteria as a neurodynamic problem. Sarajevo: Narodna štamparija; 1956. (Serbian)
11. *Gélineau JBE*. De la narcolepsie. Gaz Hôp *(*Paris*)* 1880*;* 55: 635–7.
12. *Loëwenfeld L*. Über narkolepsie. Med Wochenschr (Münch) 1902; 49: 1041−5.
13. *von Economo C*. Sleep as a problem of localization. J Nerv Ment Dis 1930; 71(3): 249−59.
14. *Šušić V*. Physical and changes in muscle tone phenomenons of the paradoxical sleep in cats [dissertation]. Belgrade: Faculty of Medicine; 1970. (Serbian)
15. *Šušić V*. Vigilance, sleep and dreaming. Belgrade: Institute for training and specialization of health workers; 1977. (Serbian)
16. *Martinović Ž*. Electrosleep and EEG forms of sleep in chronic alcoholics during abstinence. Alkoholizam 1977; 14(1−2): 38−99. (Serbian)
17. *Martinović Ž*. Dream analysis of chronic alcoholics during electrosleep procedures. In: *Wageneder F, German RH*, editors. Electrotherapeutic Sleep and Electroanesthesia. Graz: Technische Universitaet; 1978. p. 149−53.
18. *Adie WJ*. Idiopathic narcolepsy: a disease sui generis; with remarks on the mechanism of sleep. Brain 1926; 49(3): 257−306.
19. *Wilson SA*. The narcolepsies. Brain 1928; 51(1): 63−109
20. *Daniels LE*. Narcolepsy. Medicine 1934; 13(1): 1−122.

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

Naslov: Neuronauke

Urednik: prof. dr Milkica M. Nešić

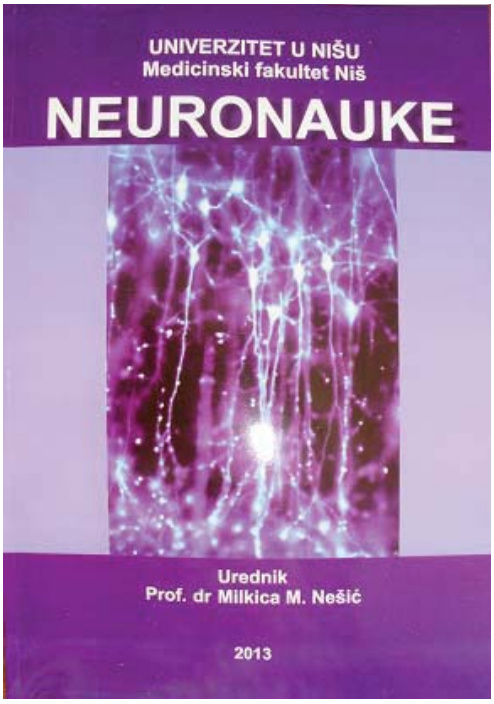
Izdavač: Medicinski fakultet Niš, Galaksija

Mesto i godina izdanja: Niš, 2013.

Štampa: „Galaksija“, Niš

Format: 29 cm

ISBN: 978-86-6322-029-1



Knjiga „Neuronauke“, urednika profesorke dr sci Milkice Nešić, fiziologa i neuropsihijatra, nastala je u okviru izbornog predmeta Neuronauke, koji je počev od februara 2007. godine, sadržan u programu Integrisanih akademskih studija medicine Medicinskog fakultetu u Nišu. Polazna ideja za njen nastanak inicirana je velikim interesovanjem polaznika koji je, istovremeno entuzijazmom predavača raznih oblasti i profila, prosto nametnuo potrebu za njenim štampanjem. Tokom uobličavanja konačnog koncepta, povećavao se broj saradnika zainteresovanih da iz svog profesionalnog domena daju doprinos temi, tako da je u krajnjoj verziji nastala dragocena i kompetentna knjiga o neuronaci, sagledanoj u svetlu fundamentalnih i kliničkih istraživanja.

Sadržaj rukopisa kroz više tematskih celina integrativnim pristupom, u kome dominira uredničkim majstorstvom kreativno usaglašena multidisciplinarnost, omogućava čitaocu da se prateći različite aspekte funkcionisanja nervnog sistema adekvatno i na savremen način upozna i sa poremećajem njegovih funkcija. Uvodni deo knjige, kao dostojna najava uzbudljivih naučnih tekstova, sastoji se iz dva rada koji prikazuju istorijski razvoj neuronauke od pre naše ere do najmodernijih tendencija, i podsećaju na osnovne principe interakcije strukturnih karakaterstika i funkcijskih mogućnosti centralnog nervnog sistema.

Ciljani tematski sadržaji nakon fundamentalnog uvida u suštinsko „biće“ neuronauke, demonstriranim savremenim informacijama iz anatomije, histologije, fiziologije i biohemije, skladno se nadovezuju na primenjenu kliničku neuronauku, gde su prikazana aktuelna saznanja iz neurologije, psihijatrije, farmakologije i neurohirurgije. U knjizi je ovakav tematski redosled predstavljen deskripcijom, slikom i šemama u pet poglavlja koja usmeravajućim naslovima i sadržajnom strukturom sama sebe najavljuju: Ćelijska i molekulska neuronauka, Vaskularizacija nervnog sistema i poremećaji cirkulacije, Organizacija senzornih sistema, Organizacija motornog sistema i Kognitivna i afektivna neuronauka. U slobodnom izboru poruka koje mogu poslužiti informativnoj nameni prikaza knjige, napravljenim uglavnom slučajnim izborom pominjemo značaj bioelektričnih odlika neurona i poremećaja neuromišićne transmisije, energetskog metabolizma, regulacijskih mehanizama cirkulacije, povezanosti neurofizioloških i neurohemijskih korelata sa bihevioralnom ekspresijom anksioznosti i afektivnih poremećaja...

Knjiga „Neuronauke“ nudi fundamentalan i primenjeni koncept neuronauke, kroz koji se čitalac može kompetentno obavestiti o sveobuhvatnim pitanjima kojima se neuronauka bavi. Zamišljena kao putokaz i učilo studentima osnovnih studija medicine, knjiga je već u samoj pripremi prevazišla svoju namenu i u konačnoj verziji nudi

dragoceno štivo specijalizantima i supspecijalistima iz oblasti anatomije, fiziologije, neurologije i drugih graničnih oblasti. Na kraju, što je i glavna odlika rukopisa koji ne završavaju svoj život u trenutnom izdanju, modernom koncepcijom, koja neuronauku autentično tretira kao dinamičnu, propulzivnu oblast, koja se neprestano inovira, „Neuronauka“ je otvorila nova pitanja i ostala otvorena za buduća saznanja.

dr Jelena Kostić,
Odeljenje dečje i adolescentne psihijatrije,
Klinika za zaštitu mentalnog zdravlja
Klinički Centar Niš

UPUTSTVO AUTORIMA

Vojnosanitetski pregled (VSP) objavljuje radove koji ranije nisu nigde publikovani, niti predati za publikovanje redosledom koji određuje uređivački odbor. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“ neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu potpisanu od strane svih autora da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjava o pojedinačnom doprinosu autora mora biti potpisana od strane svakog autora rada, skenirana i poslata uz rad kao dopunska datoteka. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa. Tim postupkom svi autori postaju odgovorni za ispunjavanje svih postavljenih uslova, čemu 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 adresi:

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** ili **metaanalize**, **kazuistika**, članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga, referati iz naučne i stručne literature 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 ne smeju prelaziti 16 stranica (sa prilozima); aktuelne teme – osam, kazuistika – šest, prethodna saopštenja – pet, a pisma uredniku, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

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.

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 koresponding autoru na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

1. ***Naslovna strana***

a) Naslov treba da bude kratak, jasan i informativan i da odgovara sadržaju rada. Podnaslove treba izbegavati.

b) Ispisuju se puna imena i prezimena autora.

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen i mesta u kojima se ustanove nalaze, sa jasnim obeležavanjem odakle je autor, koristeći standardne znake za fus-note.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i 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 (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi 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 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

Literatura se u radu 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 dodaje 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.

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 fusnoti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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/download/uputstvo_za_autore.pdf.

INSTRUCTIONS TO AUTHORS

Vojnosanitetski pregled (VSP) publishes only not previously published nor submitted papers in any other journals in the order determined by the Editorial Board. The following should be enclosed with the manuscript: a statement that the paper has not been submitted or accepted for publication elsewhere, a statement specifiing the actual contribution of each co-coautor, a consent signed by all the authors that the paper could be submitted; the name, exact address, phone number, and e-mail address of the first author and co-authors. VSP reserves all copyrights.

From January 1, 2012 the Vojnosanitetski pregled has been edited using the service e-Ur: Electronic Journal Editing.

All users of the system: authors, editors and reviewrs have to be registrated users with only one e-mail address. Registration should be made on the web-address:

http://scindeks-eur.ceon.rs/index.php/vsp

VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, from the **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, extensive abstracts of interesting articles from foreign language journals, and other contributions. 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. Observational and experimental articles, reviews and meta-analyses, should not exceed 16 pages (including tables and illustrations); case reports – 6; short communications – 5; letters to the Editor, reports on scientific meetings and book reviews – 2.

All measurements should be reported in the metric system in terms of the International System of Units (SI). Standard, internationally accepted terms should be used.

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**.** Avoid the use of colors in graphs.

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 first author for corrections that should be returned within 3 days. Manuscripts accepted for publication are not being returned.

Preparation of manuscript

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

1. ***Title page***

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

b) Full name of each author;

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

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

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

3. ***Text***

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

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

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

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

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

References

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

Examples of references:

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

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

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

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 typed double-spaced 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, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

Illustrations

Figures are submitted as photos which should be sharp. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

Legends for illustrations are typed on a separate page, with arabic numerals corresponding to the illustrations. Identify and explain each one clearly in the legend symbols, arrows, numbers, or letters used to identify parts of the illustrations. Explain the 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/download/instructions_to_authors.pdf.

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vmaini1@EUnet.rs
vmavsp@hotmail.com

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

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

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

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

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

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

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vmaini1@EUnet.rs
vmavsp@hotmail.com

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

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

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

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

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

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

Datum _____	Potpis _____