YU ISSN 0042-8450

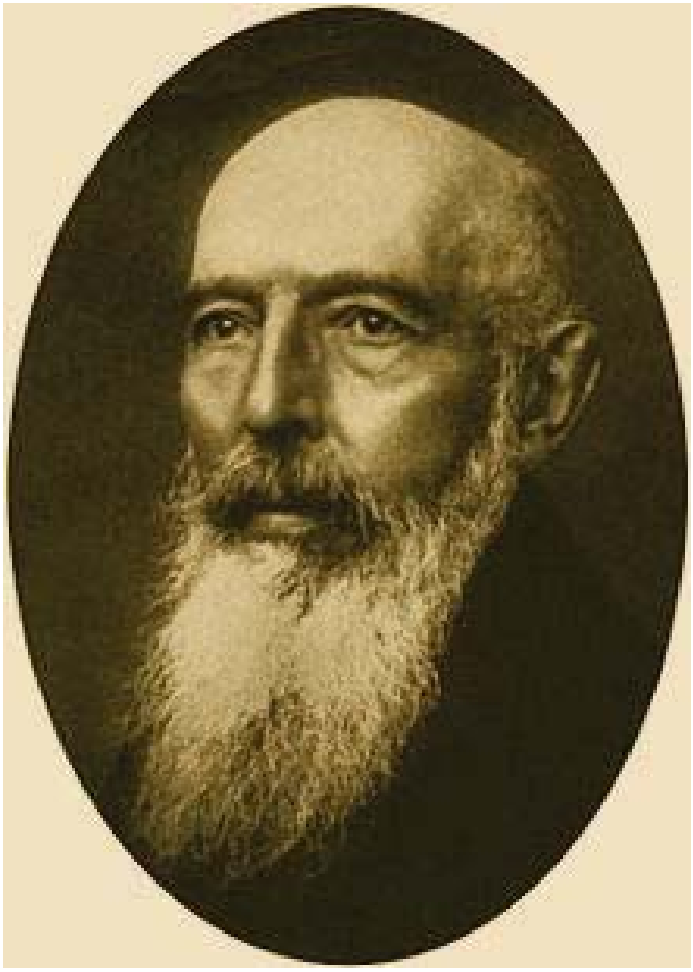
ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2013; July Vol. 70 (No. 7): p. 627-718.



YU ISSN 0042-8450 vol. 70, br. 7, 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. 7, 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

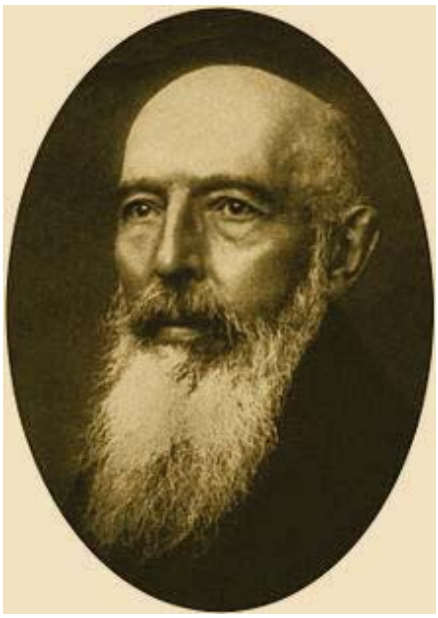
Editorial Office: Military Medical Academy, INI; Crnotravska 17, PO Box 33–55, 11040 Belgrade, Serbia. Phone: Editor-in-chief +381 11 3609 311; Main Journal Manager +381 11 3609 479; Fax: +381 11 2669 689; E-mail: **vsp@vma.mod.gov.rs**

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 Din, institutions 10,000.00 Din in Serbia, and foreign subscribers 150 €.

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

CONTENTS / SADRŽAJ



Gerhard Henrik Armauer Hansen (29 July 1841 – 12 February 1912), a Norwegian physician, remembered for his identification of *Mycobacterium leprae* in 1873 as the causative agent of leprosy, also called Hansen`s disease in his honour.
Leprosy and plague were the most widespread diseases of the Old and Middle Ages carrying away millions of human lives (see pages 706–10).

Gerhard Henrik Armauer Hansen (29. jul 1841 – 12. februar 1912), norveški lekar, upamćen je po otkriću *Mycobacterium leprae* kao uzročnika lepre koja je, njemu u čast, dobila naziv Hansenova bolest.
Lepra i kuga bile su najraširenije bolesti starog i srednjeg veka koje su odnele na milione ljudskih života (vidi str. 706–10).

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

“Eppur si muove” (“And yet it moves”)

„Ipak se kreće”

Silva Dobrić

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

Despite numerous discussions about imperfection of impact factor (IF) as a reliable indicator of scientific journals quality, it still survives in the academic and scientific communities as a more or less objective measure of their success and the influence in their scientific field [1–3]. The value of IF is published each year for the previous year in the month of June by the Journal Citation Report (JCR) publeshed by the Institute for Scientific Information (USA), now a part of the Thomson Reuters company. In general, to the journals found in this report, it is necessary to enter into a system of monitoring some of the Thomson Reuters citation databases, so-called Web of Science (WoS), because IF is calculated on the basis of citations obtained in journals that accompany these databases. As is well-known, IF is the quotient of the number of citations of articles published in a journal during the previous two years and the total number of articles published in those two years. For example, IF of a journal in 2012 is the quotient of the citations of articles published within 2010 and 2011 made in 2012, and the total number of articles published in that journal during 2010 and 2011. In this way, IF denotes an average citation rate of a single article from a given journal in a given year, in this case 2012.

This year, the JCR for 2012 was published on June 19, and, I suppose, rejoiced editorial boards of most scientific journals in Serbia included in the WoS databases, because they achieved a rise in the value of IF, very significant for some of them. In the category of biomedical journals the biggest “jump” in the value of IF was realized in the Archives of Biological Sciences and the Journal of Medical Biochemistry whose values from the last year's 0.360, and 0.610 increased to 0.791 and 1.084, respectively, being in the first case an increase of 119.7%, and in the second one 77.7% [4].

IF of the Vojnosanitetski Pregled (VSP) also increased in the last year, albeit modest, from 0.179 to 0.210 (about 17%). Considering that since the inclusion in the indexing system of the Science Citation Index Expanded (SCIE), that is one of WoS databases, in 2008, the number of citable articles published during the year in the Journal rose by around 10%, then regarding the method of IF calculating, an increase in its value resulted in a substantive increase in citations of

Uprkos brojnim raspravama o nedostacima impakt faktora (IF) kao pouzdanog pokazatelja kvaliteta naučnih časopisa, u akademskim i naučnim krugovima on i dalje opstaje kao manje-više objektivno merilo njihove uspešnosti i značaja koje ostvaruju u svojoj naučnoj oblasti [1–3]. Kao što je poznato, vrednost IF objavljuje se svake godine u junu mesecu za prethodnu godinu u *Journal Citation Report* (JCR) koji objavljuje *Institute for Scientific Information* (USA), sada u sklopu kompanije Thomson Reuters. Da bi se časopis uopšte našao u tom izveštaju neophodno je da uđe u sistem praćenja neke od citatnih baza Thomson Reuters-a, tzv. Web of Science (WoS), jer se IF izračunava na osnovu citata ostvarenih u časopisima koje prate te baze. Podsećanja radi, IF predstavlja količnik (kvocijent) broja citata članaka objavljenih u nekom časopisu u toku prethodne dve godine i ukupnog broja članaka objavljenih u te dve godine. IF časopisa za 2012. godinu, npr. predstavlja kvocijent između citata članaka objavljenih tokom 2010. i 2011. koji je postignut u 2012. godini, i ukupnog broja članaka objavljenih u tom časopisu tokom 2010. i 2011. godine. Na taj način IF označava prosečnu citiranost pojedinog članka iz datog časopisa u određenoj godini, u ovom slučaju u 2012.

Ove godine JCR za 2012. objavljen je 19. juna i, pretpostavljam, obradovao uredništva većine naučnih časopisa iz Srbije koji se nalaze u sistemu praćenja baza WoS jer je kod njih, uglavnom, došlo do porasta vrednosti IF, kod nekih i značajnog. U kategoriji biomedicinskih časopisa najveći skok vrednosti IF zabeležili su *Archives of Biological Sciences* i *Journal of Medical Biochemistry* čija je vrednost sa prošlogodišnjih 0,360, odnosno 0,610 porasla na 0,791, odnosno 1,084, što u prvom slučaju iznosi porast od 119,7%, a u drugom 77,7% [4].

IF Vojnosanitetskog pregleda (VSP) takođe je u prošloj godini zabeležio porast, iako skroman, sa 0,179 na 0,210 (oko 17%). Međutim, ako se uzme u obzir da je od ulaska u sistem praćenja baze *Science Citation Index Expanded* (SCIe) 2008. godine, broj citabilnih članaka koji se objave tokom jedne godine na stranicama časopisa porastao za oko 10%, onda je, uzimajući u obzir način izračunavanja IF, porast njegove vrednosti rezultat stvarnog porasta citiranosti članaka ob-

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

articles published in the VSP. This certainly confirms our being on the right track.

The increase of VSP's IF in 2012 is, of course, a result of dedicated work of the entire Editorial Board and the Editorial Staff of the Journal, as well as our reviewers, so on this occasion, I thank them for their cooperation and efforts to raise the quality of the Journal.

I also congratulate editorial boards of scientific journals from Serbia that made a progress in the values of their IF, and hope that we all will have even more readers and more citations in the coming years.

javljenih u VSP. Ovaj podatak raduje i pokazuje da smo na dobrom putu.

Porast IF časopisa VSP u 2012. godini rezultat je, svakako, predanog rada čitavog Uredništva i Redakcije, kao i naših recenzenata, te im se i ovom prilikom zahvaljujem na saradnji i naporima koje ulažu za podizanje kvaliteta časopisa.

Koristim priliku da čestitam i uredništvima naučnih časopisa iz Srbije koji su, takođe, u prethodnom periodu ostvarili pomake u vrednosti IF, na postignutom uspehu s nadom da će naši časopisi u narednim godinama imati još veći krug čitalaca i još veću citiranost.

REFERENCES

1. *Huth EJ*. Authors, editors, policy makers, and the impact factor. Croat Med J 2001; 42(1): 14–7.
2. *Bordons M, Fernandez MT, Gomez I*. Advantages and limitations in the use of impact factor measures for the assessment of research performance in a peripheral country. Scienrtometrics 2002; 53: 195–206.
3. *Pendlebury DA*. The use and misuse of journal metrics and other citation indicators. Arch Immunol Ther Ex 2009; 57(1): 1–11.
4. Anon. Impact factor are published for the year 2012. Available at: www.kobson.nb.rs/kobson.82.html (accessed on June 21, 2013)

ORIGINAL ARTICLES

UDC: 61:79]::612.2
DOI: 10.2298/VSP110208043M

Differences in the bioenergetic potential of athletes participating in team sports

Razlike u aerobnom bioenergetskom potencijalu sportista u timskim sportovima

Julijan Malacko*, Dragan Doder†, Slaviša Djurdjević‡, Biljana Savić†, Radoslava Doder§

***Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad, Serbia; †Provincial Institute for Sport and Sports Medicine, Novi Sad, Serbia; ‡Institute for Air Medicine, Military Medical Academy, Belgrade, Serbia; §Clinic for Infectious Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia**

Abstract

Background/Aim. In modern training technology, assessment of aerobic bioenergetic potential in athletes is commonly performed by standard laboratory procedures to determine basic or specific functional abilities for specific sport activity or discipline. The aim of study was to assess the aerobic bioenergetic potential of athletes participating in basketball, football and handball. **Methods.** The study included 87 athletes (29 basketball players, 29 football players, and 29 handball players) aged 21–24. Evaluation of the aerobic bioenergetic potential of athletes participating in basketball, football and handball was performed followed by both univariate (ANOVA) and multivariate (MANOVA) statistical methods to determine differences among the athletes in relative (VO_2 mL/kg/min) and absolute oxygen consumption (VO_2 L/min). **Results.** Statistically significant differences between absolute and relative oxygen consumption were found in basketball players (Mb), football players (Mf), and handball players (Mh) (MANOVA, $p = 0.00$). ANOVA also revealed significant differences in relative oxygen consumption (VO_2 mL/kg/min) ($p = 0.00$). The football players (55.32 mL/kg/min) had the highest relative oxygen consumption, followed by the handball players (51.84 mL/kg/min) and basketball players (47.00 mL/kg/min). The highest absolute oxygen consumption was recorded in the basketball players (4.47 L/min), followed by the handball players (4.40 L/min) and footballers (4.16 L/min). **Conclusion.** Statistically significant differences in the aerobic bioenergetic potential, expressed by the relative oxygen consumption were found among atletes participating in different team sports. It can be assumed that the player from the sports in which it is necessary to cross greater distance in total during the match have a greater need for aerobic capacity.

Key words:
athletes; football; basketball; oxygen consumption.

Apstrakt

Uvod/Cilj. Kod savremenih načina vežbanja vrši se procena aerobnih bioenergetskih mogućnosti sportista obično primenom standardnih laboratorijskih postupaka, sa ciljem da se utvrde ili osnovne ili specifične funkcionalne sposobnosti za određenu sportsku aktivnost ili disciplinu. Cilj istraživanja bio je da se izvrši procena aerobnog bioenergetskog potencijala sportista u košarci, fudbalu i rukometu. **Metode.** Istraživanjem je bilo obuhvaćeno 87 sportista (29 košarkaša, 29 fudbalera i 29 rukometaša), starosti od 21–24 godine. Izvršena je procena aerobnog bioenergetskog potencijala sportista u košarci, fudbalu i rukometu, a zatim je primenom metode multivarijantne i univarijantne analize varijanse (MANOVA/ANOVA) ispitana značajnost razlika u relativnoj (VO_2 mL/kg/min) i apsolutnoj (VO_2 L/min) potrošnji kiseonika. **Rezultati.** Multivarijantna statistička značajnost razlika između aritmetičkih sredina apsolutne i relativne potrošnje kiseonika kod košarkaša (Mk), fudbalera (Mf) i rukometaša (Mr) dobijena je na nivou $p = 0{,}00$, dok je univarijantnom analizom varijanse statistička značajnost razlika postojala samo u varijabli relativne potrošnje kiseonika (VO_2 mL/kg/min), takođe, na nivou $p = 0{,}00$. Najviše vrednosti relativne potrošnje kiseonika (VO_2 mL/kg/min) zabeležene su u grupi fudbalera (55,32 mL/kg/min), zatim rukometaša (51,84 mL/kg/min), na kraju kod košarkaša (47,00 mL/kg/min). U apsolutnoj potrošnji kiseonika najviše vrednosti zabeležene su u grupi košarkaša (4,47 L/min), zatim rukometaša (4,40 L/min) i fudbalera (4,16 L/min). **Zaključak.** Dobijene su statistički značajne razlike u aerobnim bioenergetskim potencijalima izraženim u relativnoj potrošnji kiseonika kod učesnika u različitim timskim sportovima. Rezultati sugerišu da sportisti koji tokom utakmice moraju preći ukupno veću razdaljinu imaju veće potrebe za aerobnim kapacitetom.

Ključne reči:
sportisti; fudbal; košarka; kiseonik, potrošnja.

Correspondence to: Dragan Doder, Provincal Institute for Sport, Masarikova 25/II, 21 000, Novi Sad, Serbia. Phone.: +381 21 572 224. E-mail: dodersport@yahoo.com

Introduction

In modern training technology, assessment of aerobic bioenergetic potential in athletes is commonly performed by standard laboratory procedures, to determine basic or specific functional abilities for specific sport activity or discipline. For this purpose, one may use protocols with sustained or unchanging workload, progressive workload to exhaustion, as well as submaximal, maximal and supramaximal workloads [1–3].

In sports of the aerobic and aerobic-anaerobic type, it is essential to express the actual bioenergetic potential with oxygen consumption, which is an indicator of the aerobic bioenergetic potential or capacity. The upper threshold of the body's ability to consume oxygen is represented by the maximal oxygen consumption, a net sum of physiological functions of the aerobic bioenergetic systems involving the lungs, the heart, blood, and working muscles [4].

The amount of oxygen that can be distributed to the working muscle and be utilized there is limited, and it can be estimated by a special equipment measuring the volume of the inhaled *vs* exhaled oxygen. The difference between the two volumes represents the amount of oxygen that has been used by the muscle, in the literature called maximal oxygen consumption (VO_2 L/min). It is normally expressed in liters per minute (absolute) as opposed to relative oxygen consumption (VO_2 mL/kg/min) calculated as absolute oxygen consumption in one minute per unit of body mass. This greatest volume of oxygen utilized by the body in a single minute, is essentially an indicator of the greatest amount of energy generated for physical work by all the aerobic metabolic processes.

The values of maximal oxygen consumption was expressed in absolute and relative units is critical for successful performance in all sustained activities lasting over 2–3 minutes [5, 6].

Team sport complexity is reflected in their structural, energy and neuro-muscular components. More specifically, a football player must be able to sprint, jump, change direction, be involved in physical contacts, and accurately passes the ball to a teammate or kicks it into the net. A basketball player must play aggressive defence, rebound, run a fast-break and shoot in the basket. A handball player, often in physically demanding, even rough playing conditions, has to be able to penetrate with the ball, shoot at the net from various out-of-balance positions, and eventually return to defence as quickly as possible. These kinds of motor structures would not be so demanding for an athlete, were they not performed in long-lasting, sustained fashion for at least an hour or more (during a whole game) and in mostly aerobic conditions. Accordingly, team sports consist of a number of high-intensity motion structures which, except during brief periods of rest, should be performed at a high level during a game, from its first to the last minute. If an athlete, participating in a team sport, wants to be successful, he/she must possess optimal aerobic and aerobic-anaerobic bioenergetic potentials, allowing for dealing with extreme demands of training and elite competition, delaying the onset of fatigue, and accelerating recovery processes.

The purpose of this study was to evaluate and assess aerobic bioenergetic potentials of athletes participating in basketball, football and handball. Following this, differences in absolute and relative values of oxygen consumption among athlets in the three sport disciplines were subjected to both multivariate and univariate statistical analyses of mean differences. Obtained informations can then be used for managing, modeling, diagnostics, planning, programming, and monitoring of training and competition cycles.

Methods

There were 87 male athletes (29 basketball players, 29 football players and 29 handball players), aged 21–24, 181–191 cm tall, weighing 76–90 kg, members of the first league clubs. Tests were conducted before the start of the preparatory period. Subjects were tested voluntarily (Table 1).

Table 1

Participants' characteristics

Parameters	Mb (n = 29)	Mf (n = 29)	Mh (n = 29)
Age (years)	21.6 ± 1.05	23.04 ± 2.48	24.09 ± 1.58
BH (cm)	190.46 ± 8.73	181.66 ± 5.01	189.51 ± 4.96
BM (kg)	83.56 ± 9,12	76.72 ± 7.49	89.11 ± 9.72
BMI (kg/m^2)	23.04 ± 0.41	23.25 ± 0.99	24.81 ± 1.41

Mb – basketball players; Mf – football players; Mh – handball players; BH – body height (cm); BM – body mass (kg); BMI – body mass index (kg/m^2).

Two variables were used for the assessment of the aerobic bioenergetic potential of each athlete: maximal oxygen consumption (VO_2 L/min), estimating the absolute amount of oxygen used by the body, and relative oxygen consumption (VO_2 mL/kg/min), representing absolute oxygen consumption per unit of body mass (kg).

Ergometric testing was run on a treadmill (Cosmed T150, Italy), with the use of a gas analyser (Cosmed Quark b2, Italy), through progressively increasing workloads. Testing protocol included 3 min of warm-up (at 3 km/h, without inclination), at the speed of 7 km/h that was increased by 1 km/h each minute, at the steady 1.5% incline. This was followed by an incrase of 0.5 km/h every 30 sec, with the incline remaining at 1.5%.

For each applied variable, the following statistical central and dispersion parameters were calculated: mean (M), minimal value (min), maximal value (max), and standard deviation (S). The normalcy of distribution was expressed by skewness (Sk) and kurtosis (Ku).

Both multivariate and univariate analyses of variance (MANOVA/ANOVA) were run to test the differences between mean values of the applied variables for basketball, football and handball players. Multivariate testing of the null hypothesis that group centroids are equal to the common centroid (GENERAL MANOVA) was performed with the χ-Wilks' lambda test; F-ratio, and statistical significance set at $p < 0.05$. Univariate statistical significance of mean differences was calculated with the F-test at $p < 0.05$. All the data were analysed with the Statistica 8 computer software application.

Results

Analysing data given in Table 2, it is clear that both relative (VO_2 mL/kg/min) and absolute (VO_2 L/min) oxygen consumption values do not skew significantly from normal distribution (Sk), demonstrating quality measuring techniques. The results showed that football players had the greatest relative oxygen consumption (55.32 mL/kg/min), followed by handball players (51.84 mL/kg/min) and basketball players (47.00 mL/kg/min). Conversely, the highest absolute oxygen consumption was recorded in basketball players (4.47 L/min), followed by handballer players (4.40 L/min) and footballers (4.16 L/min) (Table 2).

It can be observed, that there were statistically significant differences in both variables among basketball (Mb), football (Mf) and handball players (Mh) (p = 0.00) (MANOVA), whereas ANOVA demonstrated significant differences only in the relative oxygen consumption (p = 0.00), but not in the absolute one (p = 0.07) (Table 3).

Table 2

Statistical parameters and their discrimination

Sport	M	SD	min	max	Sk	Ku
			VO_2 (mL/kg/min)			
Basketball	47.00	2.37	42.07	51.28	-0.24*	-0.40
Football	55.32	3.60	49.60	65.73	0.59*	0.72
Handball	51.84	4.15	45.23	58.55	0.02*	-0.64
			VO_2 (L/min)			
Basketball	4.47	.50	3.53	5.49	0.23*	-0.23
Football	4.16	.41	3.23	5.06	0.17*	0.19
Handball	4.40	.68	3.21	5.45	-0.19*	-0.98

M – mean; S – standard deviation, min - minimal value; max – maximal value; Sk - skewness; Ku - kurtosis; *normalcy of distribution; VO_2 (L/kg/min) – relative oxygen consumption; VO_2 (L/min) – absolute oxygen consumption.

Table 3

Statistical parameters of univariate (ANOVA) and multivariate (MANOVA) analysis of variance

Variable	Mb	Mf	Mh	F	p
VO_2 (mL/kg/min)	47.00	55.32	51.84	4.32	0.00*
VO_2 (L/min)	4.47	4.16	4.40	2.62	0.07

λ = .44 F = 20.46 p = .00*

Mb – basketball players; Mf – football players; Mh – handball players; VO_2 (mL/kg/min) – relative oxygen consumption; VO_2 (L/min) – absolute oxygen consumption; * – statistically significante difference.

Discussion

Assessment of the aerobic bioenergetic potential of athletes represents an integral marker of functional ability of all the systems participating in delivery, transport and energy transformation of oxygen. High aerobic bioenergetic potential is essential for successful performance in many sports, including handball, basketball and football.

Football is a game requiring both anaerobic, a mix of anaerobic-aerobic, and aerobic work. Besides aerobic endurance, which is the most important in terms of the average distance covered (8–12 km), there is the need for anaerobic work as well, such as in sprints, accelerations, contact game, etc. In football, body's bioenergetic needs vary, and depend a great deal on the level of competition, game model, team position, training cycle stage, as well as the area of running covered during a game. The average relative oxygen consumption in football is about 58.2 mL/kg/min, specifically about 51 mL/kg/min for keepers, 59 mL/kg/min for defenders, 63 mL/kg/min for midfielders, and 60 mL/kg/min for attackers. Keepers are normally characterized by explosiveness, flexibility and quick reactions, defenders by endurance and coordination, while attackers typically possess extraordinary speed and explosiveness [7–9].

The greatest positive influence on the level of skill and performance in football players is typically due to a well-developed aerobic and anaerobic/glycolytic mechanism of energy generation, necessary for performing various technical-tactical tasks in situational competitive conditions [10].

It is possible to apply a similar model to handball and basketball, that essentially have comparable, but not identical physical demands. Recent rule changes in handball have significantly altered the way the game had been played up until ten years ago. The rules on passive play, a quick pivot, letting keepers quickly introduce the ball into play, are only some of the changes which have rather increased the speed of the game, as well as shortened the intervals between sprints. In modern handball, motion structures are characterized by frequent, short sprints separated by brief pauses.

Players run over between 4,500 and 5,500 meters on the match in a variety of movements (37% walking, running 31%, 25% fast running and 7% in different sprint dinamic) [11].

Relative oxygen consumption of trained senior basketball players is somewhere between 45–65 mL/kg/min [12]. The values for younger players are slightly lower (37–55 mL/kg/min). In the present study, no differences were found in relative oxygen consumption with respect to the

position of play. It is generally accepted that anaerobic functioning is crucial for high performance in basketball. Nevertheless, the role of aerobic mechanisms is important, especially during recovery time rather than having a direct influence on the game [13].

Since there are limits in utilizing aerobic mechanisms in recovery, it is reasonable to say that high-level aerobic ability is necessary for playing basketball, but further improvements of this ability will not have more significant benefits. For this reason, aerobic metabolism is thought to have a moderate effect on basketball performance.

In American college basketball, there was even a negative correlation between aerobic power and the amount of time spent in the game [14].

According to our results, it can be assumed that the differences found in the aerobic bioenergetic potential, as expressed by the relative oxygen consumption, may be due to differences in morphological characteristics (body height and mass), situational motion structures (technical and tactical elements), training routines and characteristics (training methods), intensity, duration and ways of motion (specific and situational conditions), as well as the workload in metabolic zones at the aerobic threshold (compensated acidosis).

Conclusion

This study confirmed significant differences among football, handball and basketball players in aerobic bioenergetic potential, as demonstrated by their relative oxygen consumption. It can be assumed that the players from the sports in which it is necessary to cross greater length (distance) in total during the match have a greater need for aerobic capacity due to different loads in metabolic zones at the aerobic threshold level (compensated acidosis).

REFERENCES

1. *American College of Sports Medicine*. Guidelines for exercise testing and prescription. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
2. *Wilmore JH, Costill DL*. Physiology of sport and exercise. 3rd ed. Champaign, IL: Human Kinetics; 2005.
3. *Malacko J, Rado I*. Technology of sports and sports training. Sarajevo: Fakultet sporta i tjelesnog odgoja; 2004. (Bosnian)
4. *Vučetić V, Šentija D*. Diagnosis of functional skills – why, when and how to test athletes? Kondicijski trening 2005; 2(2): 8−14. (Croatian)
5. *Guyton AC, Hall JE*. Textbook of medical physiology. 11th ed. Philadelphia: W.B. Saunders Company; 2001. (Croatian)
6. *Malacko J, Doder D*. Technology of sport training and recovery. Novi Sad: Pokrajinski zavod za sport; 2008. (Serbian)
7. *Castagna C, Impellizzeri FM, Chamari K, Carlomagno D, Rampinini E*. Aerobic fitness and yo-yo continuous and intermittent tests performances in soccer players: a correlation study. J Strength Cond Res 2006; 20(2): 320−5.
8. *Apostolidis N, Nassis GP, Bolatoglou T, Geladas ND*. Physiological and technical characteristics of elite young basketball players. J Sports Med Phys Fitness 2004; 44(2): 157−63.
9. *Krustrup P, Mohr M, Steensberg A, Bencke J, Kjaer M, Bangsbo J*. Muscle and blood metabolites during a soccer game: implications for sprint performance. Med Sci Sports Exerc 2006; 38(6): 1165−74.
10. *Reilly T, Bangsbo J, Franks A*. Anthropometric and physiological predispositions for elite soccer. J Sports Sci 2000; 18(9): 669−83.
11. *Bon M, Perš J, Šibila M. Kovačič S*. Player movements analysis on international matches. Ljubljana: Faculty of Sport; 2002.
12. *Wells GD, Norris SR*. Assessment of physiological capacities of elite athletes & respiratory limitations to exercise performance. Paediatr Respir Rev 2009; 10(3): 91−8.
13. *Ostojic SM, Mazic S, Dikic N*. Profiling in basketball: physical and physiological characteristics of elite players. J Strength Cond Res 2006; 20(4): 740−4.
14. *Sallet P, Perrier D, Ferret JM, Vitelli V, Baverel G*. Physiological differences in professional basketball players as a function of playing position and level of play. J Sports Med Phys Fitness 2005; 45(3): 291−4.

Received on February 8, 2011.
Revised on May 5, 2011.
Accepted on May 12, 2011.
OnLine-First November, 2012.

ORIGINAL ARTICLE

UDC: 616-073.75::616.831-006-079.4
DOI: 10.2298/VSP110223044R

Proton magnetic resonance spectroscopy and apparent diffusion coefficient in evaluation of solid brain lesions

Protonska magnetnorezonantna spektroskopija i prividni difuzioni koeficijent u proceni solidnih lezija mozga

Dragana Ristić Baloš, Svetlana Gavrilović, Slobodan Lavrnić, Brankica Vasić, Marija Mačvanski, Dušan Damjanović, Tatjana Stošić Opinćal

Radiology and Magnetic Resonance Imaging Center, Clinical Center of Serbia, Belgrade, Serbia

Abstract

Background/Aim. Advanced magnetic resonance techniques can provide insight in physiological changes within pathological canges and contribute to better distinquishing between different tumor types and their discrimination from non-neoplastic lesions. The aim of this study was to evaluate the role of proton magnetic resonance spectroscopy (^{1}H-MRS) and apparent diffusion coefficients (ADC) in distinguishing intracranial glial tumors from tumor like non-neoplastic lesions, as well as for differentiating high- from low-grade gliomas. **Methods.** This retrospective study included 47 patients with solid brain lesions (25 non-neoplastic, 14 low-grade and 8 anaplastic glial tumors). In all patients ^{1}H-MRS (at a TE of 135 ms and 30 ms) and diffusion-weighted imaging (DWI) were performed. The choline to creatine (Cho/Cr), choline to N-acetyl aspartate (Cho/NAA), N-acetyl aspartate to creatine (NAA/Cr) and myoinositol to creatine (mIn/Cr) ratios and the apparent diffusion coefficient (ADC) were determined. **Results.** The Cho/Cr ratio was significantly higher in glial tumors grade II than in non-neoplastic lesions (p = 0.008) and in glial tumors grade III than in non-neoplastic lesions (p = 0.001). The Cho/NAA ratio was significantly higher in glial tumors grade II than in non-neoplastic lesions (p = 0.037). ΔADC/ADC between glial tumors grade II and glial tumors grade III showed a statistical significance (p = 0.023). **Conclusion.** Our study showed that ^{1}H-MRS and apparent diffusion coefficients can help in evaluation and differentiation of solid brain lesions.

Key words:
brain neoplasms; glioma; brain ischemia; diagnosis; diagostic techniques and procedures; magnetic resonance imaging; magnetic resonance spectroscopy.

Apstrakt

Uvod/Cilj. Savremene tehnike magnetne rezonance mogu pružiti uvid u fiziološke promene unutar patoloških i doprineti boljem razlikovanju različitih tipova tumora, kao i njihovom odvajanju od bolesti koje nisu maligne. Cilj ovog istraživanja bio je da se utvrdi uloga magnetnorezonante spektroskopije i prividnog difuzionog koeficijenta u razlikovanju intrakranijalnih glijalnih tumora od netumorskih obolenja, kao i u razlikovanju visokogradusnih od niskogradusnih glioma. **Metode.** Ovom retrospektivnom studijom obuhvaćeno je 47 bolesnika sa solidnim lezijama mozga (25 netumorskih, 14 niskogradusnih i 8 anaplastičnih glijalnih tumora). Kod svih bolesnika rađena je protonska magnetnorezonantna spektroskopija (sa vremenom eha 135 ms i 30 ms) i difuziono snimanje. Određivani su odnosi holin/kreatin (Cho/Cr), holin/N-acetil aspartat (Cho/NAA), N-acetil aspartat/kreatin (NAA/Cr), mioinozitol/kreatin (mIn/Cr), kao i prividni difuzioni koeficijent (ADC). **Rezultati.** Nađena je statistički značajna razlika između indeksa Cho/Cr za glijalne tumore gradusa II i netumorske promene (p = 0,008) kao i za glijalne tumore gradusa III i netumorske promene (p = 0,001). Odnos Cho/NAA bio je statistički značajno viši kod glijalnih tumora gradusa II u odnosu na netumorske promene (p = 0,037). Odnos standardne devijacije ΔADC/ADC između glijalnih tumora gradusa II i glijalnih tumora gradusa III pokazao je statistički značajnu razliku (p = 0,023). **Zaključak.** Naše istraživanje pokazalo je da protonska magnetnorezonantna spektroskopija i prividni difuzioni koeficijent mogu biti korisni u evaluaciji i diferencijaciji solidnih lezija mozga.

Ključne reči:
mozak, neoplazme; gliom; mozak, ishemija; dijagnoza; dijagostičke tehnike i procedure; magnetna rezonanca, snimanje; magnetna rezonanca, spektroskopija.

Correspondence to: Dragana Ristić Baloš, Radiology and Magnetic Resonance Imaging Center, Clinical Center of Seriba, Pasterova 2, 11 000 Belgrade, Serbia. Phone: +381 11 361 5554. E-mail: drbgaga@gmail.com

Introduction

Conventional magnetic resonance (MR) imaging has become the gold standard for detection and morphological assessment of solid brain lesions [1–5]. However, MR imaging based differentiation of neoplastic from non-neoplastic brain masses and the establishment of tumor grade are often difficult [4, 6, 7]. Further evaluation and follow-up are often necessary, including histopathological examination of biopsy specimens [8]. When lesions cannot be treated surgically or when they are located at areas of high risk for biopsy, greater accuracy of non-invasive imaging evaluation is desirable [1, 2]. Assessment of MR images obtained after administration of a paramagnetic contrast agent must be done with caution, because any pathology associated with disruption of blood-brain barrier (BBB) results in post-contrast enhancement [9]. Advanced MR techniques, like MR spectroscopy and diffusion-weighted imaging, can provide insight in physiological changes within pathology and contribute to more successful distinguishing between tumor types and their separation from tumor mimicking lesions [1, 10].

The radiological differential diagnosis of solid brain masses varies from tumors (gliomas WHO grades I–III), benign pseudotumoral lesions to demyelinating or ischemic lesions [7]. Therefore, establishment of correct diagnosis is crucial for choosing appropriate therapeutic procedure and patient outcome [11, 12]. Gliomas are the most common primary neoplasms of brain, typically heterogeneous, varying histologically from low grade to high grade [11–14]. Although golden standard in diagnosis of brain glioma, histological evaluation can be misleading, because sampling regions may or may not correspond to increased cellularity and/or neoangiogenesis [11, 15–18]. Therefore, more accurate information about tumor physiology, such as metabolism, cellularity and microstructure are important in determining tumor grade and cannot be collected only based on conventional MR imaging [11]. Advanced MR imaging techniques, such as proton MR spectroscopy (^{1}H-MRS) and diffusion-weighted MR imaging (DWI) could provide insight in those features and hence increase accuracy of prediction of tumor histological grade [8, 9, 15]. Tracing of brain metabolites concentrations using ^{1}H-MRS can provide information about cell proliferation, degradation, energetic metabolism and appearance of ischemia or necrosis [5, 14, 16]. DWI and apparent diffusion coefficient (ADC) values obtained from DWI, provide complementary information about cellular density and tissue microstructure [16].

The aim of this study was to assess the role of proton MR spectroscopy and DWI in discrimination of gliomas from non-neoplastic mimics, as well as for differentiation of grade II from grade III of glial neoplasms.

Methods

Patients

This study was conducted in the Center for Radiology and Magnetic Resonance Imaging, Clinical Center of Serbia, Belgrade between November 2006 and August 2010. Retrospective study included 47 patients (22 women and 25 men, age range: 12–72 years, mean age 43 years) with solid brain lesions that were with 22 histopathologically (WHO classification) proven gliomas (14 grade II and 8 anaplastic tumors grade III) and 25 with non-neoplastic lesions (5 hamartomas, 11 demyelinating lesions, 9 ischemic lesions) whose diagnosis were established by clinical examination or MR imaging. All the glioma patients underwent MR imaging examination followed by surgery and histological evaluation of the lesion. Fourteen of them were assigned to be grade II (5 diffuse astrocytomas, 4 oligoastrocytomas and 5 oligodendrogliomas) and 8 as anaplastic astrocytomas grade III (Table 1).

Table 1

Demographic and diagnostic procedures characteristics in the patients with solid brain lesion

Pathologies	Total number	Sex (n)		Median of age (years)	Spectroscopy			
		M	F		SVS*	CSI*	SVS†	CSI†
Hamartoma	5	3	2	26.2	1	2	1	1
Demyelinating lesions	11	5	6	37.8	1	4	5	1
Ischemic lesions	9	5	4	49.1	2	0	6	1
Astrocytoma diffusum grade II	5	3	2	41.8	2	2	0	1
Oligoastrocytoma grade II	4	3	1	53.3	0	1	1	2
Oligodendroglioma grade II	5	4	1	44.2	1	0	2	2
Anaplastic astrocytoma grade III	8	2	6	45.9	2	4	1	1
Total	47	25	22	43	9	13	16	9

TE-echo time; *TE 30 ms; †TE 135 ms; SVS – single voxel spectroscopy; CSI – chemical shift imagin; M – male; F – female.

MR imaging

MR imaging examinations were performed on a 1.5 T MR imging device (Avanto, Siemens Medical Solutions, Erlangen, Germany) using the standard 8-channel transmit/receive head coil. The conventional MR imaging protocol consisted of a three-plane localizer sequence, axial T1 weighted spin echo (SE), repetition time eho time [(TR/TE) 550/9.4 ms, slice thickness 5 mm, gap 1 mm, matrix 512 × 256, NEX 2, FOV 24 cm], axial and sagittal turbo T2 weighted spin echo (TSE), (TR/TE 4820/94 ms, slice thickness 5 mm, gap 1 mm, matrix 512 × 256, NEX 2, FOV 24 cm), coronal fluid-attenuated inversion recovery (FLAIR), (TR/TE/TI 9900/126/2500 ms, slice thickness

5 mm, no gap, matrix 256 × 224, NEX 2, FOV 24 cm) sequences. After administration of contrast agent (gadopentetate dimeglumine, 0.1 mmoL/kg body weight; Magnevist, Schering, Germany), 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence was performed.

^{1}H-MRS

Proton MR singl evoxel spectroscopy (SVS) or chemical shift imaging (CSI) with a TE of 30 ms and TE of 135 ms, was performed immediately after completion of conventional MR imaging. SVS was used for well-circumscribed lesions and CSI for diffuse infiltrative lesions. Post contrast T1-weighted MPRAGE images were used for positioning of the volume of interest (VOI). Typical VOIs sizes were 100 × 80 mm^2. VOIs for SVS were placed at image regions to show post-contrast enhancement. For CSI, voxels which showed the greatest departure of Cho/Cr from the values for normal appearing white matter were selected. To suppress the water signal, chemical shift selective saturation was applied. The acquisition time was approximately 7 min for CSI and 4 min for SVS. Spectroscopic data were processed with the Syngo v14 software implemented on MR imaging console. The processing algorithm included the application of a Hanning filter, baseline and phase correction. Metabolite peak areas were obtained after all the observed resonances in the spectra were fitted.

At TE = 30 ms evaluated metabolites were: N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myoinositol (mIn), glutamine and glutamate (Glx); at TE 135 measured metabolites were: N-acetyl aspartate (NAA), choline (Cho) and creatine (Cr).

DWI

An echo planar (EPI) SE sequence (TR/TE 3808/89 ms, slice thickness 5 mm, matrix 128 × 128, NEX 2, FOV 24 cm) was used for obtaining diffusion-weighted images; *b* value of 0 s/mm^2, as a reference, and *b* values of 1,000 s/mm^2 were included in DWI sequences. We used DWI to calculate corresponding ADC maps. ADC values were calculated by using the equation:

$$\ln(S/S_0) = -bADC$$

where b is a diffusion sensitivity factor, S is a signal at b = 1,000, S_0 is a signal at b = 0 and ADC was previously explained.

The positions of regions of interest (ROIs) placed on the ADC maps corresponded as much as possible to the location of spectroscopic VOIs. The ROIs size varied from 20 to 44 pixels.

Statistical analysis

The SPSS 12.0 for Windows was used for statistical analysis. Unpaired two-tailed Student's *t*-test was used for comparison of ^{1}H-MRS metabolite ratios (Cho/Cr, Cho/NAA, NAA/Cr, mIn/Cr) and ADC values (ADC, ΔADC and ΔADC/ADC) between the groups (non-neoplastic lesions, glial tumors grade II and III) and all seven different pathologies (demyelinating lesions, ischemic lesions, hamartomas, diffuse astrocytomas, oligoastrocytomas, oligodendrogliomas and anaplastic astrocytomas). The significance level was set to be $p < 0.05$.

Results

The results obtained for metabolite ratios and diffusion parameters are summarized in Table 2.

Table 2

Metabolite ratios in solid brain lesions

Pathologies	Cho/Cr	Cho/NAA	NAA/Cr	mIn/Cr	ADC	ΔADC	ΔADC/ADC
Non-neoplastic lesions (hamartoma, ischemic lesions, demyelinating lesions)	1.21 ± 0.46	0.91 ± 0.49	1.59 ± 0.88	0.40 ± 0.23	1.14 ± 0.41	0.12 ± 0.05	0.13 ± 0.13
Hamartoma	1.00 ± 0.40	0.92 ± 0.64	1.38 ± 0.67	0.52 ± 0.30	1.05 ± 0.13	0.09 ± 0.03	0.09 ± 0.03
Ischemic lesions	0.99 ± 0.36	0.83 ± 0.39	1.39 ± 0.71	0.20 ± 0.08	1.12 ± 0.56	0.14 ± 0.07	0.13 ± 0.08
Demyelinating lesions	1.47 ± 0.44	0.98 ± 0.54	1.84 ± 1.08	0.41 ± 0.20	1.20 ± 0.35	0.11 ± 0.03	0.15 ± 0.19
Glial tumors grade II (diffuse astrocytoma, oligoastrocytoma, oligodendroglioma)	2.08 ± 1.18	3.60 ± 4.35	1.27 ± 0.79	0.39 ± 0.16	1.32 ± 0.42	0.12 ± 0.04	0.10 ± 0.04
Diffuse astrocytoma	1.48 ± 1.18	1.00 ± 1.05	1.89 ± 0.62	0.43 ± 0.10	1.38 ± 0.60	0.14 ± 0.05	0.11 ± 0.04
Oligoastrocytoma	2.55 ± 1.34	5.64 ± 6.37	0.90 ± 0.64	0.50 ± 0.00	1.09 ± 0.32	0.10 ± 0.02	0.10 ± 0.04
Oligodendroglioma	2.32 ± 1.02	4.57 ± 4.02	0.96 ± 0.78	0.12 ± 0.00	1.45 ± 0.22	0.11 ± 0.03	0.08 ± 0.03
Glial tumors grade III (Astrocytoma anaplasticum)	2.47 ± 0.88	3.59 ± 7.30	2.77 ± 1.77	0.78 ± 046	1.14 ± 0.34	0.10 ± 0.05	0.09 ± 0.05

Note: Values are mean ± standard deviation
Cho – choline; Cr – creatine; NAA – N-acetyl aspartate; mIn – myoinositol; ADC – apparent diffusion coefficient; ΔADC – standard deviation of ADC

Data analysis showed that the Cho/Cr ratio was significantly higher in glial tumors grade II compared to non-neoplastic lesions ($p = 0.008$) and that the Cho/Cr ratio was significantly higher in glial tumors grade III than in non-neoplastic lesions ($p = 0.001$) (Figure 1). The Cho/NAA ratio was significantly higher in glial tumors grade II than in non-neoplastic lesions ($p = 0.37$).

NAA/Cr, and mIn/Cr ratios could not differentiate between non-neoplastic lesions, glial tumors grade II and III.

Statistically significant difference was also revealed between Cho/Cr ratios of anaplastic astrocytoma and ischemic lesions ($p = 0.003$), demyelinating lesions ($p = 0.002$)

and hamartomas (p = 0.01) respectively (Figure 2). The NAA/Cr ratio was significantly different between diffuse astrocytomas and hamartomas (p = 0.046), oligoastrocytoma and demyelinating lesions (p = 0.006) (Figure 3), anaplastic astrocytoma and ischemic lesions (p = 0.048) and anaplastic astrocytoma and oligodendroglioma (p = 0.004).

were excluded from evaluation of NAA/Cr and Cho/NAA ratios in statistical analysis.

Comparison of ADC, ΔADC and ΔADC/ADC values between tumors and non-neoplastic lesions showed a statistical significance of ΔADC/ADC between glial tumors grade II and glial tumors grade III (p = 0.023).

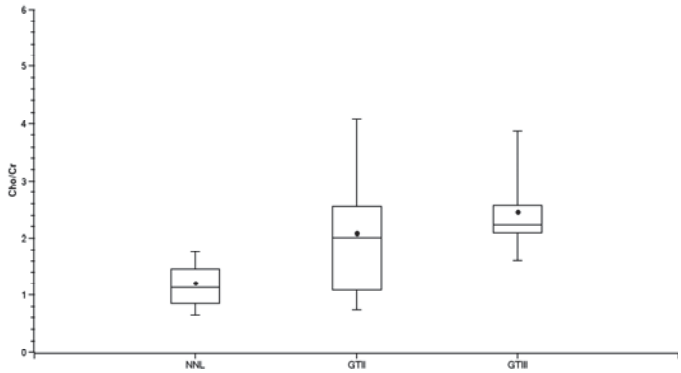


Fig. 1 – Box plot of the choline-to-creatine ratio (Cho/Cr) shows a significant difference between non-neoplastic lesions and glial tumors grade II and between non-neoplastic lesions and glial tumors grade III.

NNL – non-neoplastic lesions; GTII – glial tumors grade II; GTIII – glial tumors grade III.

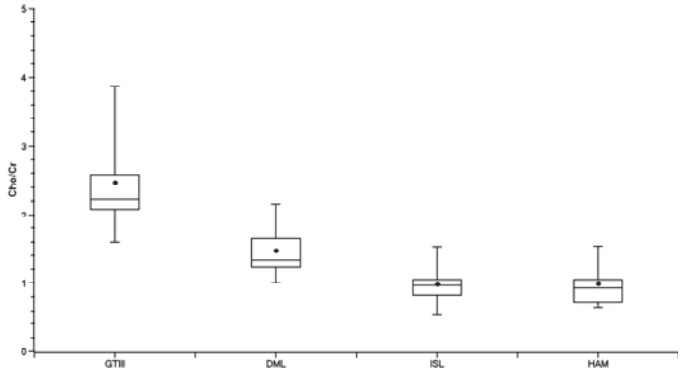


Fig. 2 – Box plot of the choline-to-creatine ratio (Cho/Cr) shows a significant difference between glial tumors grade III and demyelinating lesions, ischemic lesions and hamartomas, respectively.

GTIII – glial tumors grade III; DML – demyelinating lesions; ISL – ischemic lesions; HAM – hamatromas.

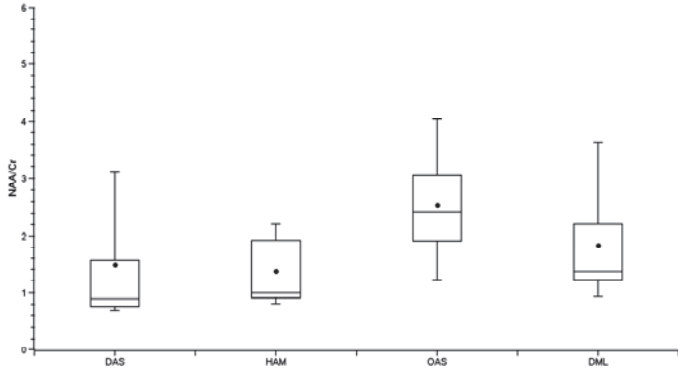


Fig. 3 – Box plot of the N-acetyl aspartate to creatine ratio (NAA/Cr) shows a significant difference between diffuse astrocytomas and hamartomas and between oligoastrocytoma and demyelinating lesions.

DAS – diffuse astrocytomas grade II; HAM – hamartomas; OAS – oligoastrocytomas grade II; DML – demyelinating lesions.

In two cases of anaplastic astrocytoma ratios NAA/Cr were increased compared to the normal values, which can be assigned to contribution of Glx resonances. Therefore, they

Axal T_2 weighted MR imaging of anaplastic astrocytoma, demyelinating lesion, ischemic lesion and hamartoma respectively shown in Figure 4a–d while metabolic ratios of

the same pathologies using proton MR spectroscopy, echo time = 30 ms are shown in Figure 4.

Axal T_2 weighteg MR images of diffuse astrocytoma, hamartoma, oligoastrocytoma and demyelinating lesion are shown in Figure 5 (a–d, respectively), while the same sesions are examined using MR spectrosopy echo time = 30 ms or echo time = 135 ms (Figure 5 – others).

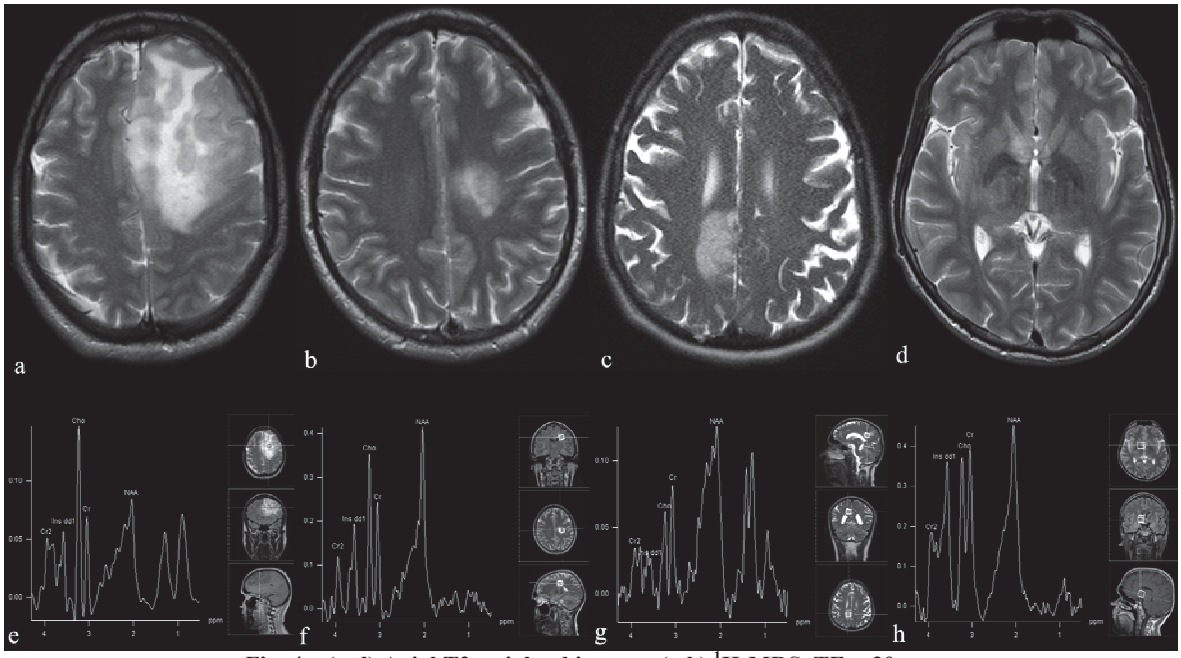


Fig. 4 – (a-d) Axial T2 weighted images; (e-h) ^{1}H-MRS, TE = 30 ms.

(a) High-grade glioma (anaplastic astrocytoma) – left sided, frontal, parasagital, heterogeneous, hyperintense extensive tumor lesion with peritumoral edema and mass efect; (b) Demyelinating lesion – left, parietal, supraventricular focal hyperintense lesion; (c) Ischemic lesion – right sided parietal, parasagital, hyperintense lesion; (d) Hamartoma – right sided, hipothalamic, parasagital, inhomogeneous hyperintense lesion; (e) Anaplastic astrocytoma – markedly increased Cho/Cr levels (2.4) and Cho/NAA, decreased NAA/Cr, prominent lactate peaks; (f) Demyelinating lesion – decreased NAA/Cr ratio, increased mIn, increased Cho/Cr and no presence of lipid and lactate peaks; (g) Ischemic lesion – normal values of Cho/Cr ratio (0.83), prominent lactate peaks, NAA/Cr level cannot be precisely determined because of considerable overlapping with resonances Glx peaks; (h) Hamartoma – moderate reduction of NAA, elevation of mIn and no elevation of Cho levels.

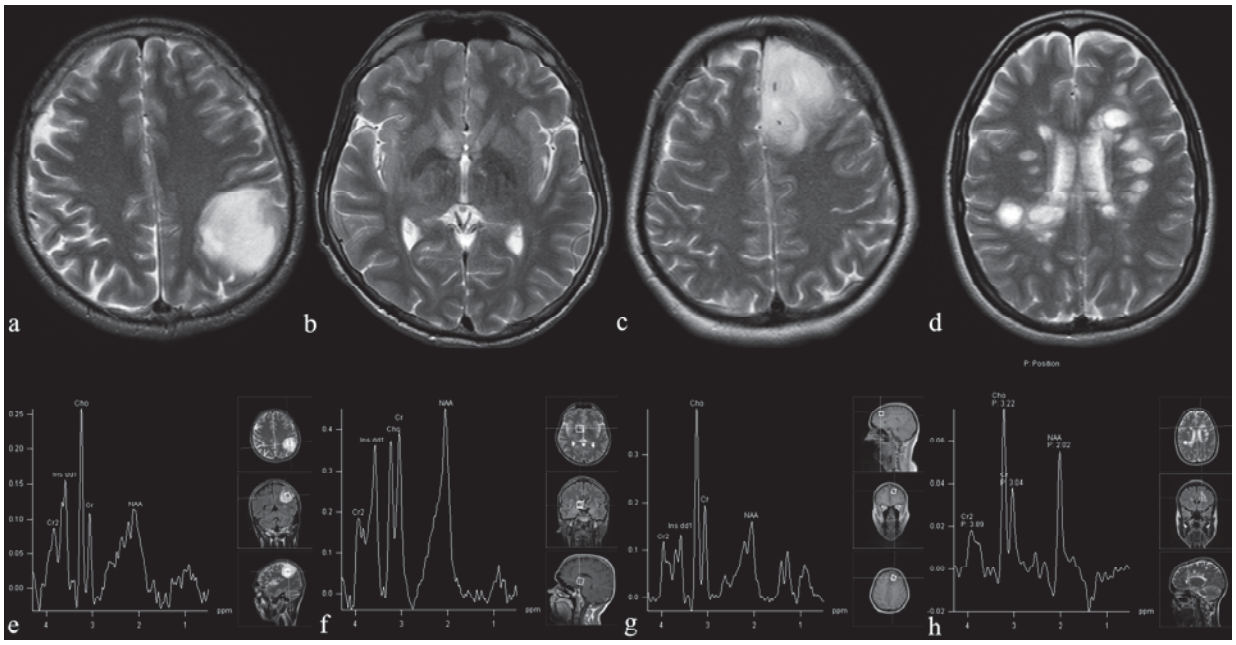


Fig. 5 – a-d) Axial T2 weighted images; (e-f) 1H-MRS, (e-g) TE = 30 ms, (f) TE = 135 ms.

(a) Diffuse astrocytoma – left sided, parietal, cortical and subcortical hyperintense extensive tumor lesion with narrow zone of peritumoral edema; (b) Hamartoma – right sided, hipothalamic, parasagital, inhomogeneous hyperintense lesion; (c) Oligoastrocytoma – left sided, frontal, cortical infiltrative hyperintense lesion with moderate mass effect; (d) Demyelinating lesion – multiple, focal, partly confluent, hyperintense lesions; (e) Diffuse astrocytoma – increased Cho/Cr levels (2.3) and mIn/Cr, decreased NAA/Cr, presence of lactate peaks; (f) Hamartoma – moderate reduction of NAA, elevation of mIn and no elevation of Cho levels; (g) Oligoastrocytoma – prominent decrease of NAA, increase of Cho level, presence of lactate peaks and no elevation of mIn; (h) Demyelinating lesion – reduction of NAA, elevation of Cho levels (1.9) and presence of lactate peaks.

Discussion

Differentiation of non-neoplastic lesions, which look similar to neoplastic, on conventional MR images presents a particular challenge regarding establishing the correct diagnosis and following treatment. Advanced MR imaging techniques, like MR spectroscopy and DWI, which show physiological status of tissue, may contribute to better characterization of those pathologies. When brain lesion is solid, without necrosis, the main diagnosis include beside glial tumors (grade I-III), pseudotumoral demyelinating disease and some ischemic lesions with atypical presentation [7].

Our research showed that the Cho/Cr ratio is higher in glial tumor grade III than in demyelinating lesions and NAA/Cr ratio is lower in oligoastrocytoma (grade II) than in demyelinating lesions (Figure 5c, d, g, h). These results can be explaned by the higher loss of funcional neuronal cells and the larger membrane turnover in glial tumors compared to demyelinating lesions [7]. Cho is a component of the phospholipid metabolism of cell membranes and its increase is related to cell membrane turnover and higher cell density from tumor proliferation [14]. NAA is a neuronal marker and a decrease of NAA levels is caused by replacement of healthy brain tissue by tumor cells [14]. Brain tumor ^{1}H-MR spectroscopy typically shows elevated Cho levels and reduced NAA levels [19]. MR imaging findings of acute demyelinating lesions can mimic glial neoplasms especialy tumefactive demyelinating lesions [2, 20]. They present as T1 hypointense and T2 and FLAIR hyperintense lesions similar to tumors and can show enhancement after administration of contrast agent because of inflammatory BBB breakdown [21]. Acute demyelinating lesions are also caracterized by the increase of Cho levels and decrease of NAA levels [2, 19]. This is due to inflammation, demyelination and intense reactive astrogliosis [21]. Bitsch et al. [22] found that elevated Cho levels correlate with glial proliferation, since Cho is a component of glial cell membranes and that there is a connection between the Cho level, neuronal dysfunction and patient's disability. The decrease of NAA is also common finding in acute demyelinating lesions. Also, Bitsch et al. [22] showed that axonal degeneration and decreased axonal density, characteristic for demyelinating process, are associated with decreased NAA. Majos et al. [7] found that elevated Cho levels and reduced NAA levels are more pronounced in brain tumors than in pseudotumoral demyelinating disease. Therefore, analysis of these metabolites values can help in differentiation between glial neoplasms and acute demyelinating lesions [7]. Our findings are in correlation with former published data.

In our research, we found that Cho/Cr ratio is higher in glial tumor grade III than in ischemic lesions. This is because of more intense cell destruction and glial proliferation in glial tumors grade III than in ischemic lesions. Based only on MR imaging examination, ischemic lesions can less frequently be a diagnostic problem for differentiation from glial neoplasms [2, 9, 18]. On ^{1}H-MR spectroscopy, infarcts typically display with the reduction of NAA level and the elevation of lactate level and a slight increase in choline [23]. In this research we did not observe lactate peaks. Loss of neuronal cells leades to decreas of NAA and increase of Cho level is due to reactive gliosis [23, 24]. The intensity of these metabolite changes reflects the severity of an ischemic process and it is related to the prognosis [24]. Moller-Hartmann et al. [18] found that Cho is a metabolite which can be used for differentiation between ischemic lesions and glial tumors since Cho level observed in glial neoplasm is significantly higher than in an ischemic process. Our findings are in accordance with the previously reported.

By analyzing the spectra obtained in our study we found higher Cho/Cr ratios in anaplastic astrocytomas than in hamartomas and lower NAA/Cr ratio in diffuse astrocytomas than in hamartomas. A lower NAA/Cr ratio in diffuse astrocytomas than in hamartomas could be due to neuronal loss that is more pronounced in glial tumors and higher Cho/Cr ratios in anaplastic astrocytomas than in hamartomas could be explaned by intense tumor glial proliferation in high grade gliomas compared to a glial component within the hamartomas as benign lesions [25]. Hamartomas, on MR imaging, appear as isointense to gray matter on T1 and T2-weighted images, but in more recent studies they have been described as T2 hyperintense lesions [26, 27]. On ^{1}H-MRS, hamartomas present with decrease in NAA/Cr and increase in Cho/Cr and mIn/Cr ratios [26, 27]. Since, NAA is a neuronal marker, its decrease is connected with neuronal loss. Reflecting gliosis is related to the increase in mIn [27]. Most commonly, elevated Cho is associated with high – grade glomas, but this also can be found in benign cerebral pathologies like hamartomas [26]. Cho level increase could be due to increasing glial component within the tumor [26]. Majos et al. [7] and Moller-Hartmann et al. [18] found that addition of spectroscopy to routine MR imaging exam helps in characterization of focal intracranial disease and improves decision making in cases suggestive of brain tumors. Our results are in accordance with data in the literature and suggest that ^{1}H-MRS is useful in evaluation of solid brain masses.

Our research showed a significant difference in ΔADC/ADC ratio between glial tumors grade II and glial tumors grade III. These findings can be due to the fact that high-grade tumors are characterised by the increased cellularity, microvascular proliferation and/or necrosis, that diffusivity of glial tumors is inversely related to the cellularity and that ADC is inversely proportional to the cellular density [28, 29]. Diffusion of a free water molecule in high grade tumors is reduced because of reduction in extracellular space by increased cellularity [30, 31]. The areas with the lowest ADC value suggest the areas with the highest cellular density and the highest tumor malignant potential [12]. Previous studies showed that DWI can be useful in differentiating benign and malignant tumors from normal parenchyma and in grading gliomas [4, 12, 30, 32]. The results of our research correspond with previous findings from the literature.

This study is limited by the small sample size (47 patients). Different tumor types in a group of glial tumors grade II (diffuse astrocytoma, oligoastrocytoma, oligodendroglioma) is another limitation of this research. Pure astrocytic tumor differs from oligoastrocytoma and oligodendroglioma in its therapeutic response to chemotherapy, so their distinction is of great importance [15].

Conclusion

Our study showed the potential use of ^{1}H-MRS and DWI in evaluation of solid brain masses. These noninvasive diagnostic techniques have the advantage over histopathologic assessment of focal brain lesions since they allow *in vivo* examination. ΔADC/ADC, Cho/Cr and NAA/Cr ratio provided additional valuable information on lesion metabolic structure that can help distinguishing brain tumors from non-neoplastic lesions and tumor grading.

Conflict of interest statement

The authors declare no conflict of interest.

REFERENCES

1. *Zonari P, Baraldi P, Crisi G.* Multimodal MRI in the characterization of glial neoplasms: the combined role of single-voxel MR spectroscopy, diffusion imaging and echo-planar perfusion imaging. Neuroradiology 2007; 49(10): 795−803.
2. *Hourani R, Horská A, Albayram S, Brant LJ, Melhem E, Cohen KJ*, et al. Proton magnetic resonance spectroscopic imaging to differentiate between nonneoplastic lesions and brain tumors in children. J Magn Reson Imaging 2006; 23(2): 99−107.
3. *Claes A, Idema AJ, Wesseling P.* Diffuse glioma growth: a guerilla war. Acta Neuropathol 2007; 114(5): 443−58.
4. *Bulakbasi N, Kocaoglu M, Ors F, Tayfun C, Uçöz T.* Combination of single-voxel proton MR spectroscopy and apparent diffusion coefficient calculation in the evaluation of common brain tumors. AJNR Am J Neuroradiol 2003; 24(2): 225−33.
5. *Howe FA, Opstad KS.* 1H MR spectroscopy of brain tumours and masses. NMR Biomed 2003; 16(3): 123−31.
6. *Al-Okaili RN, Krejza J, Woo JH, Wolf RL, O'Rourke DM, Judy KD,* et al. Intraaxial Brain Masses: MR Imaging–based Diagnostic Strategy—Initial Experience. Radiology 2007; 243(29): 539–50.
7. *Majós C, Aguilera C, Alonso J, Julià-Sapé M, Castañer S, Sánchez JJ,* et al. Proton MR spectroscopy improves discrimination between tumor and pseudotumoral lesion in solid brain masses. AJNR Am J Neuroradiol 2009; 30(3): 544−51.
8. *Lin A, Bluml S, Mamelak AN.* Efficacy of proton magnetic resonance spectroscopy in clinical decision making for patients with suspected malignant brain tumors. J Neurooncol 1999; 45(1): 69−81.
9. *Hourani R, Brant LJ, Rizk T, Weingart JD, Barker PB, Horská A.* Can proton MR spectroscopic and perfusion imaging differentiate between neoplastic and nonneoplastic brain lesions in adults? AJNR Am J Neuroradiol 2008; 29(2): 366−72.
10. *Stosić-Opinćal TL, Macvanski MV, Gavrilović SS, Gavrilov MS, Damjanović DS, Vasić BD,* et al. Diffusion and perfusion magnetic resonance imaging in evaluation of primary glial brain tumors. Acta Chir Iugosl 2009; 56(4): 25−30. (Serbian)
11. *Arvinda HR, Kesavadas C, Sarma PS, Thomas B, Radhakrishnan VV, Gupta AK,* et al. Glioma grading: sensitivity, specificity, positive and negative predictive values of diffusion and perfusion imaging. J Neurooncol 2009; 94(1): 87−96.
12. *Sugahara T, Korogi Y, Kochi M, Ikushima I, Shigematu Y, Hirai T,* et al. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. J Magn Reson Imaging 1999; 9(1): 53−60.
13. *Knopp EA, Cha S, Johnson G, Mazumdar A, Golfinos JG, Zagzag D*, et al. Glial Neoplasms: Dynamic Contrast enhanced T2*-weighted MR Imaging. Radiology 1999; 211(3): 791–8.
14. *Nagar VA, Ye J, Xu M, Ng WH, Yeo TT, Ong PL*, et al. Multivoxel MR spectroscopic imaging--distinguishing intracranial tumours from non-neoplastic disease. Ann Acad Med Singapore 2007; 36(5): 309−13.
15. *Spampinato MV, Smith JK, Kwock L, Ewend M, Grimme JD, Camacho DL*, et al. Cerebral blood volume measurements and proton MR spectroscopy in grading of oligodendroglial tumors. AJR Am J Roentgenol 2007; 188(1): 204−12.
16. *Chang SM, Nelson S, Vandenberg S, Cha S, Prados M, Butowski N,* et al. Integration of preoperative anatomic and metabolic physiologic imaging of newly diagnosed glioma. J Neurooncol 2009; 92(3): 401–15.
17. *Hakyemez B, Erdogan C, Ercan I, Ergin N, Uysal S, Atahan S.* High-grade and low-grade gliomas: differentiation by using perfusion MR imaging. Clin Radiol 2005; 60(4): 493−502.
18. *Möller-Hartmann W, Herminghaus S, Krings T, Marquardt G, Lanfermann H, Pilatus,* et al. Clinical application of proton magnetic resonance spectroscopy in the diagnosis of intracranial mass lesions. Neuroradiology 2002; 44(5): 371−81.
19. *Al-Okaili RN, Krejza J, Wang S, Woo JH, Melhem ER.* Advanced MR Imaging Techniques in the Diagnosis of Intraaxial Brain Tumors in Adults. RadioGraphics 2006; 26(Suppl 1): S173−89.
20. *Malhotra HS, Jain KK, Agarwal A, Singh MK, Yadav SK, Husain M,* et al. Characterization of tumefactive demyelinating lesions using MR imaging and in-vivo proton MR spectroscopy. Mult Scler 2009; 15(2): 193−203.
21. *Saindane AM, Cha S, Law M, Xue X, Knopp EA, Zagzag D.* Proton MR spectroscopy of tumefactive demyelinating lesions. AJNR Am J Neuroradiol 2002; 23(8): 1378−86.
22. *Bitsch A, Bruhn H, Vougioukas V, Stringaris A, Lassmann H, Frahm J,* et al. Inflammatory CNS demyelination: histopathologic correlation with in vivo quantitative proton MR spectroscopy. AJNR Am J Neuroradiol 1999; 20(9): 1619−27.
23. *Felber SR, Aichner FT, Sauter R, Gerstenbrand F.* Combined magnetic resonance imaging and proton magnetic resonance spectroscopy of patients with acute stroke. Stroke. 1992 Aug;23(8):1106-10.
24. *Ramin SL, Tognola WA, Spotti AR.* Proton magnetic resonance spectroscopy: clinical applications in patients with brain lesions. Sao Paulo Med J 2003; 121(6): 254−9.
25. *Sharma MS, Suri A, Shah T, Ralte A, Sarkar C, Gupta V,* et al. Intraventricular glioneuronal hamartoma: histopathological correlation with magnetic resonance spectroscopy. J Neurooncol 2005; 74(3): 325−8.
26. *Amstutz DR, Coons SW, Kerrigan JF, Rekate HL, Heiserman JE.* Hypothalamic hamartomas: Correlation of MR imaging and spectroscopic findings with tumor glial content. AJNR Am J Neuroradiol 2006; 27(4): 794−8.
27. *Freeman JL, Coleman LT, Wellard RM, Kean MJ, Rosenfeld JV, Jackson GD,* et al. MR imaging and spectroscopic study of epileptogenic hypothalamic hamartomas: analysis of 72 cases. AJNR Am J Neuroradiol 2004; 25(3): 450−62.
28. *Kleihues P, Louis DN, Wiestler OD, Burger PC,Scheithauer BW.* WHO grading of tumours of the central nervous system In: *Louis DN, Ohgaki H, Wiestler OD, Cavenee WK*, editors. WHO Classification of Tumours of the Central Nervous System. Lyon, France: IARC; 2007; p. 10−1.
29. *Kwee TC, Galbán CJ, Tsien C, Junck L, Sundgren PC, Ivancevic MK,* et al. Comparison of apparent diffusion coefficients and

distributed diffusion coefficients in high-grade gliomas. J Magn Reson Imaging 2010; 31(3): 531−7.

30. *Castillo M, Smith JK, Kwock L, Wilber K.* Apparent diffusion coefficients in the evaluation of high-grade cerebral gliomas. AJNR Am J Neuroradiol 2001; 22(1): 60−4.
31. *Stadnik TW, Chaskis C, Michotte A, Shabana WM, van Rompaey K, Luypaert R,*et al. Diffusion-weighted MR imaging of intracerebral masses: comparison with conventional MR imaging and histologic findings. AJNR Am J Neuroradiol 2001; 22(5): 969−76.
32. *Kono K, Inoue Y, Nakayama K, Shakudo M, Morino M, Ohata K,* et al. The role of diffusion-weighted imaging in patients with brain tumors. AJNR Am J Neuroradiol 2001; 22(6): 1081−8.

Received on February 23, 2011.
Accepted on April 8, 2011.
OnLine-First November, 2012.

ORIGINAL ARTICLE

UDC: 616.31::616.716.1/.4-053.2-071.3
DOI: 10.2298/VSP110224042S

Cephalometric assessment of maxillary length in Serbian children with skeletal class III

Kefalometrijska procena dužine maksile kod srpske dece sa skeletnom klasom III

Zdenka Stojanović*, Predrag Nikolić†, Angelina Nikodijević†, Jasmina Milić‡, Branislav Stojanović§

***Clinic for Dentistry, Military Medical Academy, Belgrade, Serbia; †Clinic for Jaw Orthopedics, School of Dental Medicine, Belgrade, Serbia;, ‡Clinic for Jaw Orthopedics, School of Dental Medicine, Pančevo, Serbia; §Military Health Department, Ministry of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Malocclusion of skeletal class III is a complex irregularity of sagittal inter-jaw relationship, which is due to irregularities of sagittal position of one or both of the jaw bones, which is often associated with disproportionate ratio of their length. The aim of this study was to determine whether the length of the jaw of children with skeletal class III in the period of mixed dentition was changed. **Methods.** Fifty children with skeletal class III and the same number of those with skeletal class I, of both sexes, have been selected on the basis of cephalometric analysis of profile tele-x-ray of the head. All the children aged 6–12 had mixed dentition, and were divided according to sex and age into three subgroups within each group. The length of maxilla, mandible and cranial base were measured. Proportions among the lengths measured within each group were found and difference significance in the measured lengths and their proportions among groups and subgroups were evaluated. **Results.** The children with skeletal class III, compared with the findings in the control group, had significantly lower values of maxillary length, total maxillary length, as well as lower values of their lengths in proportion to lengths of the front or the total length of cranial base and in proportion to mandibular lengths ($p < 0.05$). Among the patients of different sexes, both in the test and the control group, a significant difference in the values of the measured lengths was found. **Conclusion.** The children with skeletal class III have significantly shorter maxilla than those with skeletal class I.

Key words:
cephalometry; maxilla; child; serbia; malocclusion, angle class III.

Apstrakt

Uvod/Cilj. Malokluzija skeletne klase III je kompleksna nepravilnost sagitalnog međuviličnog odnosa, koji nastaje usled nepravilnosti sagitalnog položaja jedne, ili obe vilične kosti, što je često udruženo sa neproporcionalnim odnosom njihovih dužina. Cilj ove studije bio je da se utvrdi da li je kod dece sa skeletnom klasom III u doba mešovite denticije izmenjena dužina gornje vilice. **Metode.** Pedesotoro dece sa skeletnom klasom III i isto toliko sa skeletnom klasom I, oba pola, selekcionisano je na osnovu kefalometrijske analize profilnih telerendgenskih snimaka glave. Sva deca su imala mešovitu denticiju, bila su starosti 6–12 godina i podeljena su prema polu i uzrastu na tri podgrupe u svakoj grupi. Merene su dužine maksile, mandibule i kranijalne baze. Utvrđivane su proporcije između izmerenih dužina unutar svake grupe i procenjivana značajnost razlika izmerenih dužina i njihovih proporcija između grupa i podgrupa. **Rezultati.** Kod dece sa skeletnom klasom III, u poređenju sa nalazom u kontrolnoj grupi, utvrđene su značajno manje vrednosti dužine tela maksile, totalne dužine maksile, kao i manje vrednosti njihovih dužina proporcionalno dužinama prednje, odnosno totalne dužine kranijalne baze i proporcionalno dužinama mandibule ($p < 0,05$). Između ispitanika različitog pola, i u ispitnoj i u kontrolnoj grupi, utvrđena je značajnost razlike za vrednosti merenih dužina. **Zaključak.** Kod dece sa skeletnom klasom III, maksila je značajno kraća nego kod dece sa skeletnom klasom I.

Ključne reči:
kefalometrija; maksila; deca; srbija; malokluzija, anglova klasa III.

Introduction

Malocclusion of skeletal class III is a complex irregularity of sagittal inter-jaw relationship, which is due to irregularities of sagittal position of one or both of the jaw bones. Position irregularity of the jaw bones is often associated with disproportionate ratio of their length. One of the most common components present in the facial morphology

Correspondence to: Zdenka Stojanović, Clinic for Dentistry, Military Medical Academy, Crnotravska 17, 11 000, Belgrade, Serbia. Phone: +381 11 3608 429. E-mail: zdebra@sezampro.rs

of patients was insufficient development of middle face, with consequently lower maxillary length, which is why some authors suggest that the anatomical structure itself was a decisive factor for classifying patients with malocclusion class III [1–5]. During the development of human fetuses, studies show that the central part of facial complex is clearly distinguishable pretty early, in the week 9 of fetal life. The size of premaxilla is an important indicator in the development of mid-facial complex. In humans, at birth, premaxillary region remains recognizable on maxilla, separated from it by premaxillary–maxillary suture and retains the ability of active osteogenesis, which is visible on palate and floor of nose. For these reasons, the size of spine nazalis anterior depends on the time of healing premaxillary–maxillary suture, which can have an impact on the growth of middle face. Nasal septum has an important, direct role in the growth of premaxilla, and thus an indirect role in the growth of maxilla. In accordance with the hypothesis of the septomedial traction in the growth of facial massif (middle face), the development process associated with malocclusion class III, can be associated with cartilaginous growth on septopresfenoidal joint [6]. Nasal capsule and nasal septum affect the forward movement of upper parts of maxilla, the expansion of space the lateral walls of nasal cavity and the development of premaxilla. Many authors are also convinced that vomero-palatine suture is important for anterior-inferior displacement of palatal bone. Traumatic nasomaxillary complex leads to abnormalities in the growth of nasal septum, and surrounding muscle dysfunction may affect the subsequent growth of the facial massif [7]. Accordingly, the growth of nasomaxillary complex is the result of two main mechanisms: passive transfer, which is due to the growth of cranial base, which "pushes" maxilla forward and active growth of maxillary and nasal structures. A growth model requires the face to grow "below the cranium", which means that during growth and development, the jaw must be moved down and forward in relation to cranial base, thanks to the sutures by which it is attached to the cranial base. During this shift, the space to open on sutures is filled with the bone proliferation in these areas. Apposition of bone occurs on both sides of the suture, so that the bone to which maxilla is attached also becomes greater. In addition, the front structures of maxilla are subject to remodeling, so that almost its entire front surface is the resorption area [8]. Although cranial growth can affect the position of maxilla, maxillary growth takes place by translation, rotation and elongation within their skeletal dimensions. So, Marcus et al. [9] believe that maxillary growth in people is expressed in: the anterior translation of maxilla because of moving forward the anterior cranial base; pneumaticizing frontal sinuses and maxillary leaning forward; moving maxilla down; lateral shifting due to appositional growth on medium-palatal suture. Having said thath, the direction of growth of the upper jaw can influence the direction of eruption of upper permanent iucisors, which is an important early correction of their oral inclination [10, 11]. Deviations in the normal development of the maxillary complex can have a significant impact on the development of skeletal class III. This fact has focused our study in children with skeletal class III just on maxilla, as the anatomical structure whose prepubertal development, besides genetic predisposition, may be compromised by an often present adenoid problem and respiratory diseases that make it difficult to breath through the nose, which directly threatens the maxillary growth in children at this age.

Methods

The study used tele-x-ray profile head shots made in its natural position, with lateral teeth in maximum intercuspidity. The recordings are drawn through acetate paper, marking relevant cephalometric points, lines, planes and angles, which are used in the angular and linear measurements. The study included children with mixed dentition, aged 6–12, which were classified into two groups, 50 patients each, both sexes (25 males and 25 females). The first, test group, consisted of children with skeletal class III, selected on the basis of the value of angle ANB $\leq 0°$. The second, control group, consisted of children with skeletal class I, selected on the basis of normal values of angles of sagittal jaw position in relation to the cranial base, SNA = 80°–82°, SNB = 78°–80° and the angle of sagittal interjaw relationship, ANB = 2°–4° (Figure 1). The average age in the first group was 8 years

Fig. 1 – Angular cephalometric measurements for the selection into groups used in the study.

SNA – angle of sagittal maxillary position in relation to the cranial base anterior; SNB – angle of sagittal mandibulary position in relation to the cranial base anterior; ANB – angle of sagittal inter-jaw relationship

and 9 months, in the second group, 9 years and three months. The patients from both groups were divided into subgroups according to age: the subgroup a – children aged 6–7 years and 11 months, subgroup b – children aged 8–9 years and 11 months and subgroup c – children aged 10–11 years and 11 months. Testing did not include the children with congenital anomalies, clefts and anodontia of some teeth. The length of

maxilla, mandible and cranial base were measured (Figure 2). Proportions among the lengths measured within each

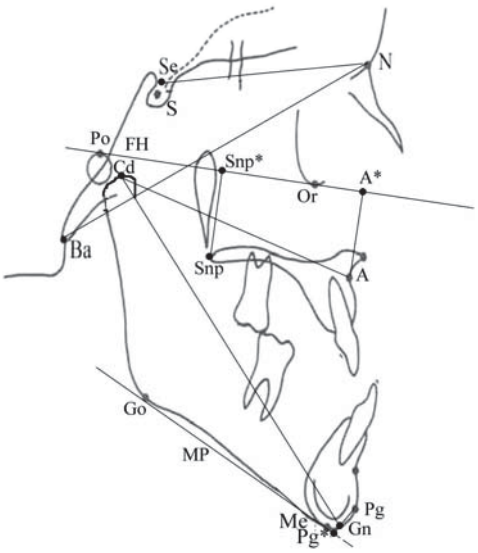


Fig. 2 – Cephalometric landmarks, reference lines and linear measurements for assessing maxillary length used in the study.

S – indicates sella (the center of sella turcica); Se – the center of apertura sella turcica; N – nasion (the most anterior limit of suture nasofrontalis); Ba – basion (the posterior inferior point of the occipital bone at the anterior margin of the foramen magnum); A – subspinale (the most posterior point on the concave anterior border of the maxillary alveolar process); A* – A point of contact of a perpendicular line drawn from point A to the FH; Snp – spina nasalis anterior (the apex of the anterior nasal spine); Snp* – a point of contact of a perpendicular line drawn from point Snp to the FH; Cd – condylion (the most posterior point on the outline of the mandibular condyle); Pg – pogonion (the most anterior point on the mandibular symphysis); Pg* – a point of contact of a perpendicular line drawn from point Pg to the MP; Gn – gnathion (the most anteroinferior point on the mandibular symphysis); Go – gonion (the most outward point on the angle formed by the junction of the ramus and body of the mandible on its posterior. inferior aspect); Me – menton (the lowermost point on the shadow of the mandibular symphysis); Po – porion (the superior aspect of the external auditory meatus); Or – orbite (the lower border of the orbit of the eye); NSe – length of anterior cranial base (Schwarz); NBa – total length of the cranial base;. CdA – total length of the upper jaw; A*Snp* – length of maxilla body; CdGn – total mandibular length; Pg*Go – mandibular body length.

group were found and difference significance in the measured lengths and their proportions among the groups and subgroups were evaluated. The results of measurement in children with skeletal class III were compared to the results of measuring the same parameters in children with skeletal class I, and to determine the difference significance of the obtained values test method Multiple Comparisons, Mann-Whitney and Wilcoxon test were used. Statistical interpretation was accepted on the probability level *$p \leq 0.05$ - significant, **$p \leq 0.01$ - highly significant, NS – $p > 0.05$ - no significant.

Results

Length of anterior cranial base (Nse) (Tables 1, 2)

Measuring the length of anterior cranial base in the group I of the patients, its average length of 72.20 mm was found in the range of values from 64.00 mm to 84.00 mm. In the group II of the patients, its length ranged from 64.00 mm to 82.00 mm, an average of 73.52 mm. The difference in the measured average values in the groups I and II was not statistically significant ($p > 0.05$). Significance of the difference in the values of this parameter in males and females, in the same age subgroups was not found in either of the groups ($p > 0.05$).

Total length of the cranial base (Nba) (Tables 1, 2)

Total length of the cranial base in the group I of the patients ranged from minimum 96.00 mm to a maximum of 121.00 mm, an average of 106.53 mm. Significance of the difference in the measured values in the patients of different sex was found only in the oldest subgroup ($p \leq 0.05$). In the group II of the patients, the measured total lengths of the cranial base ranged from 99.00 mm to 120.00 mm, an average of 109.06 mm. These values were significantly different from those measured in the patients of the group I ($p \leq 0.05$). The average values of the parameter NBa measured in the males and females of the same age subgroups were not significantly different ($p > 0.05$).

Total length of the upper jaw (CdA) (Tables 1, 2)

Total length of the upper jaw in the group I, ranged from minimum 75.00 mm to a maximum of 97.00 mm, an average value was 82.82 mm. The difference in average values of this parameter males and females of the same age subgroups was not significant ($p > 0.05$). In the group II of the patients, the values of total length of the upper jaw were measured, ranging from 75.00 mm to 99.00 mm, an average of 87.46 mm. These values were significantly different from those set forth in the group I ($p \leq 0.05$). The values of this parameter in the patients of different sexes were significantly different only in the oldest subgroup ($p \leq 0.05$).

*Length of maxilla body (A*Snp*) (Tables 1, 2)*

In the patients of the group I the values of the body length of the upper jaw were set in the interval from 39.00 mm to 52.00 mm, an average of 44.44 mm. Significance of the difference in the values of the measured parameter in the patients of different sexes of the same age subgroups was found only in the subgroup of the oldest ($p \leq 0.05$). Body length of the upper jaw in the group II of the patients was 47.52 mm on average, ranging from 41.00 mm to 54.00 mm, which was significantly different from the value of this parameter in the group I ($p \leq 0.05$). There was significant difference in its value in patients of different sexes of middle age subgroup ($p \leq 0.05$).

Total mandibular length (CdGn) (Tables 1, 2)

The average total length of mandible in the group I was 116.52 mm, and the measured values ranged from 105.00 mm to 141.00 mm. A significant difference of average total mandibular lengths in the patients of different sexes was not proven in either of the age groups ($p > 0.05$). In the patients of the group II, the average total mandibular length of 115.76 mm was found with ranging from 105.00 mm to 127.00 mm. These values were not significantly different from those in

Table 1

The parameters measured in the study and the results of the groups comparison

Parameters	Group	Min	Med	Max	$\bar{x} \pm SD$	*p*
A*Snp*	1	39.00	44.50	52.00	44.44 ± 2.98	-3.08*
	2	41.00	47.00	54.00	47.52 ± 2.66	
CdA	1	75.00	82.00	97.00	82.82 ± 4.74	-4.64*
	2	75.00	88.00	99.00	87.46 ± 4.90	
Pg*Go	1	68.00	75.50	95.00	75.86 ± 4.94	-0.12
	2	66.00	76.00	85.00	75.98 ± 4.10	NS
CdGn	1	105.00	116.50	141.00	116.52 ± 7.10	0.76
	2	105.00	115.00	127.00	115.76 ± 5.69	NS
NSe	1	64.00	72.00	84.00	72.20 ± 4.18	-1.32
	2	64.00	73.50	82.00	73.52 ± 3.75	NS
NBa	1	96.00	106.00	121.00	106.53 ± 5.15	0.04*
	2	99.00	109.00	120.00	109.06 ± 4.71	

NS – no significant difference; * – significant difference (Method of multiple comparisons);
A*Snp*/NSe – Proportional relationships of body length of the upper jaw to the anterior cranial base;
A*Snp*/Pg*Go – Proportional relationship of body lenght of the upper jaw to mandibulan body length;
CdA/CdGn – Proportional relationship of the total lenght of the upper jaw to the total length of the lower jaw;
CdA/Nba – Proportional relationship of the total lenght of the upper jaw to the total length of the cranial base;
Min – minimal value; Med – median; Max – maximal value; $\bar{x}$ –mean; SD – standard deviation;
group 1 – children with skeletal class III (test group); group 2 – children with skeletal class I (control group).

Table 2

The parameters measured in the study and the results of the subgroups comparison

Parameters	Group	Subgroup	$\bar{x} \pm SD$		*p*
			M	F	
A*Snp*	1	a	44.67 ± 3.39	44.00 ± 2.19	0.63 (NS)
		b	44.50 ± 2.46	44.83 ± 3.71	0.89 (NS)
		c	45.78 ± 2.05	42.14 ± 3.13	0.03 *
	2	a	45.67 ± 5.03	45.75 ± 2.75	1.00 (NS)
		b	48.18 ± 2.71	46.15 ± 1.82	0.04 *
		c	49.09 ± 2.12	48.25 ± 2.12	0.48 (NS)
CdA	1	a	81.50 ± 4.55	82.00 ± 3.46	0.63 (NS)
		b	84.30 ± 3.86	82.17 ± 3.64	0.25 (NS)
		c	85.67 ± 6.50	80.00 ± 4.97	0.06 (NS)
	2	a	85.00 ± 4.58	85.50 ± 5.07	0.86 (NS)
		b	87.91 ± 5.89	85.23 ± 4.57	0.38 (NS)
		c	91.09 ± 3.96	87.38 ± 2.83	0.03 *
Pg*Go	1	a	74.33 ± 3.45	73.17 ± 3.66	0.81 (NS)
		b	76.70 ± 2.41	74.17 ± 4.39	0.10 (NS)
		c	81.11 ± 7.37	74.43 ± 1.13	0.01 **
	2	a	74.33 ± 2.08	72.50 ± 5.32	0.59 (NS)
		b	75.73 ± 4.78	74.62 ± 4.17	0.73 (NS)
		c	79.18 ± 2.44	76.50 ± 2.27	0.04 *
CdGn	1	a	112.33 ± 4.63	113.50 ± 4.76	0.75 (NS)
		b	119.60 ± 5.25	114.25 ± 7.12	0.09 (NS)
		c	121.11 ± 10.22	116.29 ± 4.07	0.43 (NS)
	2	a	114.33 ± 5.03	113.00 ± 5.10	0.72 (NS)
		b	116.27 ± 5.02	112.23 ± 5.76	0.06 (NS)
		c	121.18 ± 4.47	115.25 ± 3.41	0.01 *
NSe	1	a	73.17 ± 4.02	71.67 ± 2.07	0.63 (NS)
		b	73.90 ± 2.42	71.42 ± 3.80	0.05 *
		c	74.33 ± 6.04	68.00 ± 2.65	0.03 *
	2	a	71.00 ± 3.61	74.00 ± 2.31	0.27 (NS)
		b	73.82 ± 4.31	71.46 ± 3.82	0.21 (NS)
		c	75.91 ± 3.36	73.88 ± 2.23	0.16 (NS)
NBa	1	a	105.33 ± 4.18	103.50 ± 2.59	0.52 (NS)
		b	107.70 ± 2.98	106.67 ± 6.13	0.35 (NS)
		c	111.13 ± 6.36	103.00 ± 2.71	0.02 *
	2	a	108.33 ± 2.08	108.25 ± 4.92	0.86 (NS)
		b	109.18 ± 5.65	106.92 ± 4.66	0.50 (NS)
		c	112.09 ± 3.53	108.88 ± 4.36	0.08 (NS)

NS – no significant difference; * – significant difference; ** (Mann–Whitney and Wilcoxon test)
A*Snp* - length of maxilla body; CdA – total length of the upper jaw; Pg*Go – mandibular body length;
CdGn – total mandibular length; Nse – length of anterior cranial base; Nba – total length of the cranial base; M – male; F – female; $\bar{x}$ – mean; SD – standard deviation; group 1 – children with skeletal clas III (test group); group 2 – children with skeletal class I (control group); a – children aged 6–7 years and 11 months; b – children aged 8–9 years and 11 months; c – children aged 10–11 years.

the group I ($p > 0.05$). A significant difference in the values of this parameter in the patients of different sexes was found in the oldest subgroup ($p \leq 0.01$).

*Mandibular body length (Pg*Go) (Tables 1, 2)*

The patients of the group I had an average mandibular length of 75.86 mm, and the measured values were within the range of 68.00 mm to 95.00 mm. A significant difference of the measured values was found in the oldest subgroup of male and female patients ($p \leq 0.05$). In the patients of the group II, the measured mandibular body lengths ranged from 66.00 mm to 85.00 mm, the average of 75.98 mm. A significant difference was not found in the values of this parameter in the tested groups ($p > 0.05$). A significant difference in the measured values of this parameter in the patients of different sexes was found in the oldest subgroup ($p > 0.05$).

*Proportional relationship of body length of the upper jaw to the anterior cranial base (A*Snp*/Nse) (Tables 3, 4)*

The average value of this proportion in the group I was 0.62, and the measured values ranged from 0.54 to 0.72. In the patients of the group II, its average value of 0.65 was established with the measured values ranging from 0.60 to 0.71. These values were significantly different from those in the group I ($p \leq 0.05$). There was no significant difference in the values of this proportion in the patients of different sexes, or in any age subgroup, test or control group ($p > 0.05$).

*Proportional relationship of body length of the upper jaw to mandibular body length (A*Snp*/Pg*Go) (Tables 3, 4)*

In the patients of the group I, the measured values of this proportional relationship ranged from 0.49 to 0.71, with

Table 3

The proportional relationships measurements and the results of the groups comparison

Proportional relationships	Group	Min	Med	Max	$\bar{x} \pm SD$	*p*
A*Snp*/NSe	1	0.56	0.62	0.72	0.62 ± 0.04	0.03*
	2	0.60	0.65	0.71	0.65 ± 0.03	
A*Snp*/Pg*Go	1	0.49	0.58	0.71	0.59 ± 0.04	0.04*
	2	0.55	0.63	0.68	0.63 ± 0.03	
CdA/CdGn	1	0.65	0.72	0.77	0.71 ± 0.03	0.04*
	2	0.69	0.76	0.80	0.76 ± 0.03	
CdA/NBa	1	0.72	0.77	0.85	0.78 ± 0.03	0.02*
	2	0.72	0.80	0.87	0.80 ± 0.03	

*** significant difference (Method of multiple comparisons) A*Snp* - length of maxilla body; CdA – total length of the upper jaw; Pg*Go – mandibular body length; CdGn – total mandibular length; Nse – Length of anterior cranial base; Min – minimal value; Med – median; Max – maximal value; $\bar{x}$ –mean; SD – standard deviation; group 1 – children with skeletal class III (test group); group 2 – children with skeletal class I (control group).**

Table 4

The proportional relationships measurements and the results of the subgroups comparison

Proportional relationships	Group	Subgroup/years	$\bar{x} \pm SD$		*p*
			M	F	
A*Snp*/NSe	1	a	0.61 ± 0.03	0.62 ± 0.03	0.69 (NS)
		b	0.60 ± 0.04	0.63 ± 0.05	0.27 (NS)
		c	0.62 ± 0.04	0.62 ± 0.04	0.83 (NS)
	2	a	0.64 ± 0.06	0.62 ± 0.02	0.71 (NS)
		b	0.65 ± 0.03	0.65 ± 0.03	0.68 (NS)
		c	0.65 ± 0.03	0.65 ± 0.03	0.71 (NS)
A*Snp*/Pg*Go	1	a	0.60 ± 0.06	0.61 ± 0.06	0.87 (NS)
		b	0.58 ± 0.03	0.61 ± 0.04	0.11 (NS)
		c	0.57 ± 0.04	0.57 ± 0.04	0.56 (NS)
	2	a	0.62 ± 0.06	0.63 ± 0.04	0.86 (NS)
		b	0.64 ± 0.03	0.62 ± 0.03	0.10 (NS)
		c	0.62 ± 0.03	0.63 ± 0.04	0.26 (NS)
CdA/NBa	1	a	0.77 ± 0.03	0.79 ± 0.02	0.20 (NS)
		b	0.78 ± 0.05	0.77 ± 0.02	0.77 (NS)
		c	0.77 ± 0.04	0.78 ± 0.04	0.75 (NS)
	2	a	0.78 ± 0.04	0.79 ± 0.05	0.71 (NS)
		b	0.80 ± 0.04	0.80 ± 0.03	0.95 (NS)
		c	0.81 ± 0.03	0.80 ± 0.03	0.43 (NS)
CdA/CdGn	1	a	0.73 ± 0.03	0.72 ± 0.03	0.63 (NS)
		b	0.71 ± 0.03	0.72 ± 0.02	0.18 (NS)
		c	0.71 ± 0.02	0.69 ± 0.03	0.11 (NS)
	2	a	0.74 ± 0.03	0.76 ± 0.04	0.48 (NS)
		b	0.76 ± 0.03	0.76 ± 0.03	0.68 (NS)
		c	0.76 ± 0.02	0.76 ± 0.02	0.56 (NS)

NS – no significant difference (Mann-Whitney and Wilcoxon test) A*Snp*/Nse – Proportional relationships of body length of the upper jaw to the anterior cranial base; A*Snp*/Pg*Go – Proportional relationships of body length of the upper jaw to the mandibular body length; CdA/Nba – Proportional relationships of the total length of the cranial base; CdA/CdGn – Proportional relationships of body length of the lower jaw M – male; F – female; $\bar{x}$ – mean; SD – standard deviation; group 1 – children with skeletal clas III (test group); group 2 – children with skeletal class I (control group); a – children aged 6–7 years and 11 months; b – children aged 8–9 years and 11 months; c – children aged 10–11 years.

the average of 0.59. The patients of the group II had the average value of the proportion A*Snp*/Pg*Go of 0.63, ranging from 0.55 to 0.68. These values were significantly different from those in the patients of the ($p \leq 0.05$). A significant difference in the average values of the tested proportional relationship was not found in the patients of different sexes within the same age groups, test or control group ($p > 0.05$).

Proportional relationship of the upper – lower jaws total length (CdA/CdGn) (Tables 3, 4)

The set values of the proportional relationship in the group I of patients ranged from 0.65 to 0.77, with the average of 0.71. The results of measuring the proportion CdA/CdGn in the patients of the group II were significantly different from those found in the group I ($p \leq 0.05$). Its values in the group II ranged from 0.69 to 0.80. The average value for the whole group was 0.76. As in the group I, there was no significant difference in the measured values in the patients of different sexes, belonging to the same age subgroups ($p > 0.05$).

Proportional relationship of the total length of the upper jaw to the total length of the cranial base (CdA/Nba) (Tables 3, 4)

By measuring this proportional relationship in the group I of the patients values ranged from 0.72 to 0.85, with the average of 0.78, were found. In the group II of the patients, the same proportion ranged from 0.72 to 0.87, with the average of 0.80, which was significantly different from values in the group I ($p \leq 0.05$). A significant difference in the values of the tested proportional relationship among the members of different sexes, within the same age groups, was not found in either of the groups ($p > 0.05$).

Discussion

Data from the literature indicate that the size and proportion of the maxilla are the major etiological factor during its growth for skeletal class III development. The authors often point out that the maxillary retrognatism is usually masked and cannot be recorded through angular analysis, due to the changed length and angulations of the cranial base, which affect the position of the point nasion, directly responsible for the size of many indicators of sagittal maxillary position (SNA, FHNA, ANV...) [11–13]. Therefore, some authors prefer to use linear measures, which do not represent actual anatomical length of the jaw bones, but a linear distance from the tip of the condylary process to the body limit of the jaw bone and its alveolar processus (total length), or the distance between the farthest anatomical points, or their structures, which limit the bodies of the jaw bones. However, those sizes are very individual and depend on age, sex and physical dimensions of patients, so that their absolute values do not say much. We get much more data when we look at these sizes compared to the total length of the cranial base and the total length of the other jaw bone, thus determining its relative or proportional value. It is accepted that the proportion of length of the upper jaw body should be a 7/10 length of NSe (A*Snp*/NSe = 0.7) and 2/3 length of the lower jaw body (A*Snp*/Pg*Go = 0.67). In the literature, we find conflicting opinions on the issue of finding the total length and body length of the maxilla in patients with skeletal class III during growth. Thus, the results of maxilla length in children during primary dentition show that there is a highly significant difference in its size in children with skeletal class III, where its body is shorter, compared to children with skeletal class I [14]. The results of measurements of maxilla total length show that it is shorter in patients with skeletal class III than in those with skeletal class I, in all age groups from 6 to 16, but that the difference in its length is not statistically significant [15]. Neither the results of maxilla total length measurements in a study performed in Korean children with primary dentition indicate significant differences between the measured lengths in children with skeletal class I and those with skeletal class III [16]. The research conducted on adult patients with skeletal class III found the existence of significantly lower total maxillary length, compared to patients with skeletal class I [17]. In our study, the group with skeletal class III showed significantly lower values of the body length and total maxillary length, both their absolute values (A*Snp*, CdA), and relative values in relation to the cranial base (A*Snp*/NSe, CdA/NBa) and the mandibular length (A*Snp*/Pg*Go, CdA/CdGn) as compared to children with skeletal class I. Proportional relationships CdA/NBa, A*Snp*/NSe are, apart from a reduced maxillary length, additionally distorted by significantly lower total length of the cranial base, and the length of the anterior cranial base, which is lower in the group with skeletal class III, although the difference compared to the results of the control group is not significant. Irregularities of the tested proportions, A*Snp*/Pg*Go, CdA/CdGn can be considered to be the sole consequence of significantly lower maxillary lengths, since mandibular lengths were not significantly changed in children with skeletal class III, also shown by the results of our previous studies [18]. The degree of proportion irregularities of total maxillary and mandibular lengths is considered to be an important factor in predicting effectiveness of the therapy, so if it is very high, it is a bad prognostic sign [19]. Therefore, the application of orthodontic and orthopedic appliances to encourage sagittal maxillary growth in the period of mixed dentition is imposed as an imperative. In patients of different sex within the same age subgroups, significant differences of the values of some tested parameters were found, both in the group with skeletal class III and in the group with skeletal class I. It is interesting that a significant difference in the values of absolute and not proportional jaw lengths was found. In children with skeletal class I, the total lengths of both jawbones and mandibular body length were significantly higher in male patients in the oldest, and maxillary body length in the middle age subgroup. In children with skeletal class III, the body length of both jawbones were significantly higher in male patients in the oldest subgroup, while a significant difference in their total lengths was not found in either of the age subgroups. Data on the value of total maxillary length, measured in a longitudinal study on growth in children with normal occlu-

sion at the age of 6, 9, 12, 14, 16 and 18, indicate its greater length in male patients of all age groups, but also that the difference in length becomes significant only from the age of 14, when in female patients the growth in the length stagnates, and in male patients it continues after the tested period [20]. Similar information is also found in the longitudinal study of growth in untreated patients with skeletal class III, of the same age interval, where significantly higher total length of maxilla in the male patients can be found only at the age of 13 and over [21]. A significant difference in maxillary length among patients of different sexes also depends on ethnicity. The results of comparative studies in Chinese and Caucasian patients with normal occlusion show that in both ethnic groups in female patients a significantly lower total maxillary length and significantly greater difference between the total jaw lengths than in male patients were found [22]. In the oldest subgroups of the test and control groups of children, significantly higher mandibular body length in male patients was found. In children with skeletal class III, difference significance of the measured mandibular total lengths among patients of different sexes within the same age subgroups was not found while in the group with skeletal class I, in the oldest subgroup, a significantly higher mandibular total length in male patients was found. A significant difference of the measured average lengths of the cranial base in patients of different sexes was found only in the group with skeletal class III, for the values of anterior cranial base in the middle and oldest groups, and for the values of total length of the cranial base, in the oldest subgroup, where male patients had significantly higher length of these parameters. These findings clearly indicate the existence of sexual dimorphism in terms of linear lengths of various anatomical structures of the craniofacial complex. In the same age subgroups, in children with skeletal classes I and III, there was a significant difference in linear values of maxillary but not mandibulary lengths. This finding could be explained by the fact that mandibular growth, usually longer than the length of maxillary growth, lasts longer in patients with skeletal class III, since studies show that the pubertal peak of growth in them is also significantly longer, for about five months [23]. Therefore, a significant difference of mandibular lengths could be expected only before the end of the pubertal peak, when the results of application of functional orthodontic and orthopedic appliances for modification of the growth type are already very limited. For these reasons, the estimated lower maxillary lengths, could be considered an early indicator of development of skeletal class III and important signal for the start of its timely treatment.

Conclusion

The results obtained in the study show that children with skeletal class III have significantly lower maxillary length than children with skeletal class I. Children with skeletal class III, compared to children with skeletal class I, have a lower absolute maxillary body length, lower total maxillary length, lower maxillary body length proportional to the lengths of anterior cranial base and mandibular body, and lower total maxillary length proportional to the total lengths of the cranial base and mandible. Comparing the results of measuring the proportional maxillary lengths in patients of different sex, in the same age subgroups, a significant difference was not found, while significantly higher lengths in male patients were found for the absolute values of the measured parameters.

REFERENCES

1. *Dibbets JM*. Morphological associations between the Angle classes. Eur J Orthod 1996; 18(2): 111−8.
2. *Singh GD, McNamara JA Jr, Lozanoff S*. Localisation of deformations of the midfacial complex in subjects with class III malocclusions employing thin-plate spline analysis. J Anat 1997; 191(Pt 4): 595−602.
3. *Singh GD, McNamara JA Jr, Lozanoff S*. Finite element morphometry of the midfacial complex in subjects with Angle's Class III malocclusions. J Craniofac Genet Dev Biol 1997; 17(3): 112−20.
4. *Singh GD, McNamara JA Jr, Lozanoff S*. Morphometry of the midfacial complex in subjects with class III malocclusions: Procrustes, Euclidean, and cephalometric analyses. Clin Anat 1998; 11(3): 162−70.
5. *Park JU, Baik SH*. Classification of Angle Class III malocclusion and its treatment modalities. Int J Adult Orthodon Orthognath Surg 2001; 16(1): 19−29.
6. *Moss-Salentijn L*. Melvin L. Moss and the functional matrix. J Dent Res 1997; 76(12): 1814−7.
7. *Singh GD*. Morphologic determinants in the etiology of class III malocclusions: a review. Clin Anat 1999; 12(5): 382−405.
8. *Proffit W*. Contemporary orthodontics. 2nd ed. St. Louis: CV Mosby Co; 1993.
9. *Marcus AF, Corti M, Loy A, Naylor GJP, Slice DE*. Advances in morphometrics. New York: Plenum Press; 1996.
10. *Ostyn JM, Maltha JC, van 't Hof MA, van der Linden FP*. The role of interdigitation in sagittal growth of the maxillomandibular complex in Macaca fascicularis. Am J Orthod Dentofacial Orthop 1996; 109(1): 71−8.
11. *Tollaro I, Baccetti T, Franchi L*. Craniofacial changes induced by early functional treatment of Class III malocclusion. Am J Orthod Dentofacial Orthop 1996; 109(3): 310−8.
12. *Mouakeh M*. Cephalometric evaluation of craniofacial pattern of Syrian children with Class III malocclusion. Am J Orthod Dentofacial Orthop 2001; 119(6): 640−9.
13. *Stojanović ZM, Milić J, Nikolić P*. Radiographic cephalometry assessment of the linear and angular parameters on cranial base in children with skeletal class III. Vojnosanit Pregl 2007; 64(9): 604−10. (Serbian)
14. *Chang HP, Kinoshita Z, Kawamoto T*. Craniofacial pattern of Class III deciduous dentition. Angle Orthod 1992; 62(2): 139−44.
15. *Reyes BC, Baccetti T, McNamara JA Jr*. An estimate of craniofacial growth in Class III malocclusion. Angle Orthod 2006; 76(4): 577−84.
16. *Choi HJ, Kim JY, Yoo SE, Kwon JH, Park K*. Cephalometric characteristics of Korean children with Class III malocclusion in the deciduous dentition. Angle Orthod 2010; 80(1): 86−90.
17. *Ramezanzadeh B, Pousti M, Bagheri M*. Cephalometric Evaluation of Dentofacial Features of Class III Malocclusion in

Adults of Mashhad. Iran J Dent. Res Dent Clin Dent Prospects 2007; 1(3): 125−130.

18. *Stojanović Z, Nikodijević A, Udovicić B, Milić J, Nikolić P.* Size of lower jaw as an early indicator of skeletal class III development. Vojnosanit Pregl 2008; 65(8): 589−95. (Serbian)
19. *Zentner A, Doll GM, Peylo SM.* Morphological parameters as predictors of successful correction of Class III malocclusion. Eur J Orthod 2001; 23(4): 383−92.
20. *Ursi WJ, Trotman CA, McNamara JA Jr, Behrents RG.* Sexual dimorphism in normal craniofacial growth. Angle Orthod 1993; 63(1): 47−56.
21. *Baccetti T, Reyes BC, McNamara Jr JA.* Gender Differences in Class III Malocclusion, Angle Orthod 2004; 75(4): 510−20.
22. *Wu J, Hägg U, Rabie AB.* Chinese norms of McNamara's cephalometric analysis. Angle Orthod 2007; 77(1): 12−20.
23. *Kuc-Michalska M, Baccetti T.* Duration of the pubertal peak in skeletal Class I and Class III subjects. Angle Orthod 2010; 80(1): 54−7.

Received on Februar 24, 2011.
Revised on December 31, 2011.
Accepted on February 7, 2012.
OnLine-First November, 2012.

ORIGINAL ARTICLE

UDC: 616.314-76/-77
DOI: 10.2298/VSP1307653S

The significance of biometric parameters in determining anterior teeth width

Značaj biometrijskih parametara za određivanje širine prednjih zuba

Ljiljana Strajnić, Ivana Vuletić, Predrag Vučinić

Clinic for Dentistry of Vojvodina, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

Abstract

Background/Aim. An important element of prosthetic treatment of edentulous patients is selecting the size of anterior artificial teeth that will restore the natural harmony of one's dentolabial structure as well as the whole face. The main objective of this study was to determine the correlation between the inner canthal distance (ICD) and interalar width (IAW) on one side and the width of both central incisors (CIW), the width of central and lateral incisors (CLIW), the width of anterior teeth (ATW), the width between the canine cusps (CCW), which may be useful in clinical practice. **Methods.** A total of 89 subjects comprising 23 male and 66 female were studied. Their age ranged from 19 to 34 years with the mean of 25 years. Only the subjects with the preserved natural dentition were included in the sample. All facial and intraoral tooth measurements were made with a Boley Gauge (Buffalo Dental Manufacturing Co., Brooklyn NY, USA) having a resolution of 0.1mm. **Results.** A moderate correlation was established between the interalar width and combined width of anterior teeth and canine cusp width ($r = 0.439$, $r = 0.374$). A low correlation was established between the inner canthal distance and the width of anterior teeth and canine cusp width ($r = 0.335$, $r = 0.303$). The differences between the two genders were highly significant for all the parameters ($p < 0.01$). The measured facial distances and width of anterior teeth were higher in men than in women. **Conclusion.** The results of this study suggest that the examined interalar width and inner canthal distance cannot be considered reliable guidelines in the selection of artificial upper anterior teeth. However, they may be used as a useful additional factor combined with other methods for objective tooth selection. The final decision should be made while working on dentures fitting models with the patient's consent.

Key words:
jaw, edentulous; dental prosthesis; anthropometry; esthetics, dental; anatomy.

Apstrakt

Uvod/Cilj. Važan element protetske terapije bezubih pacijenata je odabir veličine prednjih veštačkih zuba koji će povratiti prirodnu harmoniju dentolabijalnih struktura kao i harmoniju čitavog lica. Osnovni cilj ovog istraživanja bio je da se utvrdi korelacija između interkantalnog rastojanja (IKTR) i interalarnog rastojanja (IAR) sa širinom oba centralna sekutića (ŠCS), širinom centralnih i lateralnih sekutića (ŠCLS), širinom prednjih zuba (ŠPZ), širinom između kvržica očnjaka (ŠKO) koji bi mogli biti korisni u kliničkoj praksi. **Metode.** Istraživanje je sprovedeno na 89 osoba sa očuvanom prirodnom denticijom, prosečne starosti od 25 godina (19–34 godina). Bilo je 23 pacijenata muškog pola i 66 pacijenata ženskog pola. Sva merenja na licu i intraoralno na zubima izvršena su korišćenjem Bolejevog merača (Buffalo Dental Manufecturing Co, Brooklyn NY, USA) sa preciznošću od 0,1 mm. **Rezultati.** Utvrđena je umerena korelacija između interalarnog rastojanja i širine prednjih zuba i širine kvržice očnjaka ($r = 0{,}439$; $r = 0{,}374$). Utvrđena je niska korelacija između interkantalnog rastojanja i širine frontalnih zuba i širine kvržice očnjaka ($r = 0{,}335$; $r = 0{,}303$). Utvrđena je značajna razlika za sve parametre među polovima ($p < 0{,}01$). Merena facijalna rastojanja i širina prednjih zuba veće su kod muškaraca nego kod žena. **Zaključak.** Rezultati ove studije pokazuju da ispitivano interalarno i interkantalno rastojanje ne mogu biti pouzdani vodiči za selekciju prednjih gornjih veštačkih zuba. Ipak, ona se mogu koristiti u kombinaciji sa ostalim metodama za objektivnu selekciju veštačkih zuba, a konačna odluka, svakako, treba da se donese nakon probe modela proteza uz saglasnost pacijenta.

Ključne reči:
bezubost; zubna proteza; antropometrija; zub, estetika; anatomija.

Correspondence to: Ljiljana Strajnić, Clinic for Dentistry of Vojvodina, Faculty of Medicine, Hajduk Veljkova 12, 21 000 Novi Sad, Serbia. Phone.: +381 64 132 5075. E-mail: ljstrajnic@neobee.net

Introduction

Loss of teeth, the anterior teeth in particular, leads to degradation of one's physical appearance and a esthetic qualities which can create an inferiority complex with all its concequences, often resulting in psychological trauma.

An important element of prosthetic treatment of edentulous patients is determination of size, shape and color of artificial anterior teeth that will restore the natural dentolabial harmony, as well as the dentofacial structure disturbed by teeth loss. The obligation and responsability are great for every doctor when restoring their patient's disturbed appearance. Smile design has long been considered a doctor's individual subjective skill.

It is a fact that in everyday practice various methods and indicators are used when determining the size of artificial anterior teeth for edentulous patients. Making the right choice is extremely important for both functional and physiognomic rehabilitation of these patients. It is therefore necessary to establish parameters that are as objective as possible in order to achieve optimal occlusion reconstruction in prosthetic treatment of edentulous patients.

Physiognomic prostethics develops one's ability of observing space and one's sense of plastic restitution, necessary for all clinical and technical work. According to physiognomic standards, the visibility of anterior teeth is determined by their correlation with the upper and the lower lip. When talking and smiling, the visible row of anterior teeth represents an individually different, wider or more narrow, horizontal stripe accentuated by its light colour. An especially prominent and specific detail of this stripe is its lower edge which represents the "incisive line". The shape of this line and its specific setting are of great importance for facial expression, as much as the shape of one's eyebrows or the hairline. The position of the "incisive line" in relation to the upper lip determines the visibility of the upper anterior teeth. When considering artificial teeth and the correlation between denture and physiognomy, most authors identify the idea of beauty with natural, non-intrusive, harmonious appearance and the position of artificial teeth in comparison with the entire face [1, 2].

Defining the ideal teeth size is a difficult task considering the vast variety and individuality of features. In order to obtain the "magic numbers" clinical practitioners may apply, mathematical theoremas were proposed, like the "golden ratio" based on elements of classic architecture and art. However, the first doctor who applied this formula to anterior teeth, Lombardi, discovered that it was too rigid for stomatology. Preston's [3] measurements confirm the unsustainability of the formula in this particular case. Numerous reports show that most beautiful smiles are not in correlation with the proportion of the golden ratio [4–6].

Patient's morphological and constitution type, gender, age and individuality should be respected when considering the harmony of shapes, colour and size of every artificial tooth. The shape and size of anterior teeth need to be harmonized with the individual face type, especially their position and visibility while talking and smiling. They represent the elements that, in the hands of a skillful prosthodontist can conjure up the patient's natural appearence, individual face expression and those tiny effects that are so specific and precious to all of us.

Namely, there is little scientific data in dental literature that could be used as objective guidelines for defining the appropriate size and shape of artificial anterior teeth and their interrelationships. In addition, the selection of width is a bigger problem than the selection of teeth length, especially in edentulous patients, when data on preextractional measurements of natural teeth are not available.

By comparatively analyzing the width of upper anterior natural and artificial teeth in complete denture wearers, Baer and Reynolds [7] conclude in their research that people prefer their artificial teeth's width to be less than their natural teeth's width. They also find out that the difference in width of anterior teeth between men and women is 2 mm.

Authors of many recent studies suggest observing people's facial measurements in order to obtain objective guidelines for anterior teeth width selection, and measuring distances between certain reference points of the face [5, 8–22]. These points are, as a rule, easily located, although their exact position is often defined differently by different authors. Some use digital photography and photogrammetry in their research to accurately measure distances between facial landmarks and compare them with the width of anterior teeth [5, 14, 16, 17, 20].

The use of biometric guidelines represents a way of matching the width of anterior teeth in complete dentures as closely as possible to the original. In doing so, anthropometric parameters obtained from one's own population undoubtedly play a significant role.

It is not pointless to state how bionorms based on foreign populations can be applied to our population only for general assessement but that for more delicate analysis we must use data derived from our own population.

Studies on anthropometric facial charcateristics and the jaw complex, as well as studies on their interrelations with natural teeth have given us knowledge of their mutual individual harmony. A great number of conducted studies on the human face prove the existence of significant variations in parameters among different races, nations and populations, as well as among individuals. One of the basic characteristics and laws of nature is the existence of an immense number of variations and intermediate forms, not uniformity or existence of a universal mold. Although all human faces are very similar, no two are the same.

There are several proposed anatomical parameters that would, in careful comparison with the widths of artificial teeth, lead to their correct selection. These anatomical parameters are: bizygomatic width (BZW) [5, 10, 11], interpupillary distance (IPD) [5, 8, 10, 11, 16], intercommissural distance (ICMD) [19, 22], interalar width (IAW) [5, 8, 11, 14, 16, 19–22], which is defined as the distance between the widest points of the ala of the nose, inner canthal distance (ICD) [9, 10, 12–17, 22] which is defined as the distance between the medial angles of the palpebral fissures, width of the upper lip philtrum (PHULW) [9] and nose length (NL) [20–22].

This study was carried out to determine correlations and relationships between ICD and IAW with the mesiodistal width of upper anterior teeth, which may be useful in clinical practice. In relation with the main goal of this research, tasks are set on a representative sample to determine the average of inner canthal distance, interalar width, mesiodistal width of central incisors, mesiodistal width of the central and lateral incisors, mesiodistal width of six maxillary anterior teeth, and to conclude the significance of differences in the tested parameters between the genders, to compare the determined values of the mesiodistal tooth width with the measured facial distances.

Methods

A total of 89 Serbian adults, 23 males and 66 females, between the age of 19 and 34 (the average age of 25) with no facial or dental deformity were selected. Subjects included dental students of the Faculty of Medicine, as well as regular patients of the Clinic for Dentistry of Vojvodina in Novi Sad. The selection criteria included: being part of the Serbian population of Vojvodina and age in which the craniofacial growth and development and tooth growth are already completed. All the subjects had a full complement of teeth with no history of orthodontic or prosthetic treatment, morphological deformity or any form of major conservative restoration, abrasion or attrition, diastema, postoperative periodontal treatment, signs of inflammation, hypertrophy or gingival recession, congenital or surgical defects of the face.

All measurements were made with a Boley Gauge (Buffalo Dental Manufacturing Co., Brooklyn NY, USA) having a resolution of 0.1 mm. Each parameter was measured three times and the average value was taken into account. All measurements were taken by one person. The subjects were seated with their heads in an upright position and looking straight ahead. While nose width measurements were taken, the subjects were instructed to inhale and exhale deeply and briefly stop breathing, as to avoid measuring the ala of the nose widespread.

The following face measurements were taken: ICD, measured between the medial angles of the palpebral fissures and IAW, measured between the widest points of the ala of the nose (Figure 1).

Teeth measurements included: the width between the two proximal contact points for a given distance, the width of both central incisors (CIW) – the distance between proximal contacts toward lateral incisors, the width of the central and lateral incisors (CLIW) – the distance between contact points of the lateral incisor and canine teeth, the width of anterior teeth (ATW) – the distance between contact points for canines and first premolars, the width between the canine cusp (CCW) – the distance between canine cusp (Figure 2).

Statistical analysis of the obtained data was performed by using computer programs Microsoft Excel 2000 and "SPSS 8.0 for Windows". For each of the studied parameters following values were calculated: minimum value (min) and maximum value (max), mean value ($\bar{x}$), standard deviation (SD), standard error (SE), coefficient of variation (CV%) and confidence interval (CI) representing the extent of those features that were found in 95% of cases within the selected sample.

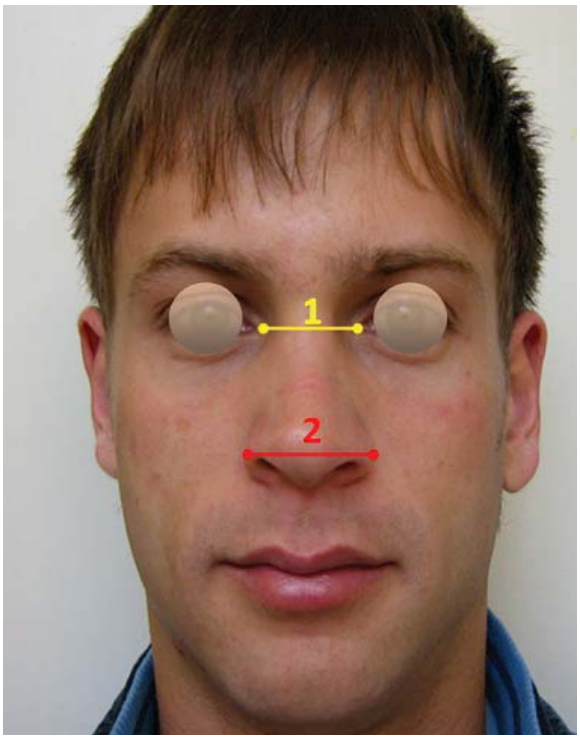


Fig. 1 – Inner canthal distance (ICD) – 1, Interalar width (IAW) – 2.

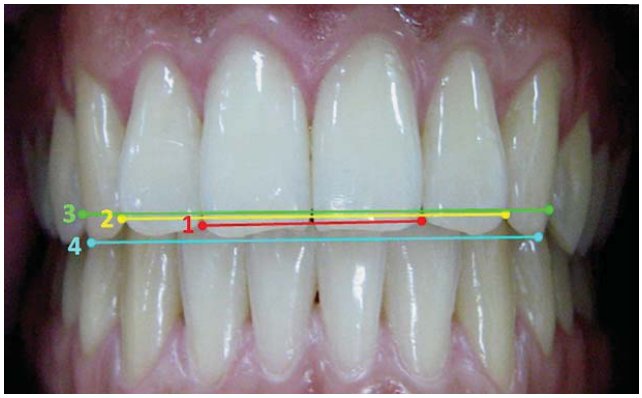


Fig. 2 – Both central incisors width (CIW) – 1; Central and lateral incisor width (CLIW) – 2; Anterior teeth width (ATW) – 3; Canine cusps width (CCW) – 4.

The interconnection between the measured parameters was determined by linear correlation analysis (Pearson's correlation analysis) and summarized numerically with the linear correlation coefficient (*r*).

In this study a statistical significance was determined by using the *t*-test (a test performed on two samples assuming unequal variance – heteroscedastic two-way test – used to asses whether the means of the two groups are statistically different from each other).

Results

CIW for women ranged from 14.5 mm to 18 mm ($\bar{x}$ = 16.3 mm). In females, the width of both central incisor ranged from 15.91 mm to 16.69 mm in 95% of cases. CLIW in females ranged from 25 mm to 32 mm in 95% of cases ($\bar{x}$ = 27.9 mm). ATW in females ranged from 34 mm to 43.5 mm ($\bar{x}$ = 37.1 mm). In females, ATW ranged 35.93 mm to 38.27 mm in 95% of cases. In females CCW ranged from 28.5 mm to 37 mm ($\bar{x}$ = 32.1 mm), and the distance between canine cusps ranged from 30.93 mm to 33.27 mm in 95% of cases. IAW in females ranged from 26 mm to 37 mm ($\bar{x}$ = 31.49 mm). In females, the interalar width ranged from 30.51 mm to 32.47 mm in 95% of cases. ICD in females ranged from 24 mm to 38 mm ($\bar{x}$ = 30.47 mm). The inner canthal distance in 95% of cases in females ranged from 29.49 mm to 31.45 mm. All the results are showed in Table 1.

The width of both central incisors in males ranged from 15 mm to 19 mm ($\bar{x}$ = 17 mm). In males, the width of both central incisor ranged from 15.6 mm to 18.37 mm in 95% of cases. CLIW in males ranged from 25.5 mm to 31 mm ($\bar{x}$ = 28.8 mm). In males, the width of central and lateral incisor ranged from 27.42 mm to 30.17 mm in 95% of cases. ATW in males ranged from 35 mm to 42 mm ($\bar{x}$ = 38.8 mm). In males, ATW ranged from 35.46 mm to 42.13 mm in 95% of cases. The distance between canines cusps ranged from 31 mm to 38 mm ($\bar{x}$ = 33.7 mm) in males (the width between canines cusps ranged from 30.76 mm to 36.64 mm in 95% of cases). IAW in males ranged from 31 mm to 40 mm ($\bar{x}$ = 35.5 mm). The interalar width in males ranged from 32.37 mm to 38.63 mm in 95% of cases. The inner canthal distance ranged from 24 mm to 35 mm ($\bar{x}$ = 32 mm) in males (the inner canthal distance ranged from 29.06 mm to 34.94 mm in 95% of cases). The results are showed in Table 1.

T-test analysis established a statistically significant difference in the values of the measured parameters between the male and female subjects. By testing the statistical significance between genders a significant difference was established for the witdth of both central incisors (CIW, p = 0.003). A statistically significant difference between genders was also established for the parameter of width of central and lateral incisors (CLIW, p = 0.020). The tested difference between genders was highly significant for the parameters of ATW (p = 0.0002). A significant diference between genders was established for the parameter of distance between canines cusps (CCW, p = 0.001). A highly significant difference between genders was established for the parameter of interalar width (IAW, p = 0.000). A significant difference between genders was also established for parameter of inner canthal distance (ICD, p = 0.01). The results are showed in Table 1.

The investigated parametral interconnection was analyzed by linear correlative analyses (Pearson's analysis) and numerically presented by the linear correlation coefficient (r) in Table 2. Linear correlative analysis resulted in a moderate correlation between interalar width and anterior teeth width

Table 1

The results of the investigated parameters in females and males

Parameter	Female			Male			p (t-test)
	$\bar{x}$ ± SD (min – max)	CV (%)	95% CI	$\bar{x}$ ± SD (min – max)	CV (%)	95% CI	
CIW (mm)	16.3 ± 0.225 (14.5–18)	13.8	15.91–16.69	17 ± 0.372 (15–19)	21.88	15.62–18.37	0.003
CLIW (mm)	27.9 ± 0.379 (25–32)	13.58	27.11–28.6	28.8 ± 0.63 (25.5–31)	21.87	27.42–30.172	0.02
ATW (mm)	37.1 ± 0.49 (34–43.5)	13.2	35.93–38.27	38.8 ± 0.84 (35–42)	21.64	35.46–42.13	0.0002
CCW (mm)	32.1 ± 0.47 (28.5–37)	14.92	30.93–33.27	33.7 ± 0.75 (31–38)	22.25	30.76–36.64	0.001
AIW (mm)	31.49 ± 0.452 (26–37)	14.35	30.51–32.47	35.5 ± 0.78 (31–40)	21.97	32.37–38.63	0.000
ICD (mm)	30.47 ± 0.449 (24–38)	14.73	29.49–31.45	32 ± 0.74 (24–35)	23.12	29.06–34.94	0.01

CIW – central incisors width; CLIW – central and lateral incisors width; ATW – anterior teath width; CCW – canine cusps width; IAW – interalar width; ICD – inner canthal distance; min – minimal value; max – maximal value; $\bar{x}$ – mean value; SD – standard deviation; CV– coefficient of variation; CI – confidence interval.

Table 2

Correlation coefficients between the investigated parameters

Parameter	CIW	CLIW	ATW	CCW	AIW	ICD
CIW	1	0.749	0.605	0.514	0.122	0.134
CLIW	0.749	1	0.738	0.690	0.235	0.232
ATW	0.605	0.738	1	0.787	0.439	0.335
CCW	0.514	0.690	0.787	1	0.374	0.303
AIW	0.122	0.235	0.439	0.374	1	0.410
ICD	0.134	0.232	0.335	0.303	0.410	1

CIW – central incisors width; CLIW – central and lateral incisors width; ATW –anterior teath width; CCW – canine cusps width; IAW – interalar width; ICD – inner canthal distance.

and canine cusps width (r = 0.439 and r = 0.374, respectively). A low level of correlation between inner canthal distance and anterior teeth width and canine cusps was established (r = 0.335 and r = 0.303, respectively).

Discussion

In case of absence of preextraction records, selection of upper anterior artificial teeth for edentulous patients is difficult. A very important aspect in the upper anterior teeth selection for complete dentures is selecting the appropriate mesiodistal width of the six maxillary anterior teeth.

According to professional sources, a scientific and universally accepted method for accurately determining the mesiodistal width of anterior artificial teeth has not yet been found [22].

Discussions on this topic are very present in the contemporary professional literature [2, 7, 8, 11–22]. Reviews of the recent scientific literature reveal studies that were carried out using different methodology and sample size, different face and natural teeth parameters [8, 10, 11–13, 15, 19, 20], casts [5, 16–18, 21, 22], or photographs [5, 14, 16, 17, 20], as well as various types of gauges which makes the comparison of results very difficult. Various face parameters such as bizygomatic [5, 10, 11], interpupillar [5, 8, 10, 11, 16], interalar [5, 8, 11, 14, 16, 19–22] and inner canthal distance [9, 10, 12–17, 22], intercommissural distance [19, 22], width of the upper lip filtrum [9] and nose lengt [20–22] have all been proposed as objective guidelines for solving this problem. In the most recent scientific literature, there are different views about the true value of these methods [5–22].

No theory is goad enough to help to select the size of artificial teeth, except when extracted natural teeth or casts of existe [19].

This study attempts to present the latest views and research in this area and investigate the possibility of using individual biological parameters in prosthetic diagnosis and treatment of our population.

This research was carried out as an attempt to better understand and analyze biometric parameters of our population. Until now there have been no similar studies conducted on our population.

The purpose of this research was to establish weather the width of upper anterior teeth is in correlation with the interalar width and the distance between the medial angle of the palpebral fissure on a representative sample of our own population, as well as to determine interrelations between these parameters that could be useful in clinical treatment of our population.

By conducting statistical analysis of Arab population, al-el Sheikh and al-Athel [8] found a significant correlation between the interalar width (♂*$\bar{x}$ = 35.54 mm, ♀† $\bar{x}$ = 31.60 mm) and the combined width of anterior teeth (♂ $\bar{x}$ = 54.87 mm, ♀ $\bar{x}$ = 50.28 mm). The authors recommend to increase the measured value of interalar width by the statistically derived magnification factor (1.56). This method is suggested as a guideline for choosing the width of anterior artificial teeth in combination with other methods. The existance of a significant difference in the examined parameters between the genders was also established.

After performing various facial and interalar measurements on members of Arab population, Al Wazan [10] determined a significant correlation (p < 0.0001) between the inner canthal distance (♂ $\bar{x}$ = 32.94 mm, ♀ $\bar{x}$ = 31.91 mm) and the 4 anterior incisors (♂ $\bar{x}$ = 30.62 mm, ♀ $\bar{x}$ = 29.52 mm). No difference in the inner canthal distance between genders was established [10]. A low correlative coefficient between the interalar width (♂ $\bar{x}$ = 39.50 mm, ♀ $\bar{x}$ = 36.11 mm) and the intercanine distance was established. Researchers recommend using facial measurements as the initial step in determining the width of anterior artificial teeth for edentulous patients [11].

While exploring the interdependence of the inner canthal distance (♂ $\bar{x}$ = 28.7 mm, ♀ $\bar{x}$ = 27.9 mm) and the width of central incisors (♂ $\bar{x}$ = 8.87 mm, ♀ $\bar{x}$ = 8.68 mm) Abdullah [12] and Abdullah et al. [13] measured facial parameters on a sample of Arab population and determined the possibility of using this distance as a guide for selection of central incisors but only after multiplying the obtained values with the coefficient of geometric progression (0.618) and then dividing the result by two.

While examining the correlation between interpupillar, bizygomatic and interalar distances (♂ $\bar{x}$ = 66.5 mm, ♀ $\bar{x}$ = 62.9 mm) in digital photographs and intercanine distance (♂ $\bar{x}$ = 60.6 mm, ♀ $\bar{x}$ = 62.8 mm) on casts on a Turkish population sample, Hasanreisoglu et al. [5] found a proportional relationship between the intercanine distance and the interalar width in women and determined a significant difference in dimensions of the upper central incisors and canine teeth between the sexes (p < 0.05, p < 0.01). According to their results interalar width can be used to determine the width of maxillary anterior artificial teeth, especially in women.

By analyzing facial and dental distances on a sample of Brazilian population in digital photographs and casts, Gomes et al. [14] conclude that the inner canthal distance (♂ $\bar{x}$ = 32.94 mm, ♀ $\bar{x}$ = 31.91 mm) and interalar width (♂ $\bar{x}$ = 34.78 mm, ♀ $\bar{x}$ = 33.76 mm) have a high correlation (p = 0.000) with intercanine distance, in photographs (♂ $\bar{x}$ = 43.10 mm, ♀ $\bar{x}$ = 41.77 mm) as well as in casts (♂ $\bar{x}$ = 54 mm, ♀ $\bar{x}$ = 53.50 mm). No difference in inner canthal distance was found between the genders.

By analyzing statistical data obtained from an Indian population sample Tandale et al. [15] established a biometric ratio of 1 : 0.271 and 1 : 1.428 by comparing inner canthal distance (♂ $\bar{x}$ = 32.16 mm, ♀ $\bar{x}$ = 31.59 mm) and intraoral measurements of the width of four incisors (♂ $\bar{x}$ = 31.62 mm, ♀ $\bar{x}$ = 30.15 mm) and all the 6 anterior teeth (♂ $\bar{x}$ = 45.81 mm, ♀ $\bar{x}$ = 45.13 mm). Significant differences between genders were found for all the measured parameters (p < 0.0001), except for inner canthal distance which showed no statistically significant difference between the genders. The authors conclude that the inner canthal distance can be used as a preliminary method for determining the width of the anterior teeth.

Within a Malaysian population sample Isa et al. [16] analyzed interpupillar distance, inner canthal distance ($\bar{x}$ = 34.36

*♂ – male; †♀ – female

mm) and interalar width ($\bar{x}$ = 39.36 mm) on digital photographs. Individual widths of the 6 anterior teeth (central incisor $\bar{x}$ = 8.54 mm, lateral incisor $\bar{x}$ = 7.09 mm, canine $\bar{x}$ = 7.94 mm) were measured on casts using a digital gauge. The authors conclude that by using regression analysis the width of anterior teeth can be predicted by combining analyzed facial parameters.

On a Brazilian population sample Lucas et al. [17] used digital photography to measure the inner canthal distance ($\bar{x}$ = 34.42 mm) and the distance between the maxillary canines tips ($\bar{x}$ = 37.45 mm) and their distal surfaces ($\bar{x}$ = 42.15 mm). They also measured the curved distance between the tips of maxillary canines ($\bar{x}$ = 43.66 mm) and their distal surfaces ($\bar{x}$ = 53.45 mm) on casts. A significant correlation was established between all determined variables (r = 0.476, r = 0.467, r = 0.285, r = 0.302). The authors conclude that inner canthal distance, when determined by photogrammetry, can be a reliable guideline for selecting the anterior teeth [17].

Ibrahimagic et al. [19] conducted a research within Croatian population in order to determine the correlation between the width of upper incisors, the width between the upper canines tips or incisor and canine width and the interalar width or the intercominsural width. The results show a statistically significant difference between males and females for all the measured variables ($p < 0.01$), and that the recorded values were higher among males. The obtained mean value for interalar width is 30.9 mm for females and 33.63 mm for males. The width between canines cusps in women is 31.021 mm and for males is 32.44 mm. Width of the nose approximates to the width between the tips of canines (1.08 : 1). The calculated values for the studied population may help in choosing the size of upper anterior teeth and their preferences in complete dentures.

Varjão and Nogueira [21] found out that the average value of interalar width for the white Brazilian male is 35.28 mm and the mean value of intercanine tooth width for the same population is 33.55 mm (measured on casts). The calculated Pearson's correlation coefficient was 0.238, which is a weak correlation between these two parameters. The authors conclude that the method of measuring the width of the base of the nose is not an accurate guideline for selecting the width of artificial teeth.

While conducting research within Croatian population, Knezovic et al. [22] conclude that using facial measurements such as face, nose and upper lip length, inner canthal, interalar and intercomisural distance for selecting anterior artificial teeth are generally inaccurate. The indexes of width/height were determined for central incisors, lateral incisors and canines. For interalar width (♂ $\bar{x}$ = 33.9 mm, ♀ $\bar{x}$ = 30.20 mm) a statistically significant difference related to gender ($p < 0.0001$) was obtained, while the inner canthal distance (♂ $\bar{x}$ = 15.41 mm, ♀ $\bar{x}$ = 15.31 mm) showed no difference.

Comparing the results of a previous researches in relation to one's own, differences rooted primarily in ethnic and morphological characteristics are observed. Given the great individual variability in human physiognomy and values of morphological parameters, the use of inaccurate standards in diagnosis and treatment planning would not only lead to wrong conclusions about the existence and severity of deviations but also to unsatisfactory results of denture therapy, both in terms of esthetics and the aspect of planning the artificial occlusion complex. Therefore, the results of specific relations of anatomic determinants and width of anterior teeth must be perceived as distinctive features of the population on which the study was performed.

Most studies, including ours, established the existance of significant differences in all values of facial and dental parametres between the genders, and show that male subjects have higher values than females. This research determines that there is very little significant difference in inner canthal distance between genders while some studies find no difference in this parameter between the two sexes whatsoever [14, 15, 22]. By taking measurements on samples of their own population, some authors do not find any significant correlation between facial and dental parameters and therefore conclude that inner canthal distance and interalar width are not reliable parameters for selecting the size of upper anterior teeth for dentures [21, 22]. Considering that no research, including ours, confirms a high correlation between facial and dental parameters, most authors reccomend using this method as a guideline in choosing the width of anterior artificial teeth but only combined with other methods.

Conclusion

The analyzed values of facial and dental parameters in our population are moving in the biometric standards contained in the relevant literature. The determined differences arise from ethnic and morphological characteristics. A moderate correlation between the interalar width and anterior teeth width and canine cusps width was established. A low correlation between the inner canthal distance and width of anterior teeth and canine cusps width was established. By testing the statistical significance between genders significant differences for all the parameters was found. The measured facial distances and anterior teeth width had higher values for men than for women.

The results of this study indicate that the investigated interalar width and inner canthal distance cannot be reliable guidelines for the selection of upper anterior artificial teeth. However, they can be used in combination with other methods for the selection of artificial teeth but the final decision should be made while testing prosthetic denture models and with patient's consent. Selection of artificial anterior teeth should be based on finding a harmonious relationship between the size and shape of teeth in relation to gender and individual constitutional characteristics.

R E F E R E N C E S

1. *Strajnic Lj.* Determination of placement of anterior teeth in removable dental prostheses. Med Pregl 2002; 55(11−12): 490−4. (Serbian)
2. *Rosenstiel SF, Ward DH, Rashid RG.* Dentists' preferences of anterior toot proportion- a web-based study. J Prosthodont 2000; 9(3): 123−36.
3. *Preston JD.* The golden proportion revisited. J Esthet Dent 1993; 5: 247–51.
4. *Ward DH.* A study of dentists' preferred maxillary anterior tooth width proportions: comparing the recurring esthetic dental proportion to other mathematical and naturally occurring proportions. J Esthet Restor Dent 2007; 19(6): 324−37.
5. *Hasanreisoglu U, Berksun S, Aras K, Arslan I.* An analysis of maxillary anterior teeth: Facial and dental proportions. J Prosthet Dent 2005; 94(6): 530−38.
6. *Ali Fayyad MA, Jamani KD, Agrabawi J.* Geometric and mathematical proportions and their relations to maxillary anterior teeth. J Contemp Dent Pract 2006; 7(5): 62−70.
7. *Baer ML, Reynolds MA.* Comparison of anterior tooth width in natural and artificial dentitions. J Prosthodon 1992; 1: 84−7.
8. *al-el-Sheikh HM, al-Athel MS.* The relationship of interalar width, interpupillary width and maxillary anterior teeth width in Saudi population. Odontostomatol Trop 1998; 21(84): 7−10.
9. *Basker R.M., Davenport J. C.* Prosthetic treatment of the Edentulous Patient. 4th ed. Oxford: Blackwell Publishing Company; 2002.
10. *Al Wazzan KA.* The relationship between intercanthal dimension and the widths of maxillary anterior teeth. J Prosthet Dent 2001; 86(6): 608−12.
11. *Al Wazzan KA, Al Haidan A, Al Madi EM, Al Murfarj A.* The relationship between facial references and mesiodistal width of maxillary anterior teeth among Saudi patients. Alexandria Dent J 1995; 20(4): 39−45.
12. *Abdullah MA.* Inner canthal distance and geometric progression as a predictor of maxillary central incisor width. J Prosthet Dent 2002; 88(1): 16−20.
13. *Abdullah MA, Stipho HD, Talic YF, Khan N.* The significance of inner-canthal distance in prosthodontics. Saudi Dent J 1997; 9(1): 36−9.
14. *Gomes VL, Gonçalves LC, do Prado CJ, Junior IL, de Lima Lucas B.* Correlation between facial measurements and the mesiodistal width of the maxillary anterior teeth. J Esthet Restor Dent 2006; 18(4): 196−205; discussion 205.
15. *Tandale UE, Dange SP, Khalikar AN.* Biometric relationship between intercanthal dimension and the widths of maxillary anterior teeth. J Indian Prosthodont Soc 2007; 7(3): 123−5.
16. *Isa ZM, Tawfiq OF, Noor NM, Shamsudheen MI, Rijal OM.* Regression methods to investigate the relationship between facial measurements and widths of the maxillary anterior teeth. J Prosthet Dent 2010; 103(3): 182−8.
17. *Lucas BL, Bernardino-Júnior R, Gonçalves LC, Gomes VL.* Distance between the medialis angles of the eyes as an anatomical parameter for tooth selection. J Oral Rehabil 2009; 36(11): 840−7.
18. *Gonçalves LC, Gomes VL, De Lima Lucas B, Monteiro SB.* Correlation between the individual and the combined width of the six maxillary anterior teeth. J Esthet Restor Dent 2009; 21(3): 182−91.
19. *Ibrahimagic L, Celebic A, Jerolimov V, Seifert D, Kardum-Ivic M, Filipovic I.* Correlation between the size of maxillary frontal teeth, the width between alae nasi and the width between corners of the lips. Acta Stomatol Croat 2001; 35(2): 169−79.
20. *Sülün T, Ergin U, Tuncer N.* The nose shape as a predictor of maxillary central and lateral incisor width. Quintessence Int 2005; 36(8): 603−7.
21. *Varjão FM, Nogueira SS.* Nasal width as a guide for the selection of maxillary complete denture anterior teeth in four racial groups. J Prosthodont 2006; 15(6): 353−8.
22. *Knezovic Zlataric D, Kristek E, Celebic A.* Analysis of width/lenght ratios of normal clinical crowns of the maxillary anterior dentition: correlation between dental proportions and facial measurements. Int J Prosthodont 2007; 20(3): 313−7.

Received on March 8, 2011.
Revised on August 25, 2011.
Accepted on September 5, 2011.

ORIGINAL ARTICLE

UDC: 617.736-003.8
DOI: 10.2298/VSP110311047R

The effect of intravitreal administration of bevacizumab on macular edema and visual acuity in age-related macular degeneration with subfoveolar choroidal neovascularisation

Uticaj intravitrealne primene bevacizumaba na edem makule i oštrinu vida kod senilne degeneracije žute mrlje sa supfoveolarnom horoidnom neovaskularizacijom

Dragana Ristić*, Miroslav Vukosavljević*†, Biljana Draganić*, Vesna Cerović*, Nenad Petrović*, Mirjana Janićijević-Petrović‡

***Ophthalmology Clinic, Military Medical Academy, Belgrade Serbia; †Faculty of Medicine of the Military Medical Academy, Universty of Defence, Belgrade, Serbia; ‡Department of Ophthalmology, Clinical Center of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Age-related macular degeneration (AMD) is a leading cause of the loss of central visual acuity in population older than 70 years. We can distinguish wet and dry form of AMD. The aim of the study was to present our early results in treatment of the wet (neovascular) form of AMD with intravitreal administration of bevacizumab. **Methods.** The study included 39 patients. Each patient underwent a complete ophthalmological examination, fluorescein angiography (FA) and optical coherence tomography (OCT). All the patients received 1.25 mg of intravitreal bevacizumab (0.05 mL of commercial phial of Avastin®). The total of three doses was given with a one-month interval between doses. **Results.** Among 39 patients, 24 were women and 15 men. The average best corrected visual acuity (BCVA) was improved from 0.09 before the therapy to 0.24 after the administration of all the three doses of bevacizumab ($p < 0.001$). The average central macular thickness (CMT) measured by OCT was improved from 474 μm in the beginning to 341 μm after the administration of all the three doses of the drug ($p < 0.001$). There were no side effects. **Conclusions.** Our short-term experience indicates that intravitreal administration of three doses of bevacizumab in one-month intervals between the doses leads to a significant reduction of macular edema and improvement of BCVA in patients with neovascular AMD.

Key words:
antibodies monoclonal; angiogenesis inhibitors; macular degeneration; choroidal neovascularisation; treatment outcome.

Apstrakt

Uvod/Cilj. Starosna degeneracija žute mrlje (*age-related macular degeneration* – AMD) glavni je uzročnik gubitka centralne oštrine vida kod osoba starijih od 70 godine. Postoje vlažna i suva forma AMD. Cilj rada bio je prikaz rezultata u lečenju vlažne (neovaskularne) forme AMD intravitrealnom primenom bevacizumaba. **Metode.** U radu je prikazano 39 bolesnika. Kod svakog bolesnika urađen je kompletan oftalmološki pregled, fluoresceinska angiografija (FA) i optička koherentna tomografija (*optical coherence tomography* – OCT). Svi bolesnici su primili intravitrealno 1,25 mg bevacizumaba (0,05 mL komercijalnog preparata Avastin®). Ukupno su date tri doze u razmacima po mesec dana između doza. **Rezultati.** Od 39 bolesnika, 24 osobe bile su ženskog, a 15 muškog pola. Prosečna najbolje korigovana vidna oštrina (*best corrected visual acuity* – BCVA) popravila se sa 0,09 pre terapije, na 0,24 nakon primene sve tri doze bevacizumaba ($p < 0,001$). Prosečna centralna debljina makule (*central macular thickness* – CMT), merena OCT aparatom, popravila se sa 474 μm na početku lečenja na 341 μm nakon primene sve tri doze leka ($p < 0,001$). Nije bilo nikakvih neželjenih efekata. **Zaključak.** Naše kratkoročno iskustvo govori u prilog tome da bevacizumab, primenjen intravitrealno u tri doze u razmacima po mesec dana, dovodi do značajnog smanjenja edema makule i poboljšanja BCVA kod bolesnika sa neovaskularnom AMD.

Ključne reči:
antitela, monoklonska; angiogeneza, inhibitori; makularna degeneracija; horoidalna neovaskularizacija; lečenje, ishod.

Correspondence to: Dragana Ristić, Ophthalmology Clinic, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia; Phone: +381 11 233 5090, +381 64 1398 813. E-mail: dadana25@yahoo.com

Introduction

Age-related macular degeneration (AMD) is a leading cause of the loss of central visual acuity in population older than 70 years. We can distinguish wet and dry form of AMD. Wet or exudative (neovascular) form is characterized by the presence of a choroidal neovascular membrane (CNV) which is related to higher retinal blood vessel permeability and neoangiogenesis. One of the underlying causes of these vascular changes is the activity of an isoform of vascular endothelial growth factor (VEGF) called VEGF-A.

VEGF-A presents in various isoforms ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$)[1]. All these isoforms can be bound by bevacizumab which is its advantage over other available VEGF-A inhibitors (pegaptanib, ranibizumab). Molecular weight of bevacizumab (149 kDa), longer half-life and ability to reach subretinal space through the circulation explain its better results in the management of neovascular AMD.

Bevacizumab (Avastin®) is a monoclonal VEGF-anti body approved for intravenous use in the management of colorectal carcinoma. Off-label intravenous administration of this medication in neovascular AMD treatment was firstly published by Michels et al. in 2005 [2]. Shortly after this many other articles about intravitreal administration of bevacizumab in neovascular AMD treatment have followed [3–8].

The aim of this article was to present our initial results in monitoring macular edema reduction and its correlation with visual acuity after intravitreal administration of bevacizumab to patients with neovascular AMD.

Methods

The participants in our study were 39 patients with subfoveolar CNV related to AMD. Intravitreal administration of bevacizumab was approved by the Ethics Committee of the Military Medical Academy (MMA). All the patients were informed about off-label usage of this medication, about possible side effects and complications but also about potential benefit from this treatment. All the patients signed a written consent form prior to drug administration. Every patient received 1.25 mg of bevacizumab (0.05 mL of commercial phial of Avastin ® intravitreally). Three doses of bevacizumab were applied at one-month intervals. The patients were followed up on first, seventh and thirtieth day. A complete ophthalmological examination was performed on each patient, including best corrected visual acuity (BCVA), fluorescein angiography (FA), and optical coherence tomography (OCT). Visual acuity and OCT were performed at each control examination, while FA was only performed during the initial examination and then one month following the third bevacizumab dose.

Best-corrected visual acuity, originally expressed in meters, was converted into logarithm of the minimum angle of resolution (LogMAR) for more accurate analyses. For statistical analysis we used the Friedman ANOVA test and Wilcoxon test with $p < 0.001$ considered highly statistically significant.

Results

We analyzed 39 patients; among them 24 were woman and 15 were man. The average age was 73.5 (64–83) years. Visual acuity and OCT were measured prior to the intervention and followed up at regular check-ups on the 7th and 30th day following the intervention. The average visual acuity before the intervention was 0.09. After three months and three monthly doses of bevacizumab applied over that period, the average visual acuity was 0.24 ($p < 0.001$). One month after the first dose, BCVA was 0.20 ($p < 0.001$) and one month after the second dose it was 0.22 ($p < 0.05$). In our study BCVA of 34 (87.2%) eyes improved and of 5 (12.8%) eyes remained the same. We had no cases of visual acuity worsening. Seven (17.95%) eyes showed visual acuity improvement of one row and 13 (33.3%) eyes improved by two rows. Eventually, 5 (12.82%) eyes showed improvement of the stunning five rows (Figure 1, Table 1).

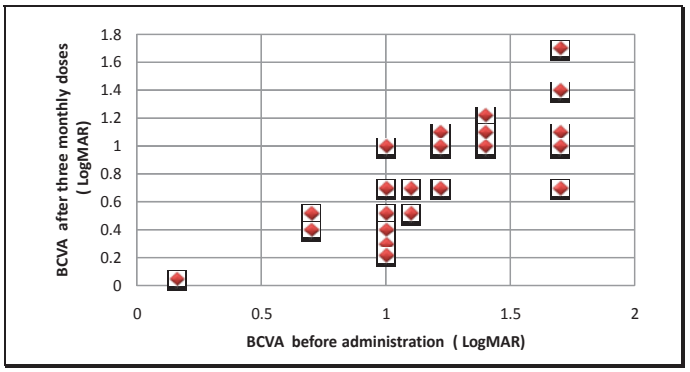


Fig. 1 – Change in best corrected visual acuity (BCVA) after the administration of three monthly doses of bevacizumab MAR – minimum angle of resolution.

Table 1
The average best corrected visual acuity (BCVA) after the administration of bevacizumab

Time (months)	Average BCVA (m)	*p*
0	0.09	
1	0.20	< 0.001
2	0.22	< 0.05
3	0.24	< 0.001

The average central macular thickness (CMT) measured by OCT prior to the intervention was 474 μm, and three months following three doses of bevacizumab it was 341 μm ($p < 0.001$). After the administration of all the three doses of bevacizumab, the average macular edema reduction was 132 μm. Every eye showed an improvement in CMT reduction, although in 5 eyes BCVA did not improve (Figures 2 and 3, Table 2).

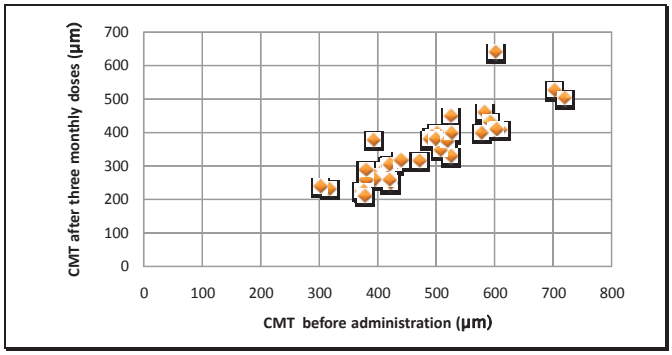


Fig. 2 – Change in central macular thickness (CMT) (μm) measured by optical coherence tomography (OCT) after the administration of three monthly doses of bevacizumab.

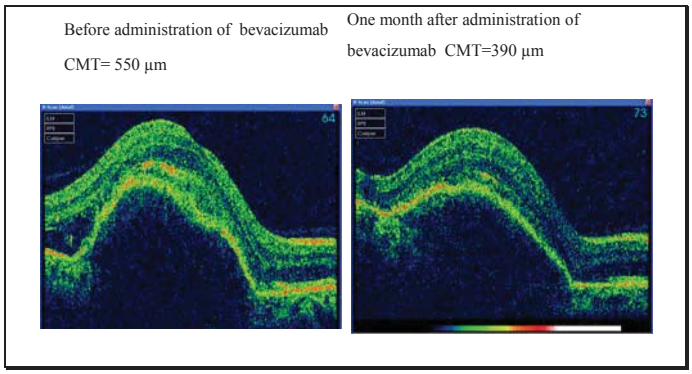


Fig. 3 – Central macular thickness (CMT) measured by optical coherence tomography (OCT) before and after administration of bevacizumab.

Table 2

Average central macular thickness (CMT) measured by optical coherence tomography (OCT) after administration of bevacizumab

Time (months)	Average CMT (μm)	*p*
0	474	
1	402	< 0.001
2	374	< 0.001
3	341	< 0.001

Discussion

Most of the eyes treated with bevacizumab in this study showed attempted improvement, both morphological and functional response. Our results confirm a significantly higher reduction of macular edema and improvement of BCVA in patients with neovascular AMD.

While systemic bevacizumab administration was related to increased risk for complications and adverse effects such as gastrointestinal bleeding, thromboembolism and arterial hypertension, intravitreal bevacizumab administration applied in our study proved to be free of any systemic and local complications.

There are several anti-VEGF medications registered for intravitreal administration worldwide. Pegaptanib was the first in this group to be accepted for wet AMD form treatment. Shortly after this one, ranibizumab was registered for intravitreal administration. Ranibizumab (Lucentis®) was approved for intravitreal administration by the American Food and Drug Administration in 2006. This decision was based on the results presented in MARINA and ANCHOR studies. These studies proved ranibizumab efficiency [3, 9].

As previously mentioned, Avastin® was introduced in ophthalmology in 2005 and soon after that several studies about its efficiency in treatment of wet AMD form followed. Fong et al. [10] reported in their study that there was no statistically significant difference regarding BCVA improvement between groups receiving bevacizumab and ranibizumab. After the reports about similar efficiency of these two medications we decided to use significantly less expensive bevacizumab in our study.

Our study was based on updated results, published worldwide, about intravitreal bevacizumab administration. A precise dose of the used medication and timeline in the treatment protocol varied among the studies. Most frequently used doses were 1.25 mg and 2.5 mg of bevacizumab. Based

on the published data that imply no statistically significant difference between the two doses in BCVA improvement and CMT reduction, we chose the lower one for our study.

Ghazi et al.[11] applied bevacizumab only once and then decided about continuing with the next dose based on clinical evaluation. In studies published by Bashshur et al. [5] and Averya et al. [4] bevacizumab was applied three times in one-month intervals between the doses. It is considered that anti VEGF effect of bevacizumab starts after approximately 24 h from administration and persists for 2–3 weeks [12]. Conrad et al. [8] in 2008 considered that bevacizumab effect might last up to 8 weeks after administration. We assume that this periodic administration provides better VEGF inhibition and, consequently, faster CNV retrieval. This was our reason to accept three doses protocol with one month between doses.

Conclusion

Today many ophthalmologists worldwide use anti-VEGF treatment, particularly bevacizumab for neovascular AMD.

In our study we used three doses of the drug with one-month period between each one. Results showed a significant difference in improving visual acuity and reducing macular edema. It is also important to point out that we did not have any complications in this study.

Still, the decision which protocol of administration is the best one remains uncertain. For this reason we believe that summing up all our experiences may bring us one step closer to more decisive criteria and most effective protocol for neovascular AMD treatment with intravitreal bevacizumab administration.

R E F E R E N C E S

1. *Churchill AJ, Carter JG, Lovell HC, Ramsden C, Turner SJ, Yeung A*, et al. VEGF polymorphisms are associated with neovascular age-related macular degeneration. Hum Mol Genet 2006; 15(19): 2955−61.
2. *Michels S, Rosenfeld PJ, Puliafito CA, Marcus EN, Venkatraman AS*. Systemicbevacizumab (Avastin) therapy for neovascular age-related macular degeneration twelve-week results of an uncontrolled open-label clinical study. Ophthalmology 2005; 112(6): 1035−47.
3. *Rosenfeld PJ, Moshfeghi AA, Puliafito CA*. Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration. Ophthalmic Surg Lasers Imaging 2005; 36(4): 331−5.
4. *Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ*. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 2006; 113(3): 363−72.e5.
5. *Bashshur ZF, Bazarbachi A, Schakal A, Haddad ZA, El Haibi CP, Noureddin BN*, et al. Intravitreal bevacizumab for the management of choroidal neovascularization in age-related macular degeneration. Am J Ophthalmol 2006; 142(1): 1−9.
6. *Rich RM, Rosenfeld PJ, Puliafito CA, Dubovy SR, Davis JL, Flynn HW Jr*, et al. Short-term safety and efficacy of intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Retina 2006; 26(5): 495−511.
7. *Spaide RF, Laud K, Fine HF, Klancnik JM Jr, Meyerle CB, Yannuzzi LA*, et al. Intravitreal bevacizumab treatment of choroidal neovascularisation secondary to age-related macular degeneration. Retina 2006; 26(4): 383−90.
8. *Conrad PW, Zacks DN, Johnson MW*. Intravitreal bevacizumab has initial clinical benefit lasting eight weeks in eyes with neovascular age-related macular degeneration. Clin Ophthalmol 2008; 2(4): 727−33.
9. *Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY*, et al. Ranibizumab versus verteporfin for neovascular age-relatedmacular degeneration. N Engl J Med 2006; 355(14): 1432−44.
10. *Fong DS, Custis P, Howes J, Hsu JW*. Intravitreal bevacizumab and ranibizumab for age-related macular degeneration a multicenter, retrospective study. Ophthalmology 2010; 117(2): 298−302.
11. *Ghazi NG, Kirk TQ, Knape RM, Tiedeman JS, Conway BP*. Is monthly retreatment with intravitreal bevacizumab (Avastin) necessary in neovascular age-related macular degeneration? Clin Ophthalmol 2010; 4: 307−14.
12. *Welch DE, Elmariah H, Peden MC, Adams SG, Ratnakaram R, Kaushal S*. Short-term response of macular oedema to intravitreal bevacizumab. Br J Ophthalmol 2009; 93(8): 1033−6.

Received on March 11, 2011.
Revised on July 18, 2011.
Accepted on July 19, 2011.
OnLine-First December, 2012.

ORIGINAL ARTICLE

UDC: 616.311.2-089.843-085.382
DOI: 10.2298/VSP1307664J

Therapeutic efficacy of connective tissue autotransplants with periosteum and platelet rich plasma in the menagement of gingival recession

Terapijski efekat plazme obogaćene trombocitima i autotransplantata vezivnog tkiva sa periostom u zbrinjavanju gingivalnih recesija

Bojan Jovičić*, Zoran Lazić*†, Milica Nedić‡, Stevo Matijević*†, Aleksandra Gostović-Špadijer§

***Clinic of Dentistry, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; ‡Faculty of Dentistry, Pančevo, Serbia; ‡Faculty of Dentistry, University in Belgrade, Belgrade, Serbia**

Abstract

Background/Aim. Gingival recession progression in clinical practice has influenced the development of various surgical procedures and techniques for solving esthetic imperfections and subjective difficulties coused by gingival recession. The aim of this study was to verify efficacy of surgical procedures and to compare both of surgical procedures through the keratinized tissue width. **Methods.** The study included 20 teeth with gingival recesion, Müller class I and II. Ten teeth with gingival recession were treated with connective tissue autotransplants with periosteum in combination with coronary guided surgical flap (CTG group). On the contralateral side 10 teeth with gingival recession were treated with the same surgical procedures but in combination with platelet-rich plasma (CTG-PRP group). We measured the keratinized tissue width. For statistical significance we used the Student's *t*-test. **Results.** The study reveled a statistical significance in reducing vertical deepress of recession by both used treatments. Root deepness in CTG and CTG-PRP group was 90% and 93.5%, respectively. With both surgical techniques we achieved larger zone of keratinized gingiva but with a wide zone of keratinized tissue in CTG – the PRP group. **Conclusion.** The concept regeneration technique with PRP and with the stimulating influence of platele activated growth factors results in the regeneration of deep periodontal tissue as an important prerequisite for the successfull treatment of gingival recession.

Key words:
gingival recession; dentistry, operative; transplantation, autologous; platelet-rich plasma; treatment outcome.

Apstrakt

Uvod/Cilj. Sve veća zastupljenost gingivalnih recesija u svakodnevnoj kliničkoj praksi uslovila je zahteve za što efikasnijim rekonstruktivnim hirurškim procedurama, u cilju rešavanja estetskih nedostataka i subjektivnih tegoba usled povlačenja gingive. Cilj rada bio je da se uporedi efikasnost lečenja autotransplantatom vezivnog tkiva (ATVT) sa periostom i plazme obogaćene trombocitima (PRP) sa ATVT sa periostom, ali bez PRP, na širinu keratinizirane gingive. **Metode.** Studijama je bilo obuhvaćeno 20 zuba sa recesijama gingive klase I i II prema Mülleru. U toku terapije 10 recesija lečeno je ATVT sa periostom u kombinaciji sa koronarno pomerenim režnjima (ATVT grupa). Na kontralateralnoj strani, isti broj recesija lečen je ATVT uz koronarno pomeren režanj i uz primenu PRP (PRP + ATVT grupa). Od kliničkih parametara praćena je širina keratinizovane gingive kao pokazatelj pokrenutih regenerativnih procesa. U statističkoj obradi rezultata korišćen je Studentov *t*-test. **Rezultati.** Analizom kliničkih parametara ustanovljeno je statistički značajno proširenje zone keratinizirane gingive, što je od izuzetnog značaja za regeneraciju s tim što je proširenje bilo izraženije u ATVT + PRP grupi. Procentualno gledano, prekrivenost korena u ATVT grupi iznosila je 90%, a u ATVT + PRP grupi 93,5%. **Zaključak.** Primenom koncepta aktivne regeneracije odnosno primenom PRP i stimulativnim dejstvom aktiviranih faktora rasta iz trombocita, postiže se regeneracija dubljeg periodontalnog tkiva što je bitan preduslov za uspešno zbrinjavanje recesije gingive.

Ključne reči:
gingiva, povlačenje; stomatologija, operativna; transplantacija, autologa; plazma bogata trombocitima; lečenje, ishod.

Correspondence to: Bojan Jovičić, Clinic of Dentistry, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.
Phone: +381 11 36 09 454.

Introduction

Gingival recession is defined as a condition when the edge of the gingival margin is located apically to the cement-enamel border, and the tooth root surface exposed towards the external surface of the tooth [1]. Such clinical conditions are commonly seen in a daily clinical practice in population of different ages, and their prevalence increases with age [2]. Most frequent etiological factors related to and responsible for the occurrence of gingival recession include mucogingival anomalies, poor oral hygiene, inadequate tooth brushing techniques, a high frenum attachment and blisters as well as various orthodontic deformities [3]. From the pathogenetic aspect, gingival recession is closely associated with tissue inflammation caused by dental plaque.

In addition to esthetic defects leading to gingival recession, unpleasant sensitivity to mechanical, chemical and technical stimuli are also the most common reasons for patients to visit dentists [4]. Elimination of pain sensation and aesthetic correction of gingival recession along with the creation of conditions for adequate maintenance of oral hygiene are only some of the aims of mucogingival plastic surgery [5].

There are numerous surgical procedures that allow for creation of a stable zone of the attached gingiva, coverage of the exposed tooth root surface and prevention of the gum from receding further [6]. The gold standard among surgical procedures for the root coverage has for long been connective tissue autograft (CTG) used with the periosteum in various combinations with coronally and laterally positioned grafts and double papilla graft, as well [7]. Apart from those methods, more advanced methods developed by modification of the existing surgical procedures were introduced into clinical practice. Some of those methods include guided tissue regeneration (GTR) where various resorbable and non-resorbable membranes are used [8], and procedures in which free gingival and pedicle grafts are used most commonly in combination with grafts in coronal position.

A method that has recently been singled out as a special one is the concept of tissue engineering, *ie* the process in which the growth factor is applied to promote the process of regeneration. A question is why there are so many diverse surgical procedures for the management of gingival recession-related problems. The answer lies in the fact that the regenerative capacity of periodontium is limited, and that surgical therapy provides only a partial regeneration in terms of long junctional epithelium, not a complete reconstruction in terms of '*restitution ad integrum*', something the modern periodontology tends to [9]. To achieve complete periodontal tissue regeneration, an additional stimulation of the regeneration process is required. Due to that the agents in blood that increase the possibility of bone and soft tissue regeneration have used [10]. Activated platelet preparations have been shown to be able to induce angiogenesis, synthesize collagen and inhibit monocytes [11]. It refers to local application of platelet-rich plasma (PRP) that proved to be very suitable for release of platelet-derived growth factors (PDGF), enabling better tissue regeneration and faster healing process. The essence of PRP are the potent bioactive growth factors, the components of platelet α-granules. Therefore, 7 specific polypeptide molecules, *ie* growth factors were included: 3 isomers of PDGF (PDGF-AA, PDGF-BB and PDGF-AB), 2 transforming growth factors beta (TGF-β), TGF-β1 and TGF-β 2, the vascular endothelial growth factor (VEGF) and the epithelial growth factor (EGF) [12]. Secreted growth factors facilitate mitosis in cells, proliferation and migration of periodontal ligament cells, stimulate their replication and matrix synthesis, initiate the vascular ingrowth, and induce cell differentiation at the site of injury when they arrive and become the predominant cells in wound [13]. The essence of PRP application is that the high concentration of platelets increases concentration of growth factors and enhances the periodontal tissue response [14].

The main parameter that testifies preservation of healthy structure of periodoncium is the keratinized gingival width. The width of attached gingiva is a variable in human population, and ranges from minimal 0.5 mm – 1 mm to maximally measured 9 mm. Its main role is to prevent free gingiva from moving under the effect of muscle force produced by closer implanted muscles. It is thought that an adequate width of attached gingiva is the one that prevents movement of the soft oral tissue onto the gingival margin. The trials conducted so far have shown that the 2-mm keratinized gingiva is a minimum that ensures healthy paradontium [15]. In case of less than 2 mm width of keratinized tissue, or a total loss of gingival margin, the marginal gingiva comes into direct contact with the movable alveolar mucosa [15]. Such a condition causes pulling of the marginal gingival away from the alveolar mucosa when speaking or laughing [16]. Continuous pulling is transferred into the area of the epithelial attachment causing thus the damage and separation of the attached epithelium from the tooth. This newly created relation allows for the accumulation of dental plaque which due to its pathogenic properties causes inflammation and damage to the periodontium. All that leads to etiopathogenetic *circulus vitiosus* and in the end to the development of gingival recession [17, 18].

The aim of the study was to compare the clinical effects of CTG and the periosteum in combination with the coronally positioned graft application and CTG and the periosteum in combination with PRP application through their effects on keratinized gingiva width.

Methods

This comparative prospective study included 20 patients of both genders in the age between 18 and 35 years. All of them were non-smokers. The criteria for their selection was the presence of bilateral gingival recession with the visible cement-enamel border at the canine and premolar sites, that corresponded to the Müller Class I and II gingival recession. All the patients were treated at the Clinic of Dentistry, Military Medical Academy in Belgrade. The mouths were divided by methodological concept, so the recession was, on one side, treated with CTG and the periosteum in combination with the coronally positioned graft and the ap-

plication of PRP (the CTG-PRP group) and, on the other side, recession was reconstructed with the CTG and the periosteum but without PRP (the CTG group).

PRP preparation was carried out following the methodology described in the works of Anitua [19] and Sonnleitner et al. [20]. So, 4.5 mL of patient's blood mixed with 0.5 mL of sodium nitrate was put in a test tube for centrifugation-based separation of blood cells. During the first spin cycle, the tube rotated at the speed of 1,200 rpm (160 g) for 20 min separating blood into the lower blood cell component layer and the upper plasma layer. The second 15-min spin cycle at the speed of 2,000 rpm enacted further blood cell separation into the upper fraction containing plasma and a small number of platelets, and the lower one containing PRP. The number of platelets in such a preparation was 1,340 × 10 9/L – 1,670 × 10 9/L, and was utilized within the next 60 minutes.

The surgical procedure involving CTG application in combination with PRP implies, prior to its application, the autograft conditioned with activated platelet concentration obtained by the special technological procedure immediately before surgical intervention. The method of CTG use includes the following procedures: after anesthesia administration, and the initial beveled incisions originating from the cement-enamel border made at the surgical site to raise the mucoperiosteal flap, the root of the tooth is completely exposed. The root surface is then mechanically cleaned with sharp curettes to remove the necrotic layer of cement. By cutting the periosteum in the apical area of the flap, the preparation of the recipient site to receive the graft is completed. The autograft is harvested from the premolar side of the palate using the technique known as 'trapdoor' technique. The sharp dissection is then completed to elevate the palate flap and ensure the access to the palatum for taking the autograft. After that, the donor site is closed with individual sutures. The autograft is positioned over the exposed surface of the root with the periosteum angled towards the root. The autograft is fixed using an individual suture, and is completely covered with the coronally positioned flap fixed with the individual silk sutures. During the recovery period, the treatment includes the use of analgesics, special diet regime and oral hygiene.

Before the surgical intervention and 6 months after the surgery, we evaluated the width of keratinized gingiva of the periodontal tissue and the effects of the applied therapy. The width of keratinized gingiva was expressed in mm.

The arithmetic means and standard deviations of all observations were calculated by using parametric prediction intervals. The differences between those parameters were calculated by using the differentiation test for small associated factors in the same sample of the observed subjects, and by using the Student'-*t* test for independent samples.

As for the non-parametric prediction intervals, the chi-square test was used as a tool to determine the frequency and the significance of the observed sample differences. The differences at the level of 0.05 considered to be significant.

Results

The results for the width of keratinized gingiva were significant indicating the efficiency of the applied surgical procedures. The mean value assessed prior to the surgical intervention in the CTG-PRP test group (Figures 1–5) was 1.00 ± 0.23 mm. Six months after the surgery, the obtained value clearly indicated a significant augmenting of the zone of keratinized gingiva of 3.70 ± 0.55 mm ($t = 7.56$; $p < 0.01$). In the CTG group (Figures 7 and 8) the value of this parameter was 1.20 ± 0.28 mm. Following six months of the surgery, the value of the obtained widening of keratinized zone was found to be 3.50 ± 0.50 mm, what was statistically very significant ($t = 5.89$; $p < 0.01$). The differences in values of those parameters ranging from 2.3 mm in the CTG group to 2.7 mm in the CTG-PRP group clearly indicated a statistically significant difference observed in both test groups particularly in the CTG PRP group, and also confirmed that regeneration processes occurred in periodontium (Table 1).

Table 1

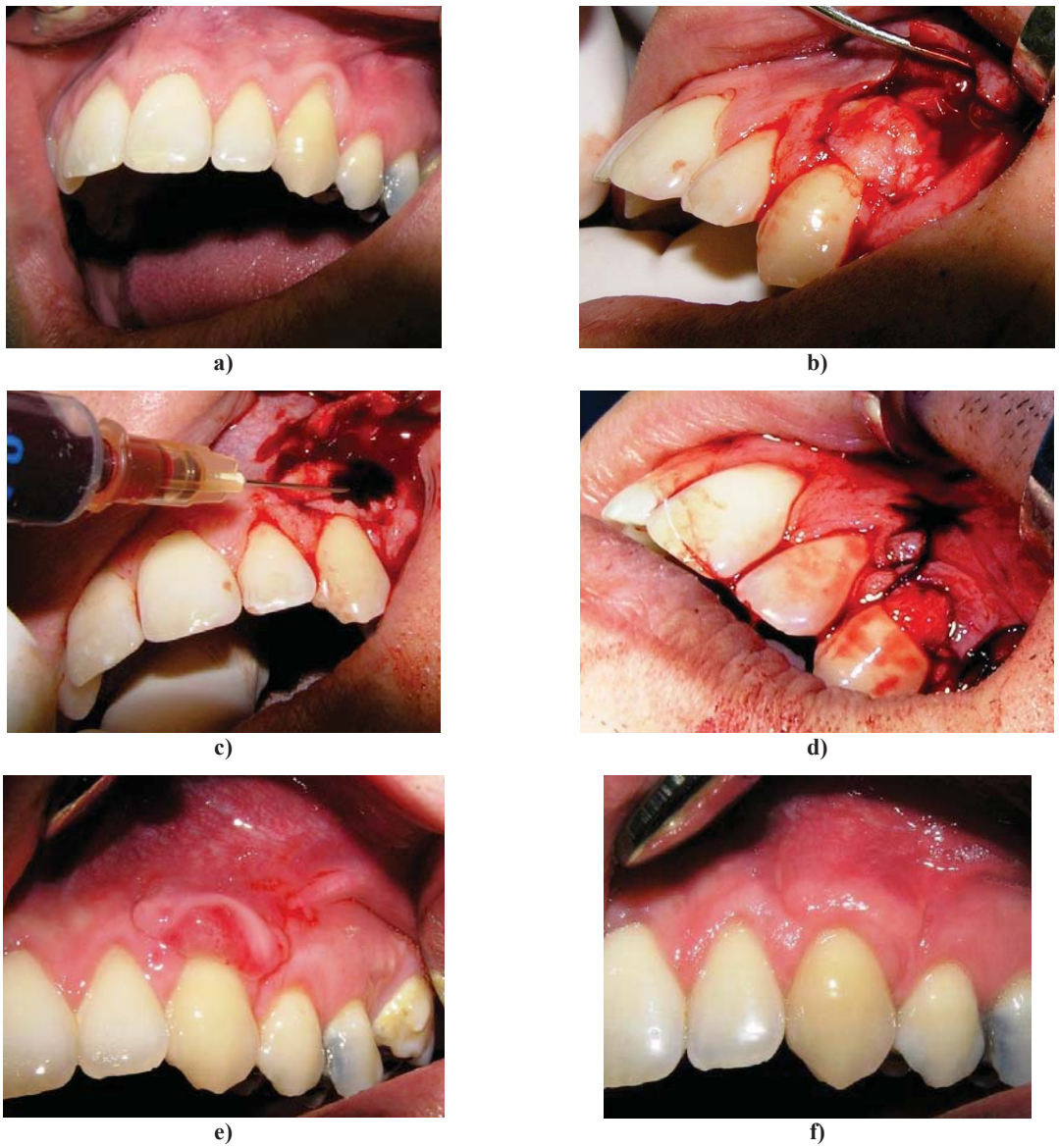
Results of keratinized tissue width prior to and 6 months after the surgery

Study group	Keratinized gingival width (mm)		*t*	*p*
	before surgery ($\bar{x} \pm SD$)	6 months after surgery ($\bar{x} \pm SD$)		
CTG – PRP	1.00 ± 0.23	3.70 ± 0.55	7.56	< 0.01
CTG	1.20 ± 0.28	3.50 ± 0.50	5.89	< 0.01

CTG – connective tissue graft; PRP – platelet rich plasma.

Discusion

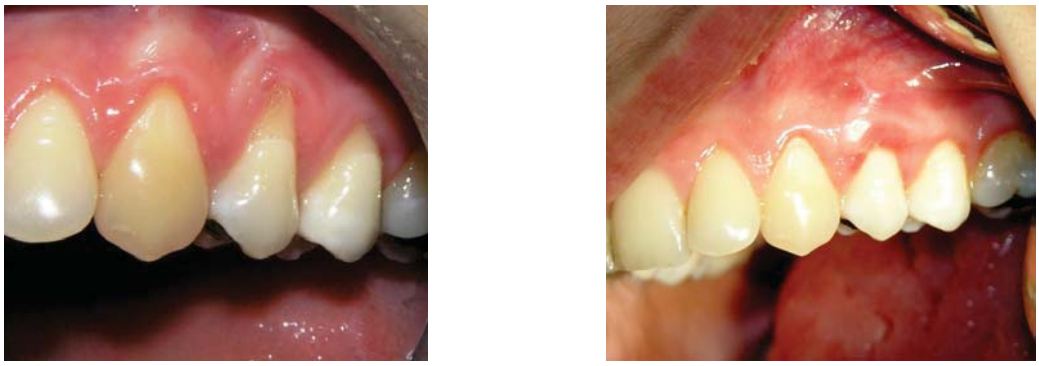
Since the 50s of the last century when the first pioneer efforts were made in the area of the treatment of gingival recession up to nowadays, there has been a lot of work done to improve surgical procedures used for managing gingival recession-related problems. Those procedures ranging in complexity from coronally and laterally positioned grafts through free gingival autografts, connective tissue grafts with periosteum to GTR along with the use of various membranes as an inductor of regeneration have given satisfactory results in terms of satisfying aesthetic criteria but without adequate functional support. The main problem with the majority of those surgical procedures was a long attached epithelium as it had histologically been confirmed in a great number of previously conducted and published studies, so the long-term preservation of the achieved outcomes could not be guaranteed [9, 21]. It was one of the crucial reasons for the introduction of the active regeneration concept into the field of paradontology. The efficacy of this concept lies in the local and continuing application of numerous bioactive growth factors and proteins that enhance the reparatory-tissue processes in the body [22].



a) **b)** **c)** **d)** **e)** **f)**

Fig. 1 – Gingival recession in tooth #23 treated with connective tissue graft (CTG) in combination with platelet rich plasma (PRP).

a) gingival recession before the treatment; b) CTG placed on the recipient site; c) application of activated PRP; d) a view on gingival recession after suture; e) two weeks after the surgery; f) six months postoperatively.



a) **b)**

Fig. 2 – Gingival recession in tooth # 24 treated with connective tissue graft (CTG) without platelet rich plasma (PRP).

a) gingival recession before the treatment; b) six months after the surgery.

The results of our study confirm the role of the 'gold standard' that CTG plays in the treatment of recession, particularly in widening of the zone of keratinized gingiva [23]. The presence of periosteum as a biological membrane increases the biological capacity of an autograft to a great extent [24]. Periosteum as a highly differential tissue of mesenchymal origin has a very important role in bone biology, and, thus, enables the regeneration of a part of the alveolar bone. All that ensure better therapeutic effects and a stable remission of the disease, what was proved by Harris [25] in his research work.

On the basis of data analyses, we could observe that widening of the zone of keratinized gingiva showed to be much more successful in the studied subjects treated with the autograft in combination with PRP as compared to those in which PRP was not applied [26]. It is presumed that, by using PRP, we stimulate the proliferative capacity in undifferentiated mesenchymal cells, their growth, migration and adhesion, thus enhancing the regenerative processes in deeper periodontal tissue structures [27].

It is obvious that most responsible for that are the PDGF and the TGF β present in a PRP fraction, as polypeptide growth factors involved in the cell proliferation, differentiation and morphogenesis of tissue [28]. Growth factors may stimulate either a mitogenic response since they enhance the proliferation of certain types of cells, or respond motogenically because they change the target cell phenotype [29]. More efficient results observed in the CTG-PRP test group can be explained by the fact that lies in the use of PRP, *ie* the high concentration of platelets increases the local concentration of secreted growth factors what thus enhances the initial response to the healing process of the soft and bone tissue. Upon the activation of the platelet fraction, the release of the growth factors important in the wound healing is enacted [30]. Thus the role of growth factors in the chemotaxis of the stem cells and differentiation into corresponding cell groups involved in the regeneration of periodontium is expressed. The fact concerning the effect of the growth factor on the type-1 collagen synthesis should not be neglected because it is the base for the formation of a new extracellular matrix of the connective and bone tissue as well as the formation of a new attachment [31].

One of the very important facts for the survival of an autograft is a good postoperative vascularization and fibrovascular invasion from the surrounding tissue combined with proper diet regime by diffusion, in which the activated plasma plays a crucial role, particularly in the first 48 hours. All that indicates that the PDGF, TGF and GF growth factors have the positive effects on the early tissue and bone healing process [32, 33].

By the elimination of subjective problems and achievement of satisfactory aesthetic results as well as the establishment of a favorable anatomic-morphological ratio, conditions for adequate oral hygiene are created, since it have shown to be one of the crucial factor in maintaining the achieved outcomes.

The obtain results of CTG application with periosteum and PRP justify the use of growth factors. Taking into account the augmentation of the zone of keratinized gingiva indicating the stimulated regeneration of periodontium, what was achieved particularly in the CTG-PRP test group, we can conclude to get more closer to our aim, the one the modern periodontology tend to, and that is restitution *ad integrum*.

Conclusion

The concept regeneration technique with PRP and with the stimulating influence of platele activated growth factors results in the regeneration of deep periodontal tissue as an important prerequisite for the successfull treatment of gingival recession.

REFERENCES

1. *American Academy of Periodontology*. Glossary of Periodontol Terms. 3rd ed. Chicago: The American Academy of Periodontology; 1992.
2. *Kassab MM, Cohen RE*. The etiology and prevalence of gingival recession. J Am Dent Assoc 2003; 134(2): 220–5.
3. *Stevanović R, Zelić O*. Coronary tissue graft for treatment of gingival recession. Stom Glas S 2003; 50(3): 144–9. (Serbian)
4. *Corauza FA, Newman C*. Clinical periodontology. 8th ed. Philadelphia: WB Sanders Company; 1996.
5. *Trombelli L*. Periodontal regeneration in gingival recession defects. Periodontol 2000 1999; 19: 138–50.
6. *Miller PD Jr, Allen EP*. The development of periodontal plastic surgery. Periodontol 2000 1996; 11: 7–17.
7. *Janković S, Dimitrijević B*. Mogućnosti savremenih procedura u terapiji gingivalnih recesija. Stom Glas S 2003; 50(1): 18–23. (Serbian)
8. *Muller HP, Stahl M, Eger T*. Dynamics of mucosal dimensions after root coverage with a bioresorbable membrane. J Clin Periodontol 2000; 27(1): 1–8.
9. *Harris R*. Histologic evaluation of connective tissue grafts in humans. Int J Periodontics Restorative Dent 2003; 23(6): 575−83.
10. *Lazić Z, Bubalo M, Petković-Ćurčin A, Duka M, Mihajlović B*. Therapeutic use of platelet-rich plasma in oral surgery. Vojnosanit Pregl 2009; 66(10): 821–5. (Serbian)
11. *Marx RE*. Platelet rich-plasma: evidence to support its use. J Oral Maxillofac Surg 2004; 62(4): 489–96.
12. *Lazić Z, Mirković Z*. Growth factors in bone regeneration. Belgrade: Zadužbina Andrejević 2007. (Serbian)
13. *Creeper F, Lichanska AM, Marshall RI, Seymour GJ, Ivanovski S*. The effect of platelet reach plasma on osteoblast and periodontal ligament cell migration, proliferation and differentiation. J Periodontal Res 2009; 44(2): 258–65.
14. *Jakse N, Tangl S, Gilli R, Berghold A, Lorenzoni M, Eskici A*, et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res 2003; 14(5): 578–83.
15. *Ainamo J, Loe H*. Anatomical characteristics of gingiva. A clinical and miscrocsope study of the free and attached gingiva. J Clin Periodontol 1996; 37(1): 5−13.
16. *Miller PD, Allen EP*. The Development of periodontal plastic surgery. Periodontology 2000; 11: 7–17.
17. *Roccuzzo M, Bunino M, Needleman I, Sanz M*. Periodontal plastic surgery for treatment of localized gingival recessions: A systematic review. J Clin Periodontol 2002; 29(Suppl. 3): 178–94.

18. *Lung NP, Loe H.* The relationship between the width of keratinized gingiva and gingival health. J Periodontol 1972; 43(10): 623–7.
19. *Anitua E.* Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999; 14(4): 529–35.
20. *Sonnleitner D, Huemer P, Sullivan DY.* A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: a technical note. Int J Oral Maxillofac Implants 2000; 15(6): 879–82.
21. *Carnio J, Camargo PM, Kenney EB, Schenk RK.* Histological evaluation of 4 cases of root coverage following a connective tissue graft combined with an enamel matrix derivative preparation. J Periodontol 2002; 73(12): 1534–43.
22. *Martínez-Zapata MJ, Martí-Carvajal A, Solà I, Bolibar I, Angel Expósito J, Rodriguez L,* et al.. Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: a systematic review. Transfusion 2009; 49(1): 44–56.
23. *Goldstein M, Boyan BD, Cochran DL, Schwarz Z.* Human histology of new attachment after root coverage using subepithelial connective tissue graft. J Clin Periodontol 2001; 28(7): 657–62.
24. *Sonick M, Hwang D.* The dependability of connective tissue grafting for the resolution of full mouth recession. Compend Contin Educ Dent 2011; 32(1): 48–53.
25. *Harris R.* A comparative Study of Root Coverage Obtained with Guided Tissue Regeneration Utilizing a Bioabsorbable Membrane Versus the Connective Tissue with Partial-Thickness Double Pedicle Graft. J Periodontol 1997; 68(8): 779–90.
26. *Huang LH, Neiva RE, Soehren SE, Giannobile WV, Wang HL.* The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: a pilot human trial. J Periodontol 2005; 76(10): 1768–77.
27. *Anilkumar K, Geetha A, Umasudhakar, Ramakrishnan T, Vijayalakshmi R, Pameela E.* Platelet-rich-fibrin: A novel root coverage approach. J Indian Soc Periodontol 2009; 13(1): 50–4.
28. *Aleksić Z, Janković S, Dimitrijević B, Pucar A, Lazić V, Leković V.* Clinical impact of platelet rich plasma in treatment of gingival recessions. Srp Arh Celok Lek 2008; 136(3–4): 95–103. (Serbian)
29. *Aleksić Z, Janković S, Dimitrijević B, Divić RT, Milinković I, Leković V.* The use of platelet rich fibrin membrane in gingival recession treatment. Srp Arh Celok Lek 2010; 138(1–2): 11–8. (Serbian)
30. *McGuire MK, Scheyer ET, Schupbach P.* Growth factor mediated treatment of recession defects a randomized controlled trial and histologic and microcomputed tomography examination. J Periodontol 2009; 80(4): 550–64.
31. *Kawase T, Okuda K, Wolff LF, Yoshie H.* Platelet rich plasma derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol 2003; 74(69: 858–64.
32. *Zucchelli G, Mele M, Stefanini M, Mazzoti C, Mounssif M, Marzadori M,* et al. Predetermination of root coverage. J Periodontol 2010; 81(7): 1019–26.
33. *Suaid FF, Carvalho MD, Santamaria MP, Casati MZ, Nociti FH Jr, Sallum AW,* et al.. Platelet-rich plasma and connective tissue grafts in the treatment of gingival recessions: a histometric study in dogs. J Periodontol 2008; 79(5): 888–95.

Received on April 12, 2012.
Revised on April 25, 2012.
Accepted on April 25, 2012.

ORIGINAL ARTICLE

UDC: 613.73:612.766.1]:613.11
DOI: 10.2298/VSP120630013V

The effects of acclimatization on blood clotting parameters in exertional heat stress

Uticaj aklimatizacije na pokazatelje hemostaze u toplotnom stresu usled fizičkog napora

Zoran Vesić*, Milica Vukašinović-Vesić†, Dragan Dinčić‡, Maja Šurbatović‡, Sonja S. Radaković‡

***Ministry of Defence, Belgrade, Republic of Serbia; †Association of Sport Medicine of Serbia, Belgrade, Serbia;, ‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Exertional heat stress is a common problem in military services. Considering the coagulation abnormalities are of major importance in development of severe heat stroke, we wanted to examine changes in hemostatic parameters in soldiers during exertional heat stress test as well as the effects of a 10-day passive or active acclimatization in a climatic chamber. **Methods.** A total of 40 male soldiers with high aerobic capacity performed exertional heat stress test (EHST) either in cool [20ºC, 16ºC wet bulb globe temperature (WBGT)], or hot (40ºC, 29ºC, (WBGT) environment, unacclimatized (U) or after 10 days of passive (P) or active (A) acclimatization. Physiological strain was measured by tympanic temperatures (Tty) and heart rates (HR). Platelet count (PC), antithrombin III (AT), and prothrombin time (PT) were assessed in blood samples collected before and immediately after the EHST. **Results.** EHST in hot conditions induced physiological heat stress (increase in Tty and HR), with a significant increase in prothrombin time in the groups U and A. Platelet counts were significantly higher after the EHST compared to the basic levels in all the investigated groups, regardless environmental conditions and acclimatization state. Antithrombin levels were not affected by EHST whatsoever. **Conclusion.** In the trained soldiers, physiological heat stress caused mild changes in some serum parameters of blood clotting such as prothrombin time, while others such as antithrombin levels were not affected. Platelet counts were increased after EHST in all groups. A 10-day passive or active acclimatization in climatic chamber showed no effect on parameters investigated.

Key words:
physical exertion; heat stress disorders; acclimatization; blood coagulation; military personnel; serbia.

Apstrakt

Uvod/Cilj. Toplotni stres usled fizičkog napora predstavlja čest problem u vojsci. Pošto su poremećaji koagulacije veoma značajni za razvoj teškog toplotnog udara, cilj istraživanja bio je da se ispitaju promene parametara koagulacije tokom toplotnog stresa usled fizičkog napora, kao i uticaj desetodnevne pasivne, odnosno aktivne aklimatizacije. **Metode.** Četrdeset vojnika muškog pola, visoke aerobne sposobnosti, izloženo je fizičkom naporu submaksimalnog intenziteta i to: grupa C u termoneutralnoj sredini: 20ºC, ili 16ºC WBGT (indeks vlažnog i globus termometra), a ostali u toploj sredini (40ºC, 29ºC WBGT), i to neaklimatizovani (U), nakon 10-dnevne pasivne (P) ili aktivne (A) aklimatizacije u klimatskoj komori. Fiziološko opterećenje određivano je preko timpanične temperature (Tty) i frekvencije srčanog rada (HR). Serumske vrednosti protrombinskog vremena (PT), antitrombina III (AT) i broja trombocita (PC) određivane su iz uzoraka krvi uzetih pre i odmah nakon testa. **Rezultati.** Fizički napor u toploj sredini izazvao je značajno povećanje vrednosti PT u neaklimatizovanoj i aktivno aklimatizovanoj grupi. Broj trombocita bio je značajno povećan nakon testa i to u svim grupama, bez obzira na temperaturne uslove tokom testa i na stanje aklimatizacije ispitanika. Vrednosti AT nisu se promenile ni u jednoj grupi. **Zaključak.** Kod utreniranih vojnika toplotni stres kombinovan sa fizičkim naporom dovodi do promene nekih parametara koagulacije (produženja PT), dok nema uticaja na druge parametre, kao što je vrednost AT. Povećanje broja trombocita nakon testa može se pripisati dejstvu fizičkog napora, bez dodatnog uticaja toplotnog stresa, a 10-dnevna pasivna ili aktivna aklimatizacija ne utiče na ove promene.

Ključne reči:
napor, fizički; stres uzrokovan toplotom; aklimatizacija; krv, koagulacija; vojnici; srbija.

Correspondence to: Sonja Radaković, Faculty of Medicine of the Military Medical Academy, University of Defence, Crnotravska 17, Belgrade, Serbia. E-mail: sonja.radakovic@vma.mod.gov.rs

Introduction

Heat stress can be a significant problem in military services. Common preventive measures, such as restriction of physical activity, taking off clothes, and moving into shade, are usually suppressed by a strong motivation to accomplish the task. Heat stress can impair both physical and mental performance, but at the same time, can influence the vital body functions, with heat stroke as the most severe consequence.

Heat stroke is a life-threatening syndrome characterized by multiple organ dysfunction, including arterial hypotension, hyperthermia, and central nervous system disorders [1, 2]. Excessive activation of systemic inflammation and hypercoagulable state may contribute to multiple organ failure and dysfunction in heat stroke [3, 4]. The heat illness considers a certain continuum, so it is of major importance to understand the processes lying underneath. The heat illness syndrome is typically depicted as a series of discrete events, characterized by pathophysiologic responses that increase in severity as one moves from the mildly impaired functioning such as heat cramps to heat exhaustion and heat stroke. Heat exhaustion is the most common heat syndrome, but, unrecognized, can progress to heat stroke [5]. Coagulation disorders are well described as part of classic heat stroke in elderly people during heat waves [6], but, considering the similar pattern of pathophysiologic response, it is also reasonable to assume that it may follow the exertional heat exhaustion, which typically occurs in healthy, young individuals undergoing strenuous physical activity in hot environments. Athletes and soldiers represent a high-risk population for this form of heat injury, in which these impairments have not been understood completely.

It is well established that acclimation to heat produced physiological adaptations which result in decreased physiological strain and increased tolerance during exercise in the heat [7, 8]. However, the effects of acclimatization on blood clotting during exertional heat stress have been of little interest so far. The aim of this study was to investigate the effect of exertional heat stress and the influence of acclimation on physiological functioning and serum blood clotting parameters in young soldiers.

Methods

Forty male soldiers (20.1 ± 0.9 years) participated in the trial after being informed of the purpose and details of the trial, any known risks and discomforts, and their right to terminate participation at will. After briefing, the soldiers gave their written informed consents to participate. The experimental protocol was approved by the Ethical Committee of the Military Medical Academy in Belgrade. Medical supervision of the subjects was conducted according to international standards [9]. Standard anthropometric measurements were conducted, baseline levels of maximal aerobic power (VO_{2max}) was indirectly determined on treadmill.

The investigation was conducted during wintertime (late November and December) in Military Medical Academy, Belgrade. The soldiers were randomly divided into four equal groups. The first group were unacclimatized controls, who performed the exertional heat-stress test (EHST) in cool environment (C). Another unacclimatized group performed the EHST in hot environment (U), and the rest two groups performed the same test, but after 10 days of acclimation in a climatic chamber (3 hours each day, at 35ºC, relative humidity 40%, wind speed < 0.1 m/s); acclimation was in one group conducted passively (P), and in the other actively (A), with 1 hour walking on a treadmill, 5.5 km/h. The EHST included walking on a motorized treadmill (5.5 km/h) either in a cool [20ºC, wet bulb globus termometer temperature (WBGT) 16ºC – group C) or hot (40ºC, WBGT 29ºC – group U, P, and A) environment, while wearing a normal combat uniform, with a backpack filled with 20 kg of sand in order to simulate regular weight burden. EHST duration was maximally 90 min; the criteria for termination were: tympanic temperature (Tty) 39.5ºC [10], heart rate (HR) 190 beats/min, or intolerable subjective discomfort. The subjects were allowed to drink tap water at will, up to 1.5 L. Blood samples were collected before the EHST and immediately after it.

The soldiers were closely monitored up to 5 hours after finishing the trial and medically examined after 2 days (ECG, blood pressure and routine blood analysis).

Environmental conditions (dry bulb-temperature, WBGT, relative humidity and wind speed) were measured by MiniLab Light Laboratories, Brighton, England. Core Tty was continuously measured using contact probes (Elektrolaboratoriet, Denmark) with a transducer introduced into the auditory canal and placed toward the eardrum. The temperature was registered every 5 minutes. Heart rate was continuously telemetrically monitored (Quinton instruments, USA), and recorded every 5 minutes. Atithrombin III (AT), prothrombin time (PT), and platelet count were assessed by standard laboratory methods in the Institute for Medical Biochemistry, Military Medical Academy, Belgrade.

Data are presented as means ± SD. The difference was assessed by the Student's *t*-test and Wilcoxon's Signed Rank test for paired samples. The normal distribution was tested by the Shapiro Wilk's test. SPSS 11.5 was used to process statistical material and the 0.05 level of significance was used.

Results

Table 1 shows the physical characteristics of the subjects. All the groups were similar in all the investigated characteristics. None of 40 soldiers showed any symptom of heat stroke or severe heat exhaustion during or after the EHST. No results of any medical exams showed any sign of serious dysfunction. All the soldiers in the group C completed the EHST. However, only one soldier in the group U successfully completed the EHST, in the rest cases tests were terminated between 45 and 70 minutes, mostly due to reaching the ethical barrier for Tty 39.5ºC, or intolerable subjective discomfort. In the acclimatized groups, most of the soldiers managed to finish the test (3 soldiers in the group P and 1 in the group A terminated the test between 60 and 80 minutes, reaching the Tty barrier). Even so, their subjective sensation of discomfort was tolerable, and they were willing to continue the test.

Table 1

Physical characteristics of the subjects

Group	Body height (m)	Body mass (kg)	Body fat content (%)	VO_{2max} (mL/kg/min)
C	1.79 ± 0.05	78.1 ± 5.3	17.1 ± 4.5	56.6 ± 5.9
U	1.82 ± 0.03	75.9 ± 6.6	16.8 ± 2.4	62.9 ± 10.1
P	1.84 ± 0.04	73.9 ± 3.4	15.7 ± 1.9	55.4 ± 5.1
A	1.79 ± 0.05	73.7 ± 9.4	16.9 ± 3.8	56.2 ± 7.7

Data presented as means ± SD; C – cool environment; U – hot environment (unacclimatized); P – passive acclimatization; A – active acclimatization.

The mean Tty and HR values are presented in Figures 1 and 2. In the first 20 minutes (before sweating onset) there was an increase in Tty in all the groups, and after that in the group C Tty remained constant. Tty raised steadily in all groups performed EHTS in hot environment, with slightly lower values recorded in the acclimatized groups. Heart rates in the group C were steady, while in hot conditions there was a permanent increase in HRs in all the groups similarly, but *vs* 267.0 ± 45.02 $\times 10^3/mm^3$ after the EHST (Z = -2.703; p = 0.007) in thr group C; 257.3 ± 38.0 $\times 10^3/mm^3$ before *vs* 300.8 ± 49.97 $\times 10^3/mm^3$ after the EHST (Z = -2.701; p = 0.007) in the group U; 225.9 ± 55.81 $\times 10^3/mm^3$ before *vs* 267.3 ± 69.43 $\times 10^3/mm^3$ after the EHST (Z = -3.156; p = 0.028) in the group P, and 252.56 ± 56.37 $\times 10^3/mm^3$ before *vs* 313.89 ± 83.26 $\times 10^3/mm^3$ after the EHST (Z = -2.666; p = 0.008) in the group A. The EHST did not influence plasma

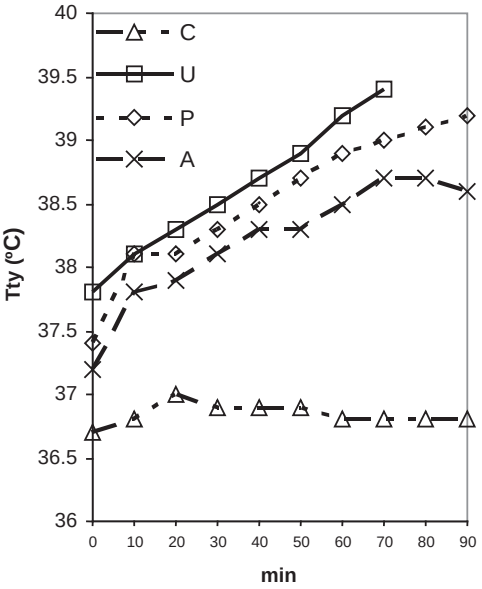


Fig. 1 – Core tympanic temperatures (Tty) during exertional heat stress test

C – cool environment; U – hot environment (unacclimatized); P – passive acclimatization; A – active acclimatization.

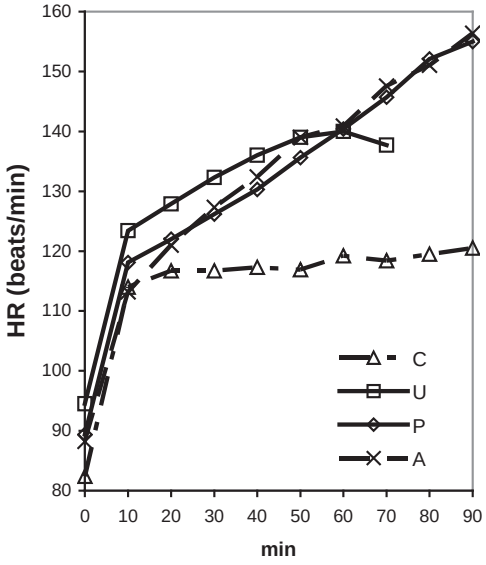


Fig. 2 – Heart rate (HR) during the exertional heat stress test

C – cool environment; U – hot environment (unacclimatized); P – passive acclimatization; A – active acclimatization.

the limit of 190 beats/min was never reached. A maximal recorded HR was 163 beats/min.

Table 2 summarizes the plasma levels of AT, PT, and platelet count (PC) for the controls, unaccclimatized, passively, and actively acclimatized soldiers performing EHST (the groups U, P, and A).

The table reveals that PT values were significantly increased after the EHST compared to the basic values in the group U and A (0.94 ± 0.08 s before *vs* 1.01 ± 0.04 s after the EHST; Wilcoxon Z = -4.583; p = 0.009, and 0.97 ± 0.04 s before *vs* 1.03 ± 0.05 s after the EHST; Z = -2.373; p = 0.018, respectively). Platelet counts were significantly increased after the EHST compared to basic levels in all investigated groups, but did not differ among the groups, neither before, nor after the EHST: 242.6 ± 42.82$\times 10^3/mm^3$ before AT levels regardless environmental conditions, and acclimatization state whatsoever.

Discussion

Impaired working efficiency is a well-known consequence of heat strain. This is particularly important in military services. Core temperature is considered a relevant indicator of thermal strain. Military training guidelines for continuous physical work times are based on achieving core temperature of 40ºC in acclimatized individuals with appropriate fluid replacement [11]. Well-trained athletes and soldiers may tolerate hyperthermia without adverse side effects due to training-induced heat acclimatization effects on cellular protective mechanisms [12].

Table 2

Serum values of blood clothing parameters before and after the exertional heat stress test

Statistical parameters for groups		Platelet count ($\times 10^3/mm^3$)		Prothrombin time (s)		Atithrombin III (U)	
		before EHST	after EHST	before EHST	after EHST	before EHST	after EHST
C	mean	242.60	267.00	1.04	1.05	1.11	1.11
	SD	42.82	45.02	0.46	0.04	0.09	0.10
	SE	13.54	14.24	0.01	0.01	0.03	0.03
	t-test	-5.036; $p = 0.001$		-0.802; p = 0.443		0.350; $p = 0.734$	
	W	Z = -2.703; $p = 0.007$		Z = -0.816; $p = 0.414$		Z = -0.051; $p = 0.959$	
U	mean	257.30	300.80	0.94	1.01	1.16	1.19
	SD	38.00	49.97	0.08	0.04	0.17	0.13
	SE	12.02	15.80	0.02	0.01	0.05	0.04
	t-test	-3.779; $p = 0.004$		-4.583; $p = 0.001$		-0.835; $p = 0.425$	
	W	Z = -2.701; $p = 0.007$		Z = -2.626; $p = 0.009$		Z = -0.818; $p = 0.413$	
P	mean	225.90	267.30	1.07	1.04	1.10	1.07
	SD	55.81	69.43	0.04	0.07	0.08	0.09
	SE	17.65	21.96	0.01	0.02	0.02	0.03
	t-test	-3.156; $p = 0.012$		1.545; $p = 0.157$		2.090; $p = 0.046$	
	W	Z = -2.194; $p = 0.028$		Z = -1.425; $p = 0.154$		Z = -1.737; $p = 0.082$	
A	mean	252.56	313.89	0.97	1.03	1.10	1.06
	SD	56.37	83.26	0.04	0.05	0.05	0.06
	SE	18.79	27.75	0.01	0.02	0.02	0.02
	t-test	-3.956; $p = 0.004$		-3.773; $p = 0.005$		1.789; $p = 0.111$	
	W	Z = -2.666; $p = 0.008$		Z = -2.373; $p = 0.018$		Z = -1.540; $p = 0.123$	

C – cool environment; U – hot environment (unacclimatized); P – passive acclimatization; A – active acclimatization; EHCT – exertional heat-stress test; W – Wilcoxon's Singed Rank test.

In our study, at high degree of heat strain, the majority of soldiers did not approach the levels at which their activity should be reduced. The HR values were well within the predicted maximum for their age, indicating that the workload had not exceeded their physical capabilities, considering their high baseline levels of VO_{2max}.

In 2002, a new definition of heat stroke was introduced suggesting that multi-organ system failure was due the combined effects of heat cytotoxicity, coagulopathies, and the systemic imflammatory response syndrome [13]. During heat stress, systemic inflammation and activated coagulation are displayed, evidenced by increased prothrombin time, activated thromboplastin time, and D-dimer, and decreased platelet count, and protein C. Hypercoagulable state, along with systemic inflammation, can result from oxidative stress and thus may contribute to organ failure and dysfunctioning in heat stress [3, 14]. The inflammatory response is an important local defence mechanism against infection and injury [15]. Because the inflammatory response is inseparable from the coagulation process, coagulation disorders are often associated with severe inflammatory disease. In experiments conducted on rodents, during heat stroke, leukocytosis, coagulation abnormalities and abnormalities of prothrombin consumption, thromboplastin generation, clotting time, one-stage prothrombin and clot retraction are common [16, 17].

The major physiological response to heat stress considers primary cardiovascular adaptation, resulting in increased skin blood flow, in order to increase heat loss. Prolonged redistribution of blood flow into the skin leads to progressive reduction of splanchnic blood flow, which is followed by nitrosative and oxidative stress, and production of reactive oxygen species. These products cause leakage at the sites of intestinal tight junctions, leading to increased permeability for Gram-positive and Gram-negative bacteria and their endotoxins. Intestinal tissue damages due to ischaemic environments contribute to local tissue inflammation and activation of inducible nitric oxide synthase and generation of reactive nitrogen species. These pathophysiological mechanisms are proposed to set a foundation to inflammation and coagulation abnormalities in heat stress [13, 14, 18].

Disseminated intravascular coagulation is a common complication of heat stroke that is initiated following thermal injury to the vascular endothelium and is regarded as an important mechanism of heat stroke morbidity and mortality. In vitro studies have shown that heat (43–44ºC) directly activates platelet aggregation and causes irreversible hyperaggregation following cooling [19]. Early in heat stroke, widespread activation of coagulation stimulates excess deposition of fibrin in the arterioles and capillaries along with platelet aggregation that leads to microvascular thrombosis. Although rapid cooling of the heat stroke patient may normalize fibrinolysis, coagulation often persists until platelets and coagulation proteins are consumed at a faster rate than they are produced. Consumptive coagulation may lead to excessive, prolonged bleeding from multiple tissue sites (venipuncture sites, gums) and is associated with fatal outcome. The host inflammatory and hemostatic responses are closely associated not only with fatal heat stroke but also with severe heat stress, especially when combined with physical strain [2, 20].

The prothrombin time provides information about the extrinsic (tissue factor) and common coagulation pathways [21]. In our study, heat stress combined with intensive physical activity led to increase in prothrombin time values in soldiers performed the EHST in hot condition. We observed no difference in the control group, which suggests that the same intensity of physical strain did not result in increasing of prothrombin time. Hence, this effect on prothrombin time can be attributed to heat stress, regardless physical activity.

These findings can indicate the tendency of prothrombin consumption when exposed to heat stress.

Antithrombin is synthesized by the liver. Human and animal studies have shown that antithrombin behaves as a negative acute-phase protein [22, 23]. According to our results, heat stress combined with strenuous physical work does not influence the antithrombin values, because we found no difference between levels before and after the EHST, regardless environmental conditions and acclimatization state. These findings suggest that the exertional heat stress of given intensity and duration could not challenge the acute-phase reaction reflected in antithrombin disturbances.

Finally, according to the results obtained in our study, exertional heat stress induced increase in platelet counts in all investigated groups, regardless of environmental conditions and acclimatization state. These findings are in disagreement with the proposed mechanisms of platelet aggregation and consumption during heat stress [3]. We suggest that inflammatory triggers for coagulation onset were not activated at the given intensity of heat stress combined with hot environmental conditions. The subjects in our investigation were healthy, fit young males, well-trained and well hydrated, with fully mobilized protective mechanisms (both acclimatization and acquired tolerance to heat), which made them resistant to endothelial injury that lays beneath the hemostatic disturbances in heat stress. The common risk factors for heat coagulopathy such as preexisting illness, drug use, alcohol, amphetamines, ecstasy abuse, were also absent in the investigated population.

Conclusion

This study demonstrated the effects of physical activity in a hot environment on physiological parameters, as shown by an increase in Tty and HR. This physiological heat stress cause mild changes in serum parameters of blood clotting such as prothrombin time, while antrithrombin values were not affected by the stress. Contrary to the expected, platelet count increased during exertional heat stress.

A 10-day acclimatization, either passive or active, showed no effect on parameters investigated, possibly due to a preexisting high level of aerobic capacity and tolerance to heat, which could not be additionally improved.

REFERENCES

1. *Simon HB.* Hyperthermia. N Engl J Med 1993; 329(7): 483−7.
2. *Bouchama A, Knochel JP.* Heat stroke. N Engl J Med 2002; 346(25): 1978−88.
3. *Lee J, Lin M, Wang N, Lin C, Chang C.* Platonin, a cyanine photosensitizing dye, causes attenuation of circulatory shock, hypercoagulable state, and tissue ischemia during heat stroke. Shock 2005; 24(6): 577−82.
4. *Bouchama A, Dehbi M, Mohamed G, Matthies F, Shoukri M, Menne B.* Prognostic factors in heat wave related deaths: A meta-analysis. Arch Intern Med 2007; 167(20): 2170−6.
5. *Leon LR. Heat stroke and cytokines.* Progress in brain research 2007; 162: 481−524.
6. *Huisse MG, Pease S, Hurtado-Nedelec M, Arnaud B, Malaquin C, Wolff M,* et al. Leukocute activation: the link between inflammation and coagulation during heatstroke. A study of patients during the 2003 heat wave in Paris. Crit Care Med 2008; 36(8): 2288−95.
7. *Radaković SS, Marić J, Surbatovic M, Raden S, Stefanova ED, Stanković N, Filipović N.* Effects of acclimation on cognitive performance in soldiers during exertional heat stress. Vojnosanit Pregl 2009; 66(5): 359−64.
8. *Cheung SS, Mclellan TM.* Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. J Appl Physiol 1998; 84(5): 1731−9.
9. ISO 12894 Ergonomics of the thermal environment - Medical supervision of individuals exposed to extreme hot or cold environment. Geneva: International Organisation for Standardisation; 2001.
10. *Selkirk GA, Mclellan TM.* Influence of aerobic fitness and body fatness on tolerance to uncompensable heat stress. J Appl Physiol 2001; 91(5): 2055−63.
11. *U.S. Army Heat Stress Control and Heat Casualty Menagment.* Department of the Army and Air Force technical bulletin. Washington, DC: Department of the Army; 2003: 48−152. Report No. TBMED 507/AFPAM (I).
12. *McClung JP, Hasday JD, He JR, Montain SJ, Cheuvront SN, Sawka MN,* et al. Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. A J Physiol Regul Integr Comp Physiol 2008; 294(1): R185−91.
13. *Leon LR, Helwig BG.* Heat stroke: role of the systemic inflammatory response. J Appl Physiol 2010; 109(6): 1980−8.
14. *Hall DM, Buettner GR, Oberley LW, Xu L, Matthes RD, Gisolfi CV.* Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am J Physiol Heart Circ Physiol 2001; 280(2): H509−21.
15. *Zimmerman GA, Mcintyre TM, Prescot SM.* The platelet-activation factor signaling system and its regulators in syndromes of inflammation ant thrombosis. Crit Care Med 2002; 30(5 Suppl): S294−301.
16. *Chen S, Niu K, Lin M.* Cerebrovascular dysfunction is an attractive target for therapy in heat stroke. Clin Experiment Pharmacol Physiol 2006; 33(8): 663−72.
17. *Khosla R, Guntupalli KK.* Heat-related illnesses. Crit Care Clin 1999; 15(2): 251−63.
18. *Dokladny K, Moseley PL, Ma TY.* Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am J Physiol Gastrointest Liver Physiol 2006; 290(2): G204−12.
19. *Gader AMA.* Al-mashhadani SA, Al-harthy SS. Direct activation of platelets by heat is the possible trigger of the coagulopathy of heat stroke. Br J Haematol 1990; 74(1): 86−92.
20. *Bouchama A, Kunzelmann C, Dehbi M, Kwaasi A, Eldali A, Zobairi F,* et al. Recombinant Activated Protein C Attenuates Endothelial Injury and Inhibits Procoagulant Microparticles Release in Baboon Heatstroke. Atheroscler Thromb Vasc Biol 2008; 28(7): 1318−25.
21. *Cheng T, Mathews K, Abrams-Ogg A, Wood D.* The link between inflammation andcoagulation: influence on the interpretation of diagnostic laboratory tests.Compend Contin Educ Vet 2011; 33(2): E1−E12.
22. *Niessen RWL.* Antithrombin acts as a negative acute phase protein as established with studies on HepG2 cells and in baboons. Thromb Haemost 1997; 78(3): 1088−92.
23. *Dhainaut JF, Marin N, Mignon A, Vinsonneau C.* Hepatic response to sepsis: interaction between coagulation and inflammatory processes. Crit Care Med 2001; 29(7 Suppl): S42−7.

Received on July 30, 2012.
Accepted on September 24, 2012.
OnLine-First March, 2012.

GENERAL REVIEW

UDC: 576.36:[616-006.04-092:616-006-08
DOI: 10.2298/VSP1307675S

Reactive oxygen species, apoptosis and cancer

Reaktivne vrste kiseonika, apoptoza i kancer

Slavica Stojnev, Ana Ristić-Petrović, Ljubinka Janković-Veličković

Faculty of Medicine, University of Niš, Niš, Serbia

Key words:
reactive oxygen species; apoptosis; neoplasms; oxidative stress.

Ključne reči:
kiseonik, reaktivne vrste; apoptoza; neoplazme; stres, oksidativni.

Introduction

Reactive oxygen species (ROS) comprise oxygen free radicals, including superoxide anion (O_2^-), hydroxyl ($HO^\bullet$), peroxy ($RO2^\bullet$) and alkoxy ($RO^\bullet$) radicals, and oxygen-derived non-radical species like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [1, 2]. As a consequence of aerobic metabolism, ROS are continuously generated in biological systems and simultaneously detoxified by complex antioxidative mechanisms [3]. Oxidative stress develops due to imbalance between the systems that generate and scavenge free radicals [4]. Persisting oxidative stress leads to the accumulation of oxidative damage of the crucial biomolecules: genomic DNA, lipids and proteins.

Reactive oxygen species in carcinogenesis

Aside from DNA-damaging function, ROS can act as second messengers and control various signaling cascades which induce and maintain the oncogenic phenotype of cancer cells [5]. The effects of ROS include anticancer activities (cell cycle arrest, senescence, apoptosis or necrosis, inhibition of angiogenesis) and pro-cancer activities (promotion of cell proliferation, invasiveness, angiogenesis, metastases and apoptosis suppression) [6].

The incidence of malignant neoplasms increases with age. Lifelong constant attacks of free radicals are considered one of the main culprits for this. The average rate of DNA oxidative products generation is about 1 in 10^5 DNA bases, but is this enough to cause the spontaneous malignancy related to age? It was found that free radicals primarily damage specific genomic sequences that are important for carcinogenesis [6, 7]. Per example, oxidative DNA damage in gastric mucosa in patients with *Helicobacter pylori* infection is unevenly distributed among the genes, with a tropism for TP53 gene [8].

Significant number of enzymatic and non-enzymatic systems protects the cell from ROS toxicity. Their impairment could also lead to deteriorating effects of ROS. Glutathione (GSH), an important guardian against the oxidative damage, accumulates abundantly within the mitochondria of cancer cells [9]. *In vivo* studies demonstrated that GSH depletion sensitizes tumor cells to oxidative cytolysis [3, 9]. The study on knockout mice with the deficiency of copper-zinc superoxide dismutase (SOD), the enzyme which is main intracellular scavenger of superoxide radicals, revealed the increased liver cancer incidence in such animals [10]. Deficiency of manganese SOD, which is main O_2^- cleaner in mitochondrial matrix, leads to mice mortality quickly after birth [11]. The animals that are heterozygotes for manganese SOD survive, but carry increased risk to develop lymphoma, adenocarcinoma and pituitary adenoma [12].

Radiation induced carcinogenesis is largely based on generation of very reactive $HO^\cdot$ radical, which interacts with DNA causing the formation of mutagenic purines, pyrimidines and oxidative deoxyribose products, such as 8-hydroxy-2-deoxyguanosine (8OHdG) [6, 13]. The defects in enzymes responsible for reparation of oxidative DNA damage increase the level of 8OHdG and other mutagenic bases, leading to higher incidence of age-related cancer in animals [14]. The DNA itself or its precursors can be altered by ROS. In mice with MTH1 enzyme deficiency, which hydrolyses DNA precursors damaged by oxidative mechanisms, the incorporation of these defective nucleotides in DNA is enabled. This increases the rate of spontaneous tumorigenesis primarily in lungs, stomach and liver [14, 15].

The role of ROS in apoptosis

It is well-established that mediators of apoptosis can induce intracellular production of ROS. Moreover, various reactive species can either initiate apoptosis or modify its course [4].

Correspondence to: Slavica Stojnev, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18 000 Niš, Serbia.
Phone: +381 64 32 333 90. E-mail: slavicastojnev@gmail.com

Apoptosis can be induced by extracellular or intracellular signals and is conducted through two major pathways: mitochondrial (intrinsic) or death receptor pathway (extrinsic). Extrinsic pathway of apoptosis is triggered by interaction of death receptor and its ligand in cellular plasma membrane [16], which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and generation of ROS. This further leads to activation of acid sphingomielinase, generation of ceramide and clustering of the receptors. The described processes form signal platform that induces apoptotic cascade [17]. Activation of NF-κB survival path increases the transcription of anti-apoptotic proteins (FLIP, MnSOD, Bcl-X, IAP) and blocks the apoptosis. In the presence of high ROS concentrations, the impossibility of NF-κB induction triggers the activation of ASK1/JNK kinases, which initiates apoptosis [18].

Intrinsic apoptotic cascade is associated with the changes in permeability of outer mitochondrial membrane, which allows the release of proapototic proteins. ROS are well known triggers of intrinsic apoptotic pathway through interaction with outer mitochondrial membrane proteins [19]. Proteins of the Bcl-2 family determine the susceptibility to apoptosis, shifting the balance between pro-apoptotic (Bax, Bak, Bad, Bim and Bid) and anti-apoptotic (Bcl-2, Bcl-XL and Bcl-w) members of the family in favour of apoptosis or against it. In the presence of apoptotic stimuli like ROS, truncated form of Bid protein causes Bax/Bak oligomerization which leads to creation of megapores in mitochondrial membrane [20]. Subsequently, apoptosome complex is formed in the cytosol, activating initiator Caspase 9 and than Caspase 3, which executes the final steps of apoptosis. Caspase activation is further enhanced due to neutralization of caspase inhibitors by proteins released from mitochondria, like Smac/Diablo and Omi/HtrA2 [19]. In addition, mitochondrial proteins like apoptesis-inducing factor and Endo G stimulate caspase-independent apoptosis via translocation into nucleus, where they mediate genomic DNA fragmentation [21].

Programmed cell death is often characterized by generation of large quantities of ROS or transient oxidative burst [22]. Catalase prevents H_2O_2 mediated apoptosis, indicating the important role of H_2O_2 in apoptosis [2]. Recently it has been found that H_2O_2 initiates caspase activation which is dependant on mitochondrial cytochrome c release [23]. However, very high doses of H_2O_2 or other ROS induce cell necrosis [13, 23]. This implies that the modus of cell death induced by oxidative stress is dose dependant. Since apoptosis represents active, energy dependant process, it seems that the intracellular ATP level is crucial determinant weather the apoptotic cascade would eventuate.

The interplay of ROS and apoptosis in cancer

Although apoptosis and cancer represent opposite entities, ROS play an important role in both of the processes. Moderate levels of ROS, where "moderate" depends on the cell type, promote apoptosis in tumor cells [24]. Nevertheless, in some malignant cells ROS can exert the opposite effect. In melanoma cell line M14, overexpression of zinc-copper SOD causes the decrease in O_2^- concentration and thus promotes apoptosis [25]. Generation of ROS by NADPH oxidase enzymatic system acts as an anti-apoptotic, pro-proliferative stimulus in pancreatic cancer cell lines [26]. The ability of mitochondrial apoptosis-inducing factor to oxidase NADH and generate superoxide anion contributes to viability of some cancer cell lines [27].

The mechanisms by which ROS exert their anti-apoptotic effect have not been completely elucidated so far. It has been hypothesized that caspase inactivation could be responsible. In some cells elevated O_2^- causes the increase in cytosolic pH value, which halts the caspase activation [28]. In opposite, H_2O_2 lowers pH in cytosol and thus acts in favor of apoptosis initiation. In addition, H_2O_2 promotes apoptosis through conversion in $HO^·$ radical that directly attacks DNA or damages mitochondria [28]. An alternative mechanism includes inactivation of PTEN protein (product of tumor suppressor gene PTEN), which increases the activation of Akt signaling pathway, thus promoting cell survival. However, Akt signaling is often attributed ambiguous roles, since some studies have indicated that the activation of this path supports cancer cell survival, but suppresses invasiveness and metastases [29].

The phenomenon of apoptosis has been investigated in many types of cancer and its significance has been well-established for urothelial carcinoma [30–32]. Recent findings have suggested that oxidative stress sensitizes tumor cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis by downregulation of anti-apoptotic proteins [33]. Moreover, TRAIL protein has gained a lot of attention as a new therapeutic target because of its property to induce apoptosis only in cancer cells, but not in normal cells [34]. However, some urothelial carcinomas develop resistance to TRAIL, therefore the reestablishment of this sensitivity is of great importance. Induction of oxidative stress with low concentration of H_2O_2 can reverse the resistance to TRAIL by significantly decreasing the expression of short living anti-apoptotic proteins such as FLIP, XIAP and Survivin [33]. Further studies are to determine the relevancy of H_2O_2 application as adjuvant intravesical agent in urothelial cancer treatment, in order to lower the threshold for initiating apoptosis in malignant cells.

Apoptosis induced by ROS in anticancer treatment

Numerous studies confirmed that generation of free radicals capable of inducing apoptosis in malignant cells is the underlying mechanism of action for numerous chemotherapeutics and radiotherapy [3, 35, 36]. Antineoplastic agents that induce generation of ROS in large quantities are anthracyclines (doxorubicin, bleomycin), platinum complexes (cisplatin), alkylating agents (cyclophosphamide), epipodophyllotoxins, camptothecin derivatives [37]. In the course of radiotherapy of malignant disease, apoptotic signal can be generated in plasma membrane through lipid peroxidation induced by radiation [13]. Antioxidants reduce ROS generation and thus contribute to preserve the integrity of healthy tissue, but also decrease the damage on tumor cells [38].

ROS generating anticancer drugs lead to depletion of intracellular antioxidative capacity and when the ROS concentration reaches a certain threshold, apoptosis is initiated [13]. In the absence of adequate antioxidative defense, damage caused by ROS induces activation of apoptosis-related genes. In addition, intracellular ROS increase can also activate redox sensitive JNK/SAPK signaling pathway, often implicated in genes transactivation and posttranslational modifications of proteins required for apoptosis [23].

Future implications

Pharmacological manipulations are able to shift the intracellular redox equilibrium toward the increase in ROS and/or the depletion of protective agents, inducing the apoptotic cascade in cancer cell. The studies investigating application of antioxidant inhibitors and/or ROS generating agents in order to induce apoptosis or to overcome the resistance to chemotherapy [39, 40] have provided significant advances, but further validation of the results is necessary.

Genomics and proteomics represent powerful and promising tools in unraveling complex molecular networks [41, 42]. Investigators have attempted to understand the subset of proteins whose expression levels are directly altered by oxidants, or those proteins that are posttranslationally modified in a redox-dependent fashion [43]. Recent proteomics based study identified gold (III) porphyrin 1a as a new, potent anticancer drug that induces apoptosis through both caspase-dependent and caspase-independent mitochondrial pathways, and demonstrated that intracellular oxidation affected gold (III) porphyrin 1a-induced apoptosis [44]. The difficulty with advanced "omics" methodologies is how to translate the provided data into a meaningful clinical context, *ie* into clinical trial design and subsequently into routine clinical use [41].

One of the most important goals in cancer studies is directed to the possibility of treating cancer by inducing apoptosis via caspase activation. ROS could play a major role in this process, acting as potent mediators. The greatest challenge remains the issue of inducing apoptosis selectively within the neoplastic cells, with maximal spearing of normal tissue. The therapeutic strategy based on triggering apoptosis by modulation of ROS levels, selectively within the neoplastic cells, carries great expectations and holds promise of a significant advance in clinical oncology.

Acknowledgements

This work was supported by the Ministry of Education Science and Technological Development of the Republic of Serbia (Grant No. 175092).

R E F E R E N C E S

1. *Circu ML, Aw TY*. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010; 48(6): 749−62.
2. *Simon HU, Haj-Yehia A, Levi-Schaffer F*. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 2000; 5(5): 415−8.
3. *Engel RH, Evens AM*. Oxidative stress and apoptosis: a new treatment paradigm in cancer. Front Biosci 2006; 11: 300−12.
4. *Matés JM, Sánchez-Jiménez FM*. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol 2000; 32 (2): 157-70.
5. *Storz P*. Reactive oxygen species in tumor progression. Front Biosci 2005; 10: 1881-96.
6. *Halliwell B*. Oxidative stress and cancer: have we moved forward? Biochem J 2007; 401(1): 1−11.
7. *Stojnev S, Golubović M, Babović P*. TP53 gene mutations – from guardian of the genome to oncogene. Acta Medica Medianae 2010; 49(1): 59−63.
8. *Choi J, Yoon SH, Kim JE, Rhee KH, Youn HS, Chung MH*. Gene-specific oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. Int J Cancer 2002; 99(4): 485–90.
9. *Nathan CF, Arrick BA, Murray HW, DeSantis NM, Cohn ZA*. Tumor cell anti-oxidant defenses. Inhibition of the glutathione redox cycle enhances macrophage mediated cytolysis. J Exp Med 1981; 153(4): 766−82.
10. *Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H,* et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene 2005; 24(3): 367–80.
11. *Melov S, Doctrow SR, Schneider JA, Haberson J, Patel M, Coskun PE*, et al. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase–catalase mimetics. J Neurosci 2001; 21(21): 8348–53.
12. *Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, et al*. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol Genomics 2003; 16(1): 29–37.
13. *Ozben T*. Oxidative stress and apoptosis: impact on cancer therapy. J Pharm Sci 2007; 96(9): 2181−96.
14. *Nakabeppu Y, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T, Nakatsu Y*. Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol Chem 2006; 387(4): 373–9.
15. *Nakabeppu Y, Tsuchimoto D, Furuichi M, Sakumi K*. The defense mechanisms in mammalian cells against oxidative damage in nucleic acids and their involvement in the suppression of mutagenesis and cell death. Free Radical Res 2004; 38(5): 423–9.
16. *Berg D, Lehne M, Muller N, Siegmund D, Munkel S, Sebald W, et al*. Enforced covalent trimerization increases the activity of the TNF ligand family members TRAIL and CD95L. Cell Death Differ 2007; 14(12): 2021–34.
17. *Zhang AY, Yi F, Jin S, Xia M, Chen QZ, Gulbins E, Li PL*. Acid sphingomyelinase and its redox amplification in formation of lipid raft redox signaling platforms in endothelial cells. Antioxid Redox Signaling 2007; 9(7): 817–28.
18. *Micheau O, Lens S, Gaide O, Alevizopoulos K, Tschopp J*. NF-kappaB signals induce the expression of c-FLIP. Mol Cell Biol 2001; 21(16): 5299–305.
19. *Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, Choi AM*. Mechanisms of cell death in oxidative stress. Antioxid Redox Signaling 2007; 9(1): 49–89.
20. *Eskes R, Desagher S, Antonsson B, Martinou JC*. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol Cell Biol 2000; 20(3): 929–35.
21. *Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, et al*. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999; 397(6718): 441–6.

22. *Carmody RJ, Cotter TG*. Signalling apoptosis: A radical approach. Redox Rep 2001; 6(2): 77–90.
23. *Haddad JJ*. Redox and oxidant-mediated regulation of apoptosis signaling pathways: Immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. Int Immunopharmacol 2004; 4(4): 475–93.
24. *Chandra J, Samali A, Orrenius S*. Triggering and modulation of apoptosis by oxidative stress. Free Radical Biol Med 2000; 29 (3−4): 323–33.
25. *Pervaiz S, Clement MV*. Tumor intracellular redox status and drug resistance: serendipity or a causal relationship? Curr Pharm Des 2004; 10(16): 1969–77.
26. *Vaquero EC, Edderkaoui M, Pandol SJ, Gukovsky I, Gukovskaya AS*. Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. J Biol Chem 2004; 279(33): 34643–54.
27. *Urbano A, Lakshmanan U, Choo PH, Kwan JC, Ng PY, Guo K, et al.* AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells. EMBO J 2005; 24(15): 2815–26.
28. *Akram S, Teong HF, Fliegel L, Pervaiz S, Clement MV*. Reactive oxygen species-mediated regulation of the Na^+-H^+ exchanger 1 gene expression connects intracellular redox status with cells' sensitivity to death triggers. Cell Death Differ 2006; 13(4): 628–41.
29. *Toker A, Yoeli-Lerner M*. Akt signaling and cancer: surviving but not moving on. Cancer Res 2006; 66(8): 3963–6.
30. *Ristić A, Janković Veličković Lj, Stokanović D*. Prognostic value of apoptotic activity in muscle-invasive bladder cancer. Vojnosanit Pregl 2011; 68(6): 511−4.
31. *Jankovic-Velickovic L, Hattori T, Stefanovic V*. Molecular markers in upper urothelial carcinoma associated to Balkan endemic nephropathy. Aristolochic acid as the major risk factor of the worldwide disease. ScientificWorldJournal 2009; 9: 1360−73.
32. *Dolićanin Z, Janković Veličković Lj, Katić V*. Biomarkers for detection, treatment decision and prognosis of the urinary bladder cancer. Facta Universitatis 2007; 14(1): 1−5.
33. *White-Gilbertson SJ, Kasman L, McKillop J, Tirodkar T, Lu P, Voelkel-Johnson C*. Oxidative stress sensitizes bladder cancer cells to TRAIL mediated apoptosis by down-regulating anti-apoptotic proteins. J Urol 2009; 182(3): 1178−85.
34. *Falschlehner C, Emmerich CH, Gerlach B, Walczak H*. TRAIL signaling: decisions between life and death. Int J Biochem Cell Biol 2007; 39(7−8): 1462–75.
35. *Kaufmann SH, Earnshaw WC*. Induction of apoptosis by cancer chemotherapy. Exp Cell Res 2000; 256(1): 42–9.
36. *Alexandre J, Batteux F, Nicco C, Chereau C, Laurent A, Guillevin L, et al.* Accumulation of hydrogen peroxide is an early and crucial step for paclitaxel-induced cancer cell death both in vitro and in vivo. Int J Cancer 2006; 119(1): 41–8.
37. *Conklin KA*. Chemotherapy-associated oxidative stress: Impact on chemotherapeutic effectiveness. Integr Cancer Ther 2004; 3(4): 294–300.
38. *Borek C*. Dietary antioxidants and human cancer. Integr Cancer Ther 2004; 3(4): 333–41.
39. *Akan I, Akan S, Akca H, Savas B, Ozben T*. Multidrug resistance-associated protein 1 (MRP1) mediated vincristine resistance: Effects of N-acetylcysteine and Buthionine sulfoximine. Cancer Cell Int 2005; 5(1): 22.
40. *Davison K, Cote S, Mader S, Miller WH*. Glutathione depletion overcomes resistance to arsenic trioxide in arsenic-resistant cell lines. Leukemia 2003; 17(5): 931–40.
41. *Stojnev S, Pejčić M, Dolićanin Z, Janković-Veličković Lj, Dimov I, Stefanović V*. Challenges of genomics and proteomics in nephrology. Ren Fail 2009; 31(8): 765−72.
42. *Pejčić M, Stojnev S, Stefanović V*. Urinary proteomics – a tool for biomarker discovery. Ren Fail 2010; 32(2): 259−68.
43. *Finkel T*. Oxidant signals and oxidative stress. Curr Opin Cell Biol 2003; 15(2): 247−54.
44. *Wang Y, He QY, Sun RW, Che CM, Chiu JF*. GoldIII porphyrin 1a induced apoptosis by mitochondrial death pathways related to reactive oxygen species. Cancer Res 2005; 65(24): 11553−64.

Received on June 7, 2011.
Accepted on July 22, 2011.

CURRENT TOPIC

UDC: 616.12-08
DOI:10.2298/VSP121024007V

Long-term ventricular assist devices in current clinical practice

Dugotrajne ventrikularne pumpe u savremenoj praksi

Lazar Velicki*†, Frazier OH‡§

***Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia; †Institute of Cardiovascular Diseases Vojvodina, Sremska Kamenica, Serbia; ‡Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas, USA; §Baylor College of Medicine, Houston, Texas, USA**

Key words:
heart failure; heart assist device; prognosis.

Ključne reči:
srce, insuficijencija; srce, implantabilni mehanički aparati; prognoza.

Introduction

Chronic heart failure (CHF) is a major healthcare issue associated with significant reduction of the quality of life, poor prognosis – high mortality rate, and still no adequate therapeutic approach available to the majority of the patients.

Statistics indicate that survival rate for patients with CHF is only 50% after 5 years, and significantly less so for those with advanced heart disease, less than 50% after one year. Incidence and prevalence of CHF are also on the increase with more than 16 million diagnosed across Europe and United States – 2.5% of total population, resulting in frequent hospital admissions and long term costs of palliative support. Further still, numbers indicate significant increase of CHF in elderly patient group (65+ years of age), population here expected to double over the next 20 years [1–3].

Although, numerous advancements in medical therapy have improved patient outcomes in CHF, prognosis is still poor and the quality of life remains limited. For patients with end-stage heart failure, heart transplant (HTX) remains to be the only long-term satisfactory option. However, the increasing demand of donor organs is not equilibrated with limited number of available hearts. Current estimates indicate that up to 100,000 patients meet criteria for HTX in the United States [4]. The scarcity of donor organs has fuelled the development of interim interventions such as different types of cardiac surgery operations aimed at restoration of left ventricle geometry and functionality, cardiac resynchronization therapy (CRT) and mechanical circulatory support (MCS). According to the Registry of the International Society for Heart and Lung Transplantation – the 25th Official Adult Heart Transplant Report – 2008, almost 29% of patients at the time of transplant were on some type of MCS modality (22% on left ventricular assist device-LVAD) [5]. Technology advancements resulting in smaller MCS units offer alternative and permanent treatment option for many patients on the heart-transplant waiting list thus mitigating mortality rate [2].

A ventricular assist device (VAD) is a mechanical pump that provides circulatory support in patients with either acute or chronic cardiac failure – when heart can no longer pump blood effectively [6, 7]. Initially, VADs offered temporary mechanical circulatory support for those patients not expected to survive until a new organ became available. Reports of long-term success using VADs indicated possibility of permanent cardiac assist [8]. Today, VADs are frequently applied to varied purpose goals including use as a bridge to transplantation (BTT), destination therapy (DT), a bridge to recovery (BTR) – aiding natural heart in the recovery process by relieving some of the pressure, and a bridge to a decision (BTD) – an evolving paradigm in management of patients who present in acute cardiogenic shock. VADs are most commonly used to support the left ventricle (LVAD), but right ventricular (RVAD) devices are also used, including biventricular support (BiVAD). The basic idea behind any kind of MCS is to provide adequate end-organ perfusion in order to avoid irreversible multi-organ system failure and, if possible, to reduce the work-load of the failing ventricle thereby allowing function recovery (although only a small portion of patients with an idiopathic cardiomyopathy have the potential to myocardial recovery). The pumps are designed to pull blood from the failing ventricle (to unload the chamber) and expel it to the corresponding large vessel and further into circulation. The pump has an inflow cannula that channels blood from the ventricle or atrium to the pump, and an outflow cannula that channels blood from the pump to the

Correspondence to: Lazar Velicki, Institute of Cardiovascular Diseases Vojvodina, Put doktora Goldmana 4, 21 204 Sremska Kamenica, Serbia. Phone.: +381 21 480 5701; Fax.: +381 21 6622 059. E-mail: lvelicki@gmail.com

aorta or pulmonary artery. Depending on the site of the pump, VADs can be classified as intracorporeal (inside the patient), extracorporeal (outside the body) or paracorporeal (immediately adjacent to the patient).

A pivotal randomized study that investigated the effect of mechanical assist devices as a DT on the outcome and quality of life in patients with CHF was the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial [3]. In this landmark trial, a total of 129 patients with end-stage heart failure – New York Heart Association (NYHA) class IV – who were ineligible for cardiac transplantation were assigned to receive a LVAD (68 patients) or optimal medical management (61 patients). "The rates of survival at 1 year were 52% in the device group and 25% in the medical-therapy group (p = 0.002), and the rates at 2 years were 23% and 8% (p = 0.09), respectively" [3]. The final conclusion of the trial was that the use of a LVAD in patients with advanced heart failure resulted in a prolonged survival period and an improved quality of life and as such, LVADs may be considered as an acceptable alternative therapy in selected patients who are not candidates for HTX. This trial was the first to establish the efficacy of device therapy for end-stage heart failure and set the standards for CHF treatment using VADs.

Depending on the level of ventricular reserve or residual volume in the left ventricle to open the aortic valve and generate a pulse, LVAD may be added to support the circulation in two ways. With total unloading of the left ventricle, LVAD is connected to the systemic circulation in a serial manner and the aortic valve is closed all the time [8]. If the failing myocardium has the capability of generating a pulse, the pump is added to the circulation in a parallel fashion – both the device and the native ventricle can pump blood into the ascending aorta. Aortic valve may open, usually during exercise when veins contract and produce increased venous return to the heart.

As a result of significant technological advancements with mechanical pumps in the last decade and their profound impact on the mainstream of our daily practice, guidelines for the CHF treatment need to be regularly updated. Guidelines are expected to be published by all major bodies, including International Society for Heart and Lung Transplantation, American Heart Association and American College of Cardiology Task Force, and the Heart Failure Society of America [9]. One may expect that these guidelines will recommend that every patient with refractory end-stage heart failure should be considered and evaluated for some kind of MCS. The Centre for Medicare and Medicaid Services requires that patients exhibit NYHA class IV symptoms (optimal medical therapy refractory patients) to qualify for MCS therapy [10]. Currently, MCS is recommended to those patients facing imminent death due to heart failure still having sustained end-organ function. In other words, candidates for long-term assist devices are those with inadequate hemodynamics despite optimized drug therapy and/or intra-aortic balloon pump assistance [11]. Hemodynamic parameters that may guide selection of patients suitable for device therapy include: pulmonary capillary wedge pressure (PCWP) of >20 mmHg, a cardiac index of $\leq$ 2 $Lmin^{-1}m^{-2}$, and a systolic blood pressure $\leq$ 80 mmHg. However, interpretation of hemodynamic profiles may be very difficult in certain cases emphasizing the need for thorough clinical assessment and careful decision making.

A study from Holman et al. [12] included a total of 420 patients with 497 implanted assist devices (314 LVADs, 5 RVADs, and 77 BiVADs). The authors found that older age, ascites, increased bilirubin, and Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) level 1 (cardiogenic shock) may be regarded as independent risk factors for mortality. A similar study using multivariate analysis on data from 47 patients receiving LVAD found that the preoperative total bilirubin value, age, and preoperative right heart dysfunction are independent predictors of unfavourable outcome and death [10]. These findings underscore the importance of proper patient selection and early implantation of LVAD [13].

This paper briefly reviews currently available VAD systems for long-term support of the left ventricle (with the focus on HeartMate II and HeartWare), their role in today's clinical practice, patient selection, observed complications, and further directions in this rapidly advancing field.

Historical notes

The history of MCS goes back into the beginning of modern cardiac surgery and provides some insight into the development of indications and corresponding clinical impact. It followed the growth of cardiac surgery procedures which established a need for developing means of extending circulatory support for patients who could not be weaned from CPB following cardiac surgery [14]. The primary focus in this field was to develop a total artificial heart (TAH), but this has had limited success and, as a result, shifted attention to VADs [2]. The first devices were invented (Dr. Hall and Dr. Liotta) and used (Dr. Crawford) in the setting of short-term support in a patient with post-cardiotomy heart failure in 1963 [15]. The patient underwent aortic valve replacement developing acute heart failure which was treated by VAD implantation that enabled him to live for 4 more days. A similar device was successfully implanted by Dr. DeBakey in 1966 in a 37 year old woman who was supported for 10 days and became the first long-term survivor after using this technology [16]. In 1969, Dr. Cooley was the first to use an artificial heart to bridge an acutely deteriorating patient to transplantation 3 days later [17]. The first implantation of an artificial heart as a permanent heart replacement was performed by Dr. DeVries in 1982. The first Clinical VAD program has been announced in the period 1975–1978 by National Heart, Lung and Blood Institute (NHLBI) through request for proposal (RPF) – devices had to be able to provide circulatory support for at least 2 years with no "break of the skin". In 1994 another RFP, this time for "Innovative Ventricular Assist Systems" was announced by the same organization. The contractors, such as: Thermo Cardiosystems, Baxter, Abiomed and Thoratec Labs, were subsequently engaged into the development process [8]. By 1986, a total of 41 patients had undergone VAD implantation, and more than

20,000 device implantations occurred worldwide since then (around 55% being HeartMate II).

An ideal VAD

An ideal VAD would be durable, capable of providing long-term reliable systemic flows, sufficient to meet metabolic needs over a substantial range of physical activity (self-adjusting operational mode), small in size, easy to implant (preferably intrapericardially) [18]. The device should produce minimal immunobiological response; it would have to be resistant to infection, with minimal risk for complications (thrombosis, bleeding, and haemolysis), possibly not requiring permanent anticoagulation therapy, consuming small amount of electrical power thus not requiring an external power source. The device should also be affordable and readily available, providing short learning curve for physicians as for the patients.

Currently, there are three generations of VADs available for clinical application (Table 1). The classification of the devices into three distinct generations does not only imply the order in which they appeared, but also functional properties, generated flow characteristics and mechanical design among other things.

Fig. 1– Thoratec HeartMate XVE (with permission from Thoratec Corporation).

Table 1

Major characteristics of different pump generations

Characteristics	First generation pumps	Second generation pumps	Third generation pumps
Operational method	Pulsatile chamber with volume displacement by external compression	Spinning rotor mounted on a central shaft	Hydrodynamic and/or electromagnetic suspended spinning rotor
Type of flow	Pulsatile	Constant, nonpulsatile	Constant, nonpulsatile
Devices	HeartMate XVE, Novacor, Thoratec PVAD and IVAD, Abiomed 5000	HeartMate II, Jarvik 2000, MicroMed DeBakey	HVAD, VentrAssist, Terumo Dura Heart, HeartQuest

First generation VADs

Initially, the first devices that were developed relied on the close imitation of the basic circulatory physiology and its inherent property – pulsatile flow. These devices (first generation VADs) were developed during 1970s and 1980s and were characterized by the use of positive volume displacement and pulsatile flow. The first generation generally consists of pumps such as the Thoratec PVAD/IVAD, the HeartMate IP/VE/XVE (Figure 1), and the Novacor LVAS. Although provided satisfactory circulatory support allowing improved survival until HTX, the first generation pumps had many limitations, such as big size that required substantial surgical dissection for the placement of the device, noisy pump operation, presence of a large diameter driveline and, most importantly, limited mechanical durability due to its mechanical construction [2]. The first generation pumps were also related to serious complications including bleeding, infections and thromboembolic events. It was the HeartMate XVE that was used in the REMATCH trial [3] to compare medical and circulatory assist device treatments for end-stage heart failure. Despite the obvious problems, a great number of patients has been successfully supported with these devices which remain in use today for selected patients.

Second generation VADs

Growing waiting lists for HTX and long waiting times of up to 1 year have been urging the need for more reliable and smaller devices [19]. Although the first generation pumps completely relied on the imitation of the physiologic property of the circulation – its pulsatile nature – introduction of the continuous flow pumps into everyday clinical practice was milestone concept that fundamentally changed the notion of human circulation physiology. Pulse is not strictly necessary despite evolutionary adaptation of the human body to pulsatile circulation. That said, continuous flow VADs are able to mimic physiologic flow only to a certain extent – a special mode of operation (pulsatility index) that permits aortic valve opening during the systole. Pulsatility index is defined as the magnitude of flow pulse provoked by the pump through each cycle [20]. Continuous flow pumps use electrical energy to rotate an axle on which a turbine or propeller system is mounted to pushing the blood through the body at a steady rate. These devices have now largely replaced use of the first generation pulsatile, volume displacement pumps. Second generation pumps have no requirements for external venting – one of the reasons for their reduced size [18]. Second generation rotary pumps are characterized by an axial blood flow path suspended by contact bearings and an internal rotor driven by an electromagnetic field [2]. The basic principle employed has been known for years – Ar-

chimedes' screw. Rotation of the rotor provides the driving force to propel the blood from the left ventricle through the pump and into the circulation. However, the system works on high rotational speeds [21], heat is generated, haemolysis with damage to the blood cells and thrombi may occur [22]. Anaemia and platelet damage along with the activation of contact coagulation system may also ensue [8]. Main advantages of these pumps are smaller size enabling easier implantation (even in small bodies), improved durability due to its design characteristics (only one moving part), less electricity consumption, improved survival and quality of life, reduction of post-implantation adverse effect (bleeding, thrombosis, infection). The control systems and power delivery mechanisms are easily portable and manageable by the patient.

Second-generation rotary pump LVADs were first introduced with the development of Hemopump. Researchers have applied its design to other circulatory assist devices (particularly HeartMate II). Subsequently, the Jarvik 2000 in 1999 and HeartMate II LVADs in 2000 have been used to support patients to HTX [14]. Up to date, the HeartMate II is the most successful second-generation pump worldwide and Food and Drug Association (FDA) approved as BTT and DT [23–25]. Eligibility criteria are essentially the same as those used to select patients for the pivotal clinical trial that included patients with shortness of breath and/or fatigue at rest or during minimal exertion despite treatment with optimal therapy for heart failure associated with a low ejection fraction (<25%) who were not candidates for HTX due to their age or comorbid conditions [26].

The physiologic response to the reduced arterial pulse or its absence during support with continuous flow pumps is not completely understood and it is unclear whether any adverse effects may surface in patients to be supported for many years [27]. Clinical experience to-date indicates that no detrimental effect of these devices on end-organ function is to be expected in the long term [28, 29]. There is a major shift among most VAD programs towards implantation of continuous flow devices though discussion about the flow modalities remains relevant [30].

HeartMate II

The HeartMate II (Thoratec Corp.) LVAD is an axial flow pump that had its origin in the early 1990s (Figure 2) and is intended for long-term support for BTT and DT in patients with CHF[31, 32]. The HeartMate II contains a rotor (spinning impeller – the only moving part) capable of producing flow rates greater than 10 L/min at resolutions ranging from 6,000 to 15,000 rpm (Figure 3). HeartMate II is approximately one seventh the size and one fourth the weight of the XVE pump. The pump can be implanted in a preperitoneal fashion or intra-abdominally, with the inflow cannula connected to the left ventricle (the apex of the heart), and the outflow graft sutured to the ascending aorta. A driveline connected to the pump should be routed transcutaneously, usually in the region of right upper quadrant of the abdomen. Power is delivered by external power sources – rechargeable batteries – that enable ambulance of the patient. The system is operated at a fixed rotational speed set by the clinician with the aim of providing optimal circulatory support for each patient. Although the internal surfaces of the device were designed to help resist the development of thrombi, anticoagulation is at present recommended to keep INR between 1.5 and 2.5.

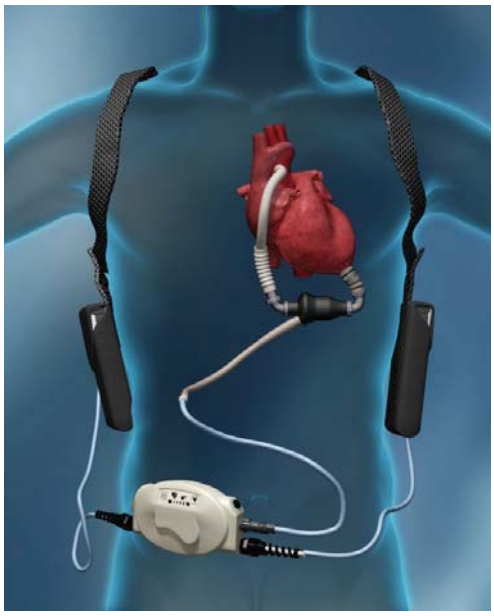


Fig. 2 – Thoratec HeartMate II device (with permission from Thoratec Corporation).

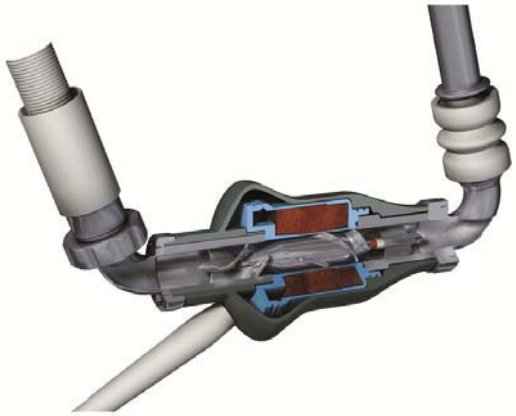


Fig. 3 – Thoratec HeartMate II device – construction design (with permission from Thoratec Corporation).

The blood pump component of the device is a titanium straight tube of 12 mm in diameter and contains the inlet stator, a pump rotor and the outlet stator (Figure 3). The rotor (the only moving part of the device) contains a magnet that fits in close proximity of the motor. A magnetic field is generated between the rotor and the motor producing torque and rotary motion that creates blood flow [33]. The blood flow is directed from the ventricle through the inlet cannula to the pump and then through the outlet cannula via an outflow graft to the aorta. The inner surfaces of the inflow conduit and the outflow graft contain a textured surface to simulate natural endothelial surface for blood flow.

Of all the continuous flow pumps currently in the use worldwide, only the Thoratec HeartMate II VAD has received approval from the US Food and Drug Administration (FDA) for use as BTT and DT in the United States [34].

The BTT pivotal multicentre, nonrandomised trial initially enrolled 133 NYHA class IV patients who were listed for cardiac transplant and were at imminent risk of dying [35]. The primary outcome of the BTT trial was survival to HTX or cardiac recovery, or being listed as United Network for Organ Sharing (UNOS) status 1A or 1B at 180 days of LVAD support. Of the 133 patients enrolled, 100 patients (75%) reached the primary end-point of HTX, cardiac recovery or survival at 180 days with ongoing mechanical support. The group of 100 patients included 56 patients who underwent HTX, 43 patients continued to receive LVAD support and were eligible for HTX, and 1 patient whose cardiac function recovered leading to LVAD explant. The overall rate of survival to HTX, recovery, or continued support was 75% at 180 days. The overall actuarial survival for patients continuing to receive HeartMate II device support was 89% at 1 month, 75% at 6 months and 68% at 12 months (the median LVAD support was 126 days).

After being FDA approved in 2008, a multi-institutional study was carried out using the INTERMACS database comprised of 169 consecutive BTT patients [36]. The study compared the effectiveness of HeartMate II and previously approved LVAD devices such as the HeartMate XVE and Thoratec IVAD. The 6 and 12-month survival of HeartMate II patients was 90% and 85% respectively whereas the control group using pulsatile devices had 6 and 12-month survival of 79% and 70% respectively. Significant reduction of device related complications was also observed for HeartMate II group (infection, neurological dysfunction, renal and respiratory dysfunction).

Another trial was carried out to evaluate effectiveness of HeartMate II device as DT in patients ineligible for HTX [25]. The study was conducted in 38 centres in the USA. Patients were randomly assigned to undergo implantation of the HeartMate II continuous flow device or HeartMate XVE pulsatile device in a 2 : 1 ratio (a total of 200 patients – 134 in the continuous flow device arm and 66 in the pulsatile device arm). The trial compared these devices with established composite end-point of survival free from disabling stroke or reoperation to repair or replace the device for mechanical failure over a 2-year period (46% and 11% for HeartMate II and HeartMate XVE respectively). Overall 2-year survival was 58% and 24%, respectively; again with significant reduction of adverse events in HeartMate II group. In total, 21 pump replacements (and 3 device explantations) were required in the pulsatile LVAD cohort as opposed to only 13 pump replacements in the continuous flow device group ($p < 0.001$). After enrolment of the initial 200 patients in the DT trial, hundreds of additional patients have been enlisted part of continued access protocol [34].

Despite a dramatic improvement in survival with the HeartMate II, there still remains the burden of morbidity associated with the device, including infection, bleeding, and thromboembolic events [36]. According to the INTERMACS annual report, the event rates (events per 100 patient months) during the first 12 months of HeartMate II therapy in BTT patients were 17.41 for bleeding, 11.8 for infection, and 1.93 for neurologic dysfunction [23].

Third generation VADs

During their extensive clinical use, it became apparent that implantation of second generation pumps in a form of BiVAD is extremely challenging if not impossible in cases of miniscule patients. An idea of developing even smaller devices that can be implanted within pericardial cavity emerged as an option to tackle the problem of BiVAD implantation and unsuitable patient's anatomy.

Further advancement in design and construction of the continuous flow pumps has led to development of bearingless devices, which in theory ought to be more durable than the previous generation pumps, and due to their smaller size allow intra-pericardial placement. Third generation VADs are continuous flow pumps broadly classified based on pump design into centrifugal and axial flow devices, and on whether the impeller is hydrodynamically or magnetically levitated. Levitation systems of third-generation rotary blood pumps suspend the moving impeller in pump thereby removing mechanical contact [2]. Centrifugal flow pumps have cone-shaped or cylindrical rotors that drive the blood flow using the centrifugal force generated from the centre of the rotor to its circumference [37]. One anticipated benefit is that the centrifugal design results in a flatter and more sensitive pressure flow curve at lower rounds-per-minute (RPM) compared to axial flow devices [17]. Examples from this heterogeneous group are the DuraHeart (Terumo Heart, Inc., Michigan), VentrAssist (Ventracor, Australia), CorAide (Arrow International, Pennsylvania), HeartWare HVAD (HeartWare, Inc., Massachusetts) and Levacor (World Heart, Inc., Oakland, CA) systems.

HVAD

The HVAD (HeartWare Inc.) is a small third-generation continuous flow rotary pump with a centrifugal and bearingless design (Figure 4). What distinguishes this pump from other of its generation is the size – the pump is small enough to be placed inside the pericardial cavity (no need for pump

Fig. 4 – HVAD (with permission from Heartware Inc.).

pocket) at the apex of the heart or left ventricle inferior wall. As a consequence, surgical trauma to the surrounding tissue is significantly reduced while the implantation procedure is simplified – not requiring abdominal incision for implantation. It usually operates at a speed of 2,400–3,500 RPMs and can provide up to 10L/min of flow [13]. The impeller is suspended in place by a combination of passive magnetic and hydrodynamic bearing systems, avoiding mechanical contact and wear (Figure 5) [18]. Physical contact between the casing and the impeller is prevented by a thin blood film generated by the hydrodynamic bearings [38–40]. The device was implanted for the first time in humans in 2006 and, since then, it has been clinically evaluated in Europe (approved for BTT) and Australia with an ongoing bridge-to-transplantation trial in the US.

Fig. 5 – HVAD – internal design (with permission from Heartware Inc.).

The HVAD is implanted through a median sternotomy with the assistance of cardiopulmonary bypass. An integrated inflow cannula is inserted into the left ventricle through the apex and is held in position by an adjustable sewing ring while pump is positioned in the pericardial cavity. Afterwards, the 10 mm outflow graft is anastomosed to the ascending aorta (Figure 6) [41].

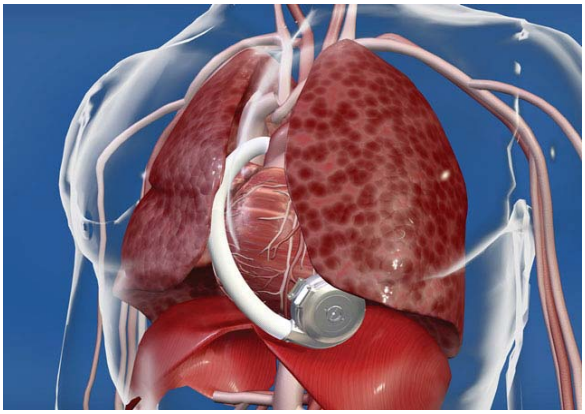


Fig. 6 – HVAD position inside the chest (with permission from Heartware Inc.).

The HeartWare device has been evaluated in several clinical trials in terms of patient survival and quality of life. A recent multicentre trial in Europe and Australia found that the actuarial survival after 6 and 12 months following HVAD implantation was 91% and 86%, respectively [42]. Another multicentre, non-randomized trial that included NYHA IV class patients, evaluated the safety of HVAD [43]. Fifty patients were included in the trial and they were supported using HVAD for 180 days until HTX, myocardial recovery, device explantation or death with following actual survival at 6, 12, and 24 months: 90%, 84%, and 79% respectively. Death as an end-point was reached in 9 cases (18%) – 3 patients died as a result of sepsis, 3 from multi-organ failure, and 3 from haemorrhagic stroke. The most frequent adverse events were infection and bleeding.

The ongoing Evaluate the HeartWare Ventricular Assist System (ENDURANCE) trial may demonstrate an important clinical advantage of this device.

Complications

Since growing number of patients are being supported with VADs for extended period of time, the interaction between the body and the VAD during long-term or even lifelong period and the management of complications have gained the interest of clinicians and biomedical engineers [44].

Thrombosis, thromboembolic events and the risk of bleeding

After the implantation of the device blood is exposed to a foreign surface requiring the use of systemic anticoagulation. Manufacturer guidelines for continuous flow devices recommend use of both antiplatelet and anticoagulation therapy in order to reduce the risk of pump thrombosis and consequent thromboembolism [37]. Although the first recommended range of the international normalized ratio (INR) for the patients with HeartMate II was 2.5 to 3.5, the target INR has been recently decreased due to greater risk of bleeding [35, 45].

It has been noted that some patients may develop thrombosis of the aortic root and the ascending aorta – especially when the outflow graft is implanted into the descending aorta [44]. In these settings, HTX should be performed on an emergency basis, if possible. In the case of pump thrombosis, immediate pump exchange is mandatory. All other thromboembolic events should be dealt with in the usual manner.

The incidence of any-cause bleeding (requiring red blood cells transfusion or necessitating surgery) with different types of VAD ranges between 0.16 and 2.45 events per patient per year while the incidence of thromboembolic events is 0.05 to 0.28 events per patient per year [42, 46]. Overdosing of anticoagulant therapy has been the major factor for elevating the risk of bleeding in patients on VADs. However, there has been growing number of reports of an increased incidence of gastrointestinal mucosal arteriovenous malformations (AVM) – pre-existing or newly developed due to loss of physiological pulsatility, and acquired von Willenbrad disease associated with axial flow devices [37]. It remains unclear whether gastrointestinal bleeding is related to the need for anticoagulation or whether it is linked to continuous flow effect (loss of pulsatility) [47]. In some patients, severe

haemolysis may occur after VAD implantation. This is usually seen when the rotor speed in the continuous flow pumps is set to higher operation mode or as a result of postoperative complications (malposition of the apical cannula, pump thrombosis, outflow graft kinking) [44, 48, 49].

Infections

Infectious complications associated with VAD placement are often encountered, although the rate has improved with the new generation devices [50]. Infection can involve any portion of a VAD – surgical site, driveline, pocket or the pump itself. Most infections involve the percutaneous driveline [51, 52]. Length of device support was associated with more than 50% of 1-year survivors developing a driveline-related infection [53]. The rate of driveline infections appears to have reduced after the introduction of newer generation pumps most likely due to smaller drivelines used by these pumps as well as the reduction in movement of the device within a surgically fashioned pocket [25]. Another potential site of infection is the pump pocket usually originating from the driveline infection or secondary to surgical trauma or hematoma formation [54]. The infections is most likely to be caused by Staphyloccocus aureus, Corynebacterium or Pseudomonas aeruginosan [44].

Aortic valve pathology

Patients treated with long-term continuous flow devices are at higher risk of developing aortic insufficiency (AI) or some degree of aortic valve degeneration [37]. It is believed that this may be the result of reduced (limited) or absent opening of the aortic valve mostly seen when the device is working in the serial fashion with continuous load of the left ventricle. The fusion of the aortic leaflets may be seen as early as 6 months following implantation. This complication is encountered in approximately 25% patients, and several risk factors have been designated: aortic root diameter, female sex, non-pulsatile flow [55]. After 18 months on device support, up to 50% of patients present with moderate or severe AI [56].

Mechanical complications

Although rare, complications such as device malfunction, inflow conduit rupture, and driveline break may occur. It is important to establish the correct cause of malfunction which, in most cases, requires device exchange. Despite modern technology and the use of high resistant materials, cable damage [57], due to kinking and twisting, or as a result of a suicide attempt occurs with an incidence of 5%–9% or up to 0.06 events per patient per year [27, 44, 58, 59].

Conclusion

Currently, long-term circulatory support with VADs offers viable choice for end-stage heart failure patients, either as BTT option or as DT. Survival rate along with the quality of life of these patients have been significantly improved. Patients supported with VADs continue to be affected by a variety of complications – the fact that only emphasizes the need to further improve this technology. Meticulous risk-benefit evaluation by a multidisciplinary team is mandatory for each patient in order to achieve optimal survival and minimize the risk of morbidity.

Grant support

The author (VL) worked on ventricular assist device systems in the Texas Heart Institute in St. Luke's Hospital, Houston, Texas, USA (2012–13) as a Fulbright Visiting Scholar.

REFERENCES

1. *Gaddam KK, Ventura H.* Developments in heart failure 2011. Congest Heart Fail 2012; 18(2): 112−26.
2. *Garbade J, Bittner HB, Barten MJ, Mohr FW.* Current trends in implantable left ventricular assist devices. Cardiol Res Pract 2011; 2011: 290561.
3. *Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W,* et al. Long-term use of a left ventricular assist device for end-stage heart failure. N Engl J Med 2001; 345(20): 1435−43.
4. *Stevenson LW, Rose EA.* Left ventricular assist devices: bridges to transplantation, recovery, and destination for whom? Circulation 2003; 108(25): 3059−63.
5. *Taylor DO, Edwards LB, Aurora P, Christie JD, Dobbels F, Kirk R,* et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fifth official adult heart transplant report--2008. J Heart Lung Transplant 2008; 27(9): 943−56.
6. *Frazier OH, Rose EA, McCarthy P, Burton NA, Tector A, Levin H,* et al. Improved mortality and rehabilitation of transplant candidates treated with a long-term implantable left ventricular assist system. Ann Surg 1995; 222(3): 327−36; discussion 336−8.
7. *Frazier OH, Rose EA, Oz MC, Dembitsky W, McCarthy P, Radovancevic B,* et al. Multicenter clinical evaluation of the HeartMate vented electric left ventricularassist system in patients awaiting heart transplantation. J Thorac Cardiovasc Surg 2001; 122(6): 1186−95.
8. *Vural KM.* Ventricular assist device applications. Anadolu Kardiyol Derg 2008; 8(Suppl 2): 117−30.
9. *Moazami N, Feldman D.* Rethinking the terminology of mechanical circulatory support. J Thorac Cardiovasc Surg 2012; 144(1): 2−3.
10. *Shiga T, Kinugawa K, Hatano M, Yao A, Nishimura T, Endo M,* et al. Age and preoperative total bilirubin level can stratify prognosis after extracorporeal pulsatile left ventricular assist device implantation. Circ J 2011; 75(1): 121−8.
11. *Frazier OH, Delgado RM.* Mechanical circulatory support for advanced heart failure: where does it stand in 2003? Circulation 2003; 108(25): 3064−8.
12. *Holman WL, Kormos RL, Naftel DC, Miller MA, Pagani FD, Blume E,* et al. Predictors of death and transplant in patients with a mechanical circulatory support device: a multi-institutional study. J Heart Lung Transplant 2009; 28(1): 44−50.
13. *Neragi-Miandoab S.* A ventricular assist device as a bridge to recovery, decision making, or transplantation in patients with advanced cardiac failure. Surg Today 2012; 42(10): 917−26.
14. *Clegg AJ, Scott DA, Loveman E, Colquitt J, Royle P, Bryant J.* Clinical and cost-effectiveness of left ventricular assist devices as destination therapy for people with end-stage heart failure: a systematic review and economic evaluation. Int J Technol Assess Health Care 2007; 23(2): 261−8.

15. *Hall, CW, Liotta D, Henly WS, Crawford ES, DeBakey ME*. Development of artificial intrathoracic circulatory pumps. Am J Surg 1964; 108:685-92.
16. *DeBakey ME*. Left ventricular bypass pump for cardiac assistance. Clinical experience. Am J Cardiol 1971; 27(1): 3−11.
17. *Tang DG, Oyer PE, Mallidi HR*. Ventricular assist devices: history, patient selection, and timing of therapy. J Cardiovasc Transl Res 2009; 2(2): 159−67.
18. *Krishnamani R, DeNofrio D, Konstam MA*. Emerging ventricular assist devices for long-term cardiac support. Nat Rev Cardiol 2010; 7(2) :71−6.
19. *Lahpor JR*. State of the art: implantable ventricular assist devices. Curr Opin Organ Transplant 2009; 14(5): 554−9.
20. *Wilson SR, Givertz MM, Stewart GC, Mudge GH Jr*. Ventricular assist devices the challenges of outpatient management. J Am Coll Cardiol 2009; 54(18):1647-1659.
21. *Nose Y*. Design and development strategy for the rotary blood pump. Artif Organs 1998; 22(6): 438−46.
22. *Zhang Y, Zhan Z, Gui XM, Sun HS, Zhang H, Zheng Z,* et al. Design optimization of an axial blood pump with computational fluid dynamics. ASAIO J 2008; 54(2): 150−5.
23. *Kirklin JK, Naftel DC, Kormos RL, Stevenson LW, Pagani FD, Miller MA,* et al. Second INTERMACS annual report: more than 1,000 primary left ventricular assist device implants. J Heart Lung Transplant 2010; 29(1): 1−10.
24. *Rogers JG, Aaronson KD, Boyle AJ, Russell SD, Milano CA, Pagani FD,* et al. Continuous flow left ventricular assist device improves functional capacity and quality of life of advanced heart failure patients. J Am Coll Cardiol 2010; 55(17): 1826−34.
25. *Slaughter MS, Rogers JG, Milano CA, Russell SD, Conte JV, Feldman D,* et al. Advanced heart failure treated with continuous-flow left ventricular assist device. N Engl J Med 2009; 361(23): 2241−51.
26. *Rector TS, Taylor BC, Greer N, Rutks I, Wilt TJ*. Use of Left Ventricular Assist Devices as Destination Therapy in End-Stage Congestive Heart Failure: A Systematic Review. Washington (DC): Department of Veterans Affairs; 2012.
27. *Slaughter MS*. Long-term continuous flow left ventricular assist device support and end-organ function: prospects for destination therapy. J Card Surg 2010; 25(4): 490−4.
28. *Frazier OH, Benedict CR, Radovancevic B, Bick RJ, Capek P, Springer WE*, et al. Improved left ventricular function after chronic left ventricular unloading. Ann Thorac Surg 1996; 62(3): 675−81.
29. *Radovancevic B, Vrtovec B, de KE, Radovancevic R, Gregoric ID, Frazier OH*. End-organ function in patients on long-term circulatory support with continuous- or pulsatile-flow assist devices. J Heart Lung Transplant 2007; 26(8): 815−8.
30. *Sansone F, Zingarelli E, Flocco R, Dato GM, Parisi F, Punta G*, et al. Pulsed or continuous flow in long-term assist devices: a debated topic. Transplant Rev (Orlando) 2012; 26(4): 241−5.
31. *Frazier OH, Myers TJ, Westaby S, Gregoric ID*. Clinical experience with an implantable, intracardiac, continuous flow circulatory support device: physiologic implications and their relationship to patient selection. Ann Thorac Surg 2004; 77(1): 133−42.
32. *Frazier OH, Gemmato C, Myers TJ, Gregoric ID, Radovancevic B, Loyalka P*, et al. Initial clinical experience with the HeartMate II axial-flow left ventricular assist device. Tex Heart Inst J 2007; 34(3): 275−81.
33. *Sheikh FH, Russell SD*. HeartMate(R) II continuous-flow left ventricular assist system. Expert Rev Med Devices 2011; 8(1): 11−21.
34. *Milla F, Pinney SP, Anyanwu AC*. Indications for heart transplantation in current era of left ventricular assist devices. Mt Sinai J Med 2012; 79(3): 305−16.
35. *Miller LW, Pagani FD, Russell SD, John R, Boyle AJ, Aaronson KD,* et al. Use of a continuous-flow device in patients awaiting heart transplantation. N Engl J Med 2007; 357(9): 885−96.
36. *Starling RC, Naka Y, Boyle AJ, Gonzalez-Stawinski G, John R, Jorde U*, et al. Results of the post-U.S. Food and Drug Administration-approval study with a continuous flow left ventricular assist device as a bridge to heart transplantation: a prospective study using the INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support). J Am Coll Cardiol 2011; 57(19): 1890−8.
37. *Patel CB, Rogers JG*. Durable mechanical circulatory support devices. Prog Cardiovasc Dis 2011; 54(2):132−43.
38. *Slaughter MS, Sobieski MA, Tamez D, Horrell T, Graham J, Pappas PS*, et al. HeartWare miniature axial-flow ventricular assist device: design and initial feasibility test. Tex Heart Inst J 2009; 36(1): 12−6.
39. *Slaughter MS*. Implantation of the HeartWare left ventricular assist device. Semin Thorac Cardiovasc Surg 2011; 23(3): 245−7.
40. *Tuzun E, Roberts K, Cohn WE, Sargin M, Gemmato CJ, Radovancevic B,* et al. In vivo evaluation of the HeartWare centrifugal ventricular assist device. Tex Heart Inst J 2007; 34(4): 406−11.
41. *Larose JA, Tamez D, Ashenuga M, Reyes C*. Design concepts and principle of operation of the HeartWare ventricular assist system. ASAIO J 2010; 56(4): 285−9.
42. *Wieselthaler GM, Driscoll O, Jansz P, Khaghani A, Strueber M*. Initial clinical experience with a novel left ventricular assist device with a magnetically levitated rotor in a multi-institutional trial. J Heart Lung Transplant 2010; 29(11): 1218−25.
43. *Strueber M, Meyer AL, Malehsa D, Haverich A*. Successful use of the HeartWare HVAD rotary blood pump for biventricular support. J Thorac Cardiovasc Surg 2010; 140(4): 936−7.
44. *Potapov EV, Stepanenko A, Krabatsch T, Hetzer R*. Managing long-term complications of left ventricular assist device therapy. Curr Opin Cardiol 2011; 26(3): 237−44.
45. *Boyle AJ, Russell SD, Teuteberg JJ, Slaughter MS, Moazami N, Pagani FD*, et al. Low thromboembolism and pump thrombosis with the HeartMate II left ventricular assist device: analysis of outpatient anti-coagulation. J Heart Lung Transplant 2009; 28(9): 881−7.
46. *Slaughter MS, Pagani FD, Rogers JG, Miller LW, Sun B, Russell SD*, et al. Clinical management of continuous-flow left ventricular assist devices in advanced heart failure. J Heart Lung Transplant 2010; 29(4 Suppl): S1−39.
47. *John R, Kamdar F, Liao K, Colvin-Adams M, Boyle A, Joyce L*. Improved survival and decreasing incidence of adverse events with the HeartMate II left ventricular assist device as bridge-to-transplant therapy. Ann Thorac Surg 2008; 86(4): 1227−34.
48. *Bhamidipati CM, Ailawadi G, Bergin J, Kern JA*. Early thrombus in a HeartMate II left ventricular assist device: a potential cause of hemolysis and diagnostic dilemma. J Thorac Cardiovasc Surg 2010; 140(1): e7−8.
49. *Meyer AL, Kuehn C, Weidemann J, Malehsa D, Bara C, Fischer S*, et al. Thrombus formation in a HeartMate II left ventricular assist device. J Thorac Cardiovasc Surg 2008; 135(1): 203−4.
50. *Califano S, Pagani FD, Malani PN*. Left ventricular assist device-associated infections. Infect Dis Clin North Am 2012; 26(1): 77−87.
51. *Argenziano M, Catanese KA, Moazami N, Gardocki MT, Weinberg AD, Clavenna MW*, et al. The influence of infection on survival and successful transplantation in patients with left ventricular assist devices. J Heart Lung Transplant 1997; 16(8): 822−31.
52. *Bentz B, Hupcey JE, Polomano RC, Boehmer JP*. A retrospective study of left ventricular assist device-related infections. J Cardiovasc Manag 2004; 15(1): 9−16.
53. *John R, Pagani FD, Naka Y, Boyle A, Conte JV, Russell SD*, et al. Post-cardiac transplant survival after support with a continu-

ous-flow left ventricular assist device: impact of duration of left ventricular assist device support and other variables. J Thorac Cardiovasc Surg 2010; 140(1): 174−81.

54. *Akay MH, Gregoric I, Cohn WE, Frazier OH*. HeartMate-II Left Ventricular Assist Device Infections Resulting from Gastrointestinal-Tract Fistulas. J Card Surg 2012; 27(5): 643−5.
55. *Cowger J, Pagani FD, Haft JW, Romano MA, Aaronson KD, Kolias TJ*. The development of aortic insufficiency in left ventricular assist device-supported patients. Circ Heart Fail 2010; 3(6): 668−74.
56. *Mudd JO, Cuda JD, Halushka M, Soderlund KA, Conte JV, Russell SD*. Fusion of aortic valve commissures in patients supported by a continuous axial flow left ventricular assist device. J Heart Lung Transplant 2008; 27(12): 1269−74.
57. *Jafar M, Gregoric ID, Radovancevic R, Cohn WE, McGuire N, Frazier OH*. Urgent exchange of a HeartMate II left ventricular assist device after percutaneous lead fracture. ASAIO J 2009; 55(5): 523−4.
58. *Birks EJ, Tansley PD, Yacoub MH, Bowles CT, Hipkin M, Hardy J*, et al. Incidence and clinical management of life-threatening left ventricular assist device failure. J Heart Lung Transplant 2004; 23(8): 964−9.
59. *Pelenghi S, Colombo T, Montorsi E, Newcomb A, Frigerio M, Martinelli L*. Failure and off-pump replacement of Incor LVAD system. ASAIO J 2009; 55(1): 121−2.

Received on October 24, 2012.
Revised on November 16, 2012.
Accepted on November 19, 2012.
OnLine-First February, 2013.

CASE REPORTS

UDC: 618.831.9-002.1::616-002.52
DOI: 10.2298/VSP1307690P

Acute meningoencephalitis in a patient with systemic lupus erythematosus

Akutni meningoencefalitis kod bolesnice sa sistemskim eritemskim lupusom

Aleksandra Perić-Popadić*†, Mirjana Bogić*†, Vesna Tomić-Spirić*†, Vojislav Djurić*†, Jasna Bolpačić*†, Branko Milošević*‡, Sanja Spasić†, Sanvila Rašković*†

***Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Clinic of Allergology and Immunology, ‡Institute of Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade, Serbia**

Abstract

Introduction. Infections in patients with systemic lupus erythematosus (SLE) are a significant factor of morbidity and mortality. Although central nervous system infections, including septic meningitis, are rare in patients with SLE, they can be significant causes of mortality inspite of the prompt and accurate diagnosis and proper management. **Case report.** We presented a woman with the diagnosis of SLE and diffuse proliferative lupus nephritis. Because of disease activity we introduced cytostatic immunosuppressive therapy, cyclophosphamide and then azathioprine. Meningoencephalitis, staphylococcal sepsis and abscess of the brain, with resulting seizures developed. **Conclusion.** This case alerts to the need of careful examination of patients with SLE, collection of adequate cultures and evaluation of predisposition towards ifnections, before the introduction of immunosuppressants due to potentially fatal infection.

Key words:
lupus erythematosus, systemic; central nervous system, infections; meningoencephalitis; immunosuppression.

Apstrakt

Uvod. Infekcije kod bolesnika sa sistemskim eritemskim lupusom (SEL) značajan su faktor morbiditeta i mortaliteta. Mada su infekcije centralnog nervnog sistema uključujući septični meningitis, retke kod bolesnika sa SEL, one mogu biti značajan uzrok mortaliteta i pored brze dijagnoze i adekvatne terapije. **Prikaz bolesnika.** Prikazali smo bolesnicu sa SEL kod koje je došlo do razvoja meningoencefalitisa i apscesa mozga pri pokušaju uvođenja citostatske imunosupresivne terapije ciklofosfamidom, a potom azatioprinom. Kao posledica apscesa ostala je encefalomalacija temporoparijetalnog dela mozga sa posledičnim epi napadima. **Zaključak.** Ovaj slučaj ukazuje na potrebu pažljivog pregleda svakog bolesnika sa SEL, kao i procene sklonosti prema infekcijama pre uvođenja terapije drugim imunosupresivnim agensima (pored glikokortikosteroida), zbog potencijalno fatalnih infekcija koje se kod njih mogu razviti.

Ključne reči:
lupus, eritematozni, sistemski; nervni sistem, centralni, infekcije; meningoencefalitis; imunosupresija.

Introduction

It is known that viral and bacterial infections may be the trigger of development or exacerbation of systemic lupus erythematosus (SLE). The patients with SLE are more prone to infections either due to the nature of disease or the applied immunosuppressive therapy [1]. About 80% of infections in SLE patients are caused by bacteria. Common acute infections in these patients are: pneumonia, urinary infections, cellulitis and sepsis [2]. Of chronic infections, the most frequent is tuberculosis [3], while fungal infections, and those caused by parasites and protozoa are most often opportunistic infections [4–6].

Central nervous system (CNS) infections, including septic meningitis, are rare bacterial complications in SLE, but they can be significant cause of mortality [7]. These infections are most commonly the consequence of a long-term immunosuppressive therapy, and can be a diagnostic problem by mimicking activity of lupus and neurolupus. We presented the instructive and difficult case of CNS infection in a SLE patient.

Correspondence to: Aleksandra Perić-Popadić, Faculty of Medicine, University of Belgrade, Prvomajska 6/88, 11 000 Zemun, Serbia. Phone: +381 64 616 41 34. E-mail: popealeksandra@yahoo.com

Case report

A 33-year-old female patient, has been treated for SLE since 2004. The onset of disease was sudden in March 2004, with edemas, pains and stiffness of joints and febrile condition which was followed by cervical and axillary lymphadenopathy, hepatosplenomegaly and anemia (hematological disease was ruled out on the basis of bone marrow puncture). On admission, bilateral neck and axillary lymph nodes were enlarged, the patient had hepatosplenomegaly. We observed an abscess on the left gluteus.

Laboratory results showed elevated erythrocyte sedimentation rate (ESR 80) and leukocytosis (11.4×10^9/L), anemia [hemoglobin (Hb) 97 g/L] and slight thrombocytopenia (131×10^9/L). Biochemistry was normal (with hypoalbuminemia of 28 g/L). Immunological analyses showed high immunoglobulin G (21.9 g/L), consumption of complements (C3 = 0.43 g/L and C4 < 0.04 g/L), positive antinuclear antibodies (ANA) homogenous 1 : 640, ds DNA ++++, positive anticardiolipin antibodies (ACLA) and positive Coombs test. The urine sediment showed 8–10 fresh, 10–12 pale erythrocytes. Urine culture was negative, 24-hour proteinuria bellow 0.5 g. Gluteal wound swab: *Escherichia coli* X-ray of the heart and lungs and heart ultrasonography showed minor pericardiac effusion.

The patient was diagnosed with SLE on the basis of American College of Rheumatology (ACR) criteria: polyarthralgia, cytopenia, positive ANA, ds DNA, ACLA, pericarditis, and erythrocyturia. The therapy included parenteral glucocorticosteroids (GCS), followed by oral prednisone of 1 mg/kg body weight (bw) in decreasing doses, antibiotics, antiaggregation therapy, H2 blockers. Later, after improving, the patient was on maintenance prednisone dose of 30 mg.

In November 2004, the patient developed the signs of iatrogenic Cushing's syndrome. Due to increase in proteinuria (up to 3.5 g/24 h) and massive erythrocyturia, kidney biopsy was performed and revealed diffuse-proliferative glomerulonephritis with activity and chronicity indexes of 4/24 and 3/12, respectively.

Further treatment included two-time pulse therapy with the intravenous (*iv*) methylprednisolone, 500 mg, followed by pulse therapy of *iv* cyclophosphamide, 800 mg. The patients was advised to take prednisone 20 mg, azathioprine 50 mg, and drugs for gastric mucosal protection.

Eighteen days after *iv* cyclophosphamide pulse therapy, the patient developed massive left-side effusion, hypertension, lower leg edemas, hepatosplenomegaly. During the same evening, the patient's condition worsened, she was febrile (38°C) with a headache. We evacuated 1,400 mL of serous pleural exudation. Proteinuria was 9.39 g/24 h. Parenteral quinolones and cephalosporins were empiricaly introduced in full doses, as well as *iv* methylprednisolone, 3 × 40 mg.

Neurological findings revealed positive meningeal signs without lateralization. Lumbal puncture was performed, and the cerebrospinal fluid was turbid, pouring out under intense pressure. The analysis revealed cerebrospinal fluid (CSF) with 820 cellular elements (98% neutrophils, 2% lymphocytes), hypoglycorrhachia, 1.94 mmol/L, and hyperproteinorrhachia, 1.78 g/L. Etiological examinations of CSF were negative.

The patient was transferred to the Clinic of Infectious Diseases.

Antimicrobial therapy was continued by: ampicilin 3 × 3.0 g *iv*, gentamicin 120 mg, rifampicin 600 mg. After 6 days, isoniazid 300 mg and pirazinamide 1,500 mg were added, because specific CNS infection was suspected. Although antimicrobial and antiedematous therapy was continued, her condition became aggravated, manifested by generalized epileptic seizures. The introduction of antiepileptics (phenobarbiton and carbamazepine) made convulsions stop. Endocranial computed tomography (CT) was carried out and the right temporoparietal hypodense area was evident. At that time, ampicilin was ruled out and vancomycin, 3 × 500 mg, was introduced (in condition of sufficient diuresis), because of cerebritis. Few days later, endocranial magnetic resonance imaging (MRI) demonstrated meningoencephalitis of the right temporo-basic region together with encephalomalacia (Figure 1) and *Staphylococcus aureus* was isolated

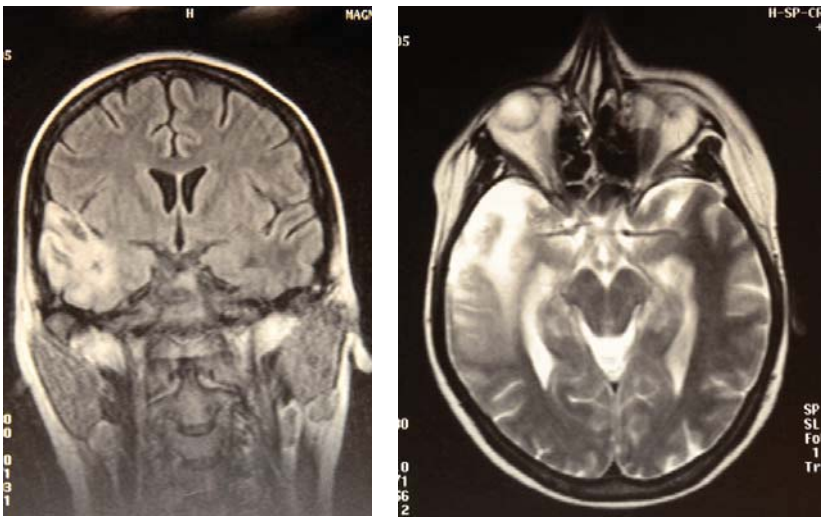


Fig. 1 – Endocranial magnetic resonance imaging meningoencephalitis of the right temporo-basic region together with encephalomalacia.

from two blood cultures. At that time, middle-grade renal failure was registered (creatinine clearance 27.6 mL/min). According to therapeutic algorithm, we introduced meropenem (but in doses of 3 × 1.0 g) with fluconasol. After few days, antituberculotic therapy was excluded. Three weeks later, when the patient's clinical improvement was evident and renal function was repaired (proteinuria 0.89 g/24 h), vancomycin and meropenem were ruled out and antimicrobial therapy was continued with gentamicin 120 mg and cefotaxim 2 × 3.0 g.

Anemia was corrected by the substitution therapy. Control endocranial MRI showed a significant regression of above-described pathological changes but, in further course, agranulocytosis was noted and bone marrow puncture was done. Filgrastim, a colony- stimulating factor, was added, resulting in the retrieval of white blood cell count and afebrile condition. In the meantime, the patient experienced abdominal difficulties, with clinical and radiologic picture of ileus and underwent surgical interventions (laparotomy, ascites evacuation and drainage). Postoperative course was uneventful. Further on, the patient was better, proteinuria was about 1 g/daily. A gluteal abscess developed again, in the right region, and was treated (Figure 2).

In June 2005, worsening of urine sediment and proteinuria occurred, so we introduced mycophenolate-mofetil in increasing doses to 2 g/day. After several days, the patient developed fever and acute bronchitis, followed by nausea, and laboratory inflammatory syndrome. Because of that, mycophenolate therapy was discontinued.

In further course there has been no attempt to introduce immunosuppressive therapy other than GCS. Control endocranial MRI (Figure 3), showed right temporoparietal hypodense area. During the last hospital stay in October 2011, the patient felt mostly well, with occasional events of bronchitis, rare epileptic seizures (approximately 1 per month) and without significant proteinuria.

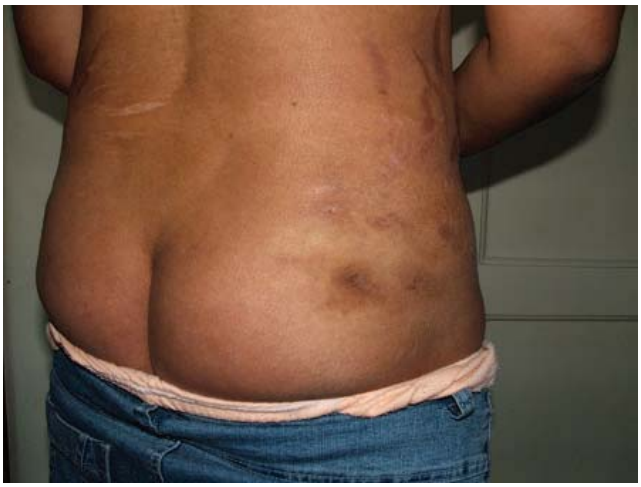


Fig. 2 – An abscess in the right gluteal region.

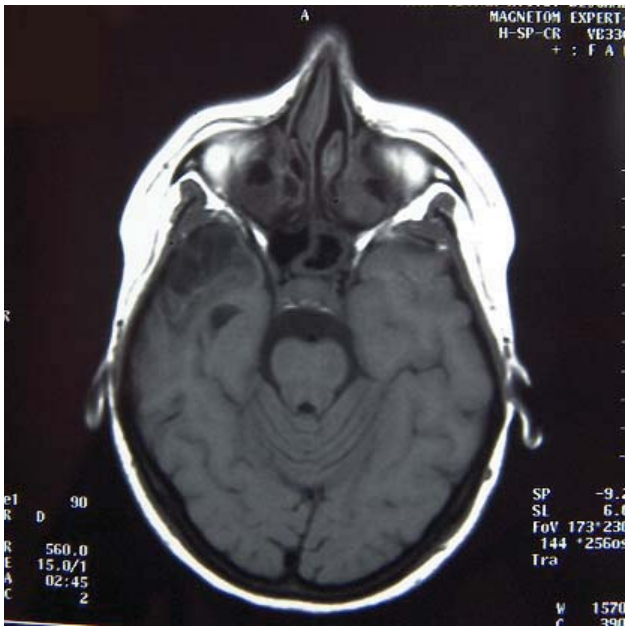


Fig. 3 – Control endocranial magnetic resonance imaging findings: right temporoparietal hypodense area, and chronic sinusitis (opacification of paranasal cavities).

Discussion

One of the most severe complications during SLE condition may be CNS infection, which is most commonly the consequence of a long-term use of GCS and other immunosuppressive agents, as well as immune disorder due to the illness itself. The majority who died from infection were on high dose prednisolone plus at least 1 other immunosuppressive agent and had serologically active disease [8]. CNS infection symptoms may mimic the activity of the disease or, conversely, may be camouflaged by GCS therapy [9].

Reviewing the causes/focus of CNS infection, the possibilities in the presented case were multiple: predisposition to infections due to conditions with complement deficit and reduced bacterial clearance *via* reticuloendothelial system; higher exposure to respiratory infections within her environment (pre-school and school children at home, poor economic conditions); the existing chronic sinusitis (CT-viewed opacification of paranasal cavities) with the possibility of infection spread and the presence of "silent foci" (such as gluteal abscess) which became reactivated upon immunosuppression and could reached the CNS through circulation. In addition, the risk factors of infections described in the literature [10–14] such as: nephritis, activity of the disease, leukopenia, positive dsDNA > 20 IU/mL, prednison in daily dose higher than 10 mg, application of cyclophosphamide, together with hypocomplementemia presenting the independent predictive factor, were all found in our patient.

Due to GCS side effects and the activity of the disease, the presented patient received *iv* pulse cyclophosphamide therapy followed by oral azathioprine [15], which produced many side effects, among them life-threathening CNS infection.

At that time, the question of differential diagnosis was raised: whether it was about SLE exacerbation and development of neurolupus, or even manifestation of antiphospholipid syndrome (APLS), or CNS infection. The following examinations were performed: lumbal puncture, CT scanning and MRI imaging, which confirmed the infection of CNS and ruled out CNS vasculitis and APLS (ACA were, except for the first result, several times negative). We concluded that it was the case of acute neuroinfection in the immunosuppressed patient.

Clinical picture and course of disease with positive blood culture results indicated *Staphylococcus aureus* infection. This is supported by the fact that epileptic seizures first occurred during meningoencephalitis and occasionally kept on appearing as the consequence of encephalomalacia.

The patient's condition demanded antibiotic therapy from the beginning. After neuroradiologic diagnostics and positive blood culture, anticerebritic therapy was necessary: combination of the third generation cephalosporines with vancomycin, and consenquently, the combination of meropenem and vancomycin were used [16, 17]. From the beginning, the patient was on full-dose antibiotic therapy. At the moment of decission about meropenem dosage, we were guided by the two facts: the patient had middle-stage renal failure, and meningoencephalitis had already achieved clinical improvement, so the dose of meropenem was adapted (from the recommended dose of 3×2.0 g to 3×1.0 g).

We consider this report instructive and interesting because of many aspects. CNS infections are rare in patients with SLE, but they can be significant causes of mortality. Kim et al. [7] found 1,420 Korean patients with SLE out of whom 20 (1.4%) had meningitis.

During a 20-year review period, among 3,165 Taiwanese SLE patients, Hung et al. [18] identified 17 patients with CNS infections. *Cryptoccocus neoformans* was the causative microorganism in 10 patients and bacterial meningitis was found in 7 of then. Most patients (94%) had active SLE at the time of CNS infection. A total of 15 patients received corticosteroid therapy, and of these, 7 in combination with immunosuppressive agents. The mortality rate was extremely high (41.2%) [18]. The presented patient also had active SLE at the time of infection, and received GCS therapy in conjunction with immunosuppressive agents.

Yang et al. [19] described 38 SLE patients with CNS infections (*Mycobacterium tuberculosis* was identified in 19 of the patients, *Listeria monocytogenes* in 3, *Klebsiella pneumoniae* in 1, *Staphylococcus aureus* in 1, *Cryptococcus neoformans* in 12 patients, and *Aspergillus fumigatus* in 1 patient).

In 2009, Baizabal-Carvallo et al. [20] reported 23 patients with SLE and meningitis among 1,411 SLE patients in Mexico.

Conclusion

Since SLE patients are at higher risk of infections, before utilization of any immunosuppressive therapy, it is necessary to identify infections. Complete and careful examination of a patient, collection of throat, nasal and sputum swabs, urine culture as well as monitoring of CNS manifestations are required for choosing the therapy. Also, care is required about additional risk factors (mentioned above), and individial disposibility for infection, and to be aware that infection in immunosuppressed patients can be unpredictible.

Acknowledgements

We want to thank Academician Professor Miodrag Čolić (Faculty of Medicine of the Military Medical Academy in Belgrade University of Defence) for useful suggestions, advices, and well-intentioned criticism during preparing this paper.

REFERENCES

1. *Zandman-Goddard G, Shoenfeld Y*. Infections and SLE. Autoimmunity 2005; 38(7): 473−85.
2. *Gladman DD, Hussain F, Ibanez D, Urowitz MB*. The nature and outcome of infection in systemic lupus erythematosus. Lupus 2002; 11(4):234–9.

3. *Erdozain JG, Ruiz-Irastorza G, Egurbide MV, Martinez-Berriotxoa A, Aguirre C*. High risk of tuberculosis in systemic lupus erythematosus? Lupus 2006; 15(4): 232−5.
4. *Chen HS, Tsai WP, Leu HS, Ho HH, Lion LB*. Invasive fungal infection in systemic lupus erythematosus: an analysis of 15 cases and a literature review. Rheumatology 2007; 46(3): 539−44.
5. *Atzeni F, Bendtzen K, Bobbio-Pallavicini F, Conti F, Cutolo M, Montecucco C*, et al. Infections and treatment of patients with rheumatic diseases. Clin Exp Rheumatol 2008; 26(1 Suppl 48): 67–73.
6. *Amital H, Govoni M, Maya R, Meroni PL, Ori B, Shoenfeld Y*, et al. Role of infectious agents in systemic rheumatic diseases. Clin Exp Rheumatol 2008; 26(1 Suppl 48): 27–32.
7. *Kim JM, Kim KJ, Yoon HS, Kwok SK, Ju JH, Park KS*, et al. Meningitis in Korean patients with systemic lupus erythematosus: analysis of demographics, clinical features and outcomes; experience from affiliated hospitals of the Catholic University of Korea. Lupus 2011; 20(5): 531−6.
8. *Duffy KN, Duffy CM, Gladman DD*. Infection and disease activity in systemic lupus erythematosus: a review of hospitalized patients. J Rheumatol 1991; 18(8): 1180–4.
9. *Zandman-Goddard G, Berkun Y, Barzilai O, Boaz M, Ram M, Anaya JM*, et al. Neuropsychiatric lupus and infectious triggers. Lupus 2008; 17(5): 380–4.
10. *Walport MJ*. Complement deficiency and disease. Br J Rheumatol 1993; 32(4): 269−73.
11. *Noël V, Lortholary O, Casassus P, Cohen P, Généreau T, André MH,* et al. Risk factors and prognostic influence of infection in a single cohort of 87 adults with systemic lupus erythematosus. Ann Rheum Dis 2001; 60(12): 1141–4.
12. *Fessler BJ*. Infectious diseases in systemic lupus erythematosus: risk factors, management and prophylaxis. Best Pract Res Clin Rheumatol 2002; 16(2): 281–91.
13. *Bosch X, Guilabert A, Pallarés L, Cervera R, Ramos-Casals M, Bové A,* et al. Infections in systemic lupus erythematosus: a prospective and controlled study of 110 patients. Lupus 2006; 15(9): 584–9.
14. *Ng WL, Chu CM, Wu AK, Cheng VC, Yuen KY*. Lymphopenia at presentation is associated with increased risk of infections in patients with systemic lupus erythematosus. QJM 2006; 99(1): 37–47.
15. *Grootscholten C, Ligtenberg G, Hagen EC, van den Wall Bake AW, de Glas-Vos JW, Bijl M,* et al. Azathioprine/ methylprednisolone versus cyclophosphamide in proliferative lupus nephritis. A randomized controlled trial. Kidney Int 2006; 70(4): 732–42.
16. *Moorthy KR, Rajsheklar V*. Management of brain abscess: an overwiev. Neurosurg Focus 2008; 24(6): E3.
17. *Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM,* et al. Practice Guidelines for the Management of Bacterial Meningitis. Clin Infect Dis 2004; 39(9): 1267–84.
18. *Hung JJ, Ou LS, Lee WI, Huang JL*. Central nervous system infections in patients with systemic lupus erythematosus. J Rheumatol 2005; 32(1): 40–3.
19. *Yang CD, Wang XD, Ye S, Gu YY, Bao CD, Wang Y*, et al. Clinical features, prognostic and risk factors of central nervous system infections in patients with systemic lupus erythematosus. Clin Rheumatol 2007; 26(6): 895–901.
20. *Baizabal-Carvallo JF, Delgadillo-Marquez G, Estanol B, Garcia-Ramos G*. Clinical characteristics and outcomes of the meningitides in systemic lupus erythematosus. Eur Neurol 2009; 61(3): 143–8.

Received on October 26, 2011.
Revised on February 2, 2012.
Accepted on February 10, 2012.

CASE REPORT

UDC: 616-006.04:[616.67+616.688
DOI: 10.2298/VSP1307695V

Liposarcoma of the paratesticular tissue and spermatic cord: A case report

Liposarkom paratestikularnog tkiva i funikulusa

Filip Vukmirović*, Nihad Zejnilović†, Jovan Ivović†

***Department of Pathology, Clinical Center of Montenegro, Podgorica, Montenegro;**
†Department of Urology, General Hospital, Bar, Montenegro

Abstract

Introduction. Liposarcomas are malignant tumors derived from fat tissues. Liposarcoma of the paratesticular tissue is rare. **Case report.** We presented a 51-year-old man with liposarcoma of paratesticular tissue and the spermatic cord, mimicking a testicular tumor. Ultrasound examination of this scrotal mass was hyperechogenic and homogeneous and separated from the testis and epididymis. The patient was operated, and the orchidectomy was performed. Histology revealed well-differentiated lipoma-like liposarcoma of the paratesticular tissue and spermatic cord. After a 6 month follow-up the patient did not show any evidence of tumor-progression or recurrence. **Conclusion.** Liposarcomas of the paratesticular tissue and seminal cord represent a rare type of tumors, often misdiagnosed preoperatively. Therapy should include radical surgical excision, usually radical inguinal orchiectomy. If the margin status is in doubt, adjuvant radiation should be performed. Local relapse is common and may occur after several years, so follow-up period has to be sufficiently long.

Key words:
liposarcoma; spermatic cord; diagnosis; urologic surgical procedures; treatment outcome.

Apstrakt

Uvod. Liposarkom je maligni tumor koji nastaje iz masnog tkiva. Paratestikularni liposarkom se rijetko javlja. **Prikaz bolesnika.** U radu je prikazan bolesnik, star 52 godine, sa liposarkomom koji je bio lociran paratestikularno i u tkivu funikulusa, sa kliničkom slikom nalik tumoru testisa. Ultrazvučno, tumorsko tkivo bilo je hiperehogeno, nehomogeno i odvojeno od testisa i epididimisa. Kod bolesnika je urađena operacija orhiektomije. Histološki, nađeno je tkivo dobro diferentovanog *lipoma-like* liposarkoma u dijelu paratestikularnog tkiva i tkiva oko funikulusa. Nakon praćenja tokom 6 mjeseci nijesu uočeni znaci progresije tumora niti recidiva. **Zaključak.** Liposarkomi koji zahvataju paratestikularno tkivo i funikulus su rijetki i obično se preoperativno pogrešno dijagnostikuju. Terapija podrazumijeva radikalnu hirušku eksciziju, najčešće radikalnu ingvinalnu orhiektomiju. Ukoliko postoji sumnja o statusu ivica resekcije, potrebno je primijeniti adjuvantnu radioterapiju. Lokalni relapsi su česti i mogu se javiti i nakon više godina, pa je potrebno dugogodišnje praćenje ovih bolesnika.

Ključne reči:
liposarkom; semevod; dijagnoza; hirurgija, urološka, procedure; lečenje, ishod.

Introduction

Liposarcoma of the spermatic cord is a rare condition, representing about 7% of paratesticular sarcomas [1]. Rhabdomyosarcoma is the most common paratesticular malignant lesion [2].

Liposarcomas are malignant tumors derived embryologically from mesodermal tissues. They represent the most common soft-tissue sarcomas, and they can occur in any part of the body that contains fatty tissue. Liposarcomas of the spermatic cord usually begin to grow directly below the external inguinal ring, so when the tumors reach a large size, they present as scrotal rather than inguinal mass [3]. We report a rare case of a liposarcoma of the paratesticular tissue and spermatic cord, mimicking a testicular tumor, which was treated surgically. Examining the existing literature, we found little similar cases reported in the literature, but our case is a very rare finding where tumor is located paratesticularly with the expansion in the funiculus tissue.

Case report

A 51-year-old patient presented with a scrotal mass. He reported gradual enlargement of this painless scrotal mass during the previous year. Clinical examination revealed a painless scrotal mass adjacent to the external inguinal ring

Correspondence to: Filip Vukmirović, Department of Pathology, Clinical Center of Montenegro, 81 000 Podgorica, Montenegro. Phone/Fax: +382 20 241 976, Phone: +382 69 302 696. E-mail: filip.vukmirovic@kccg.me

but separated from the normal testis and epididymis was palpated. Testicular tumor markers were within the normal limits. Ultrasound examination of this scrotal mass was hyperechogenic and inhomogeneous and separated from the testis and epididymis, which showed no pathological finding. No malignancy was suspected.

In surgery, a mass was found to be not of testicular origin, but it was growing from the spermatic cord, descending into the scrotum, around the upper pole of the testis. Wide excision and radical orchectomy were performed. The tumor mass was measuring about 13 cm, it had a yellowish lipoma-like texture. This mass had a bunch of grape appearance and consisted of several masses of various sizes surrounding the spermatic cord, and could be separated from the right epididymis and the testis. The patient's postoperative course was uncomplicated, and he was discharged on the 2 postoperative day. Histological examination revealed lipoma-like well-differentiated liposarcoma (Figure 1–3). The tumor did not show any signs of infiltration into the testis or epididymis. All resection margins were found to be free of tumor. Following surgical resection of the tumor with suspicious margins adjuvant hemiotherapy was conducted. After a 6-month follow-up the patient did not show evidence of tumor-progression or recurrence and felt well.

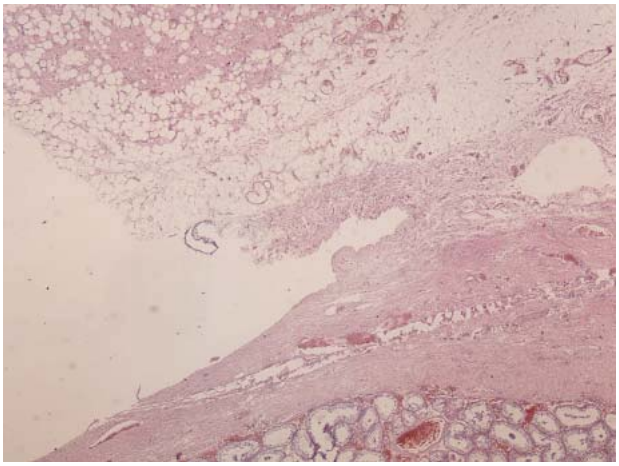


Fig. 1 – Testicular tissue with peritesticular tumor tissue (HE, ×20).

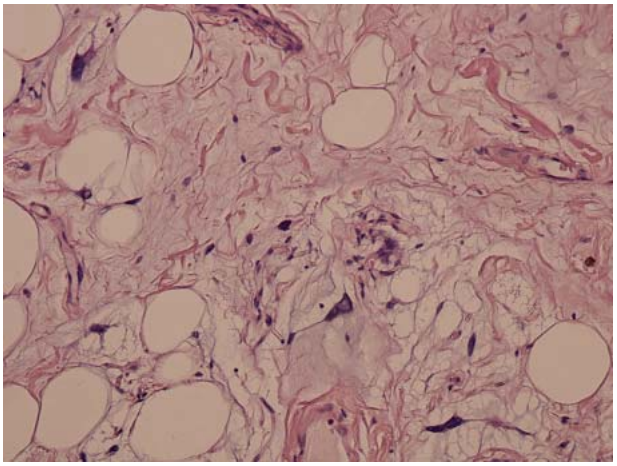


Fig. 2 – Well-differentiated (lipoma-like) liposarcoma with numeorus lipoblasts (HE, ×200).

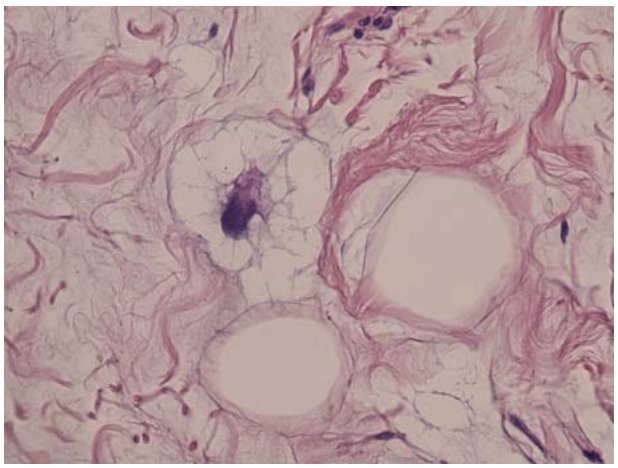


Fig. 3 – Well-differentiated (lipoma-like) liposarcoma showing multivacuolated lipoblast (HE, ×400).

Discussion

Liposarcomas are malignant tumors derived embryologically from mesodermal tissues. They are classified in 4 histology subtypes (well differentiated, myxoid, pleomorphic, and dedifferentiated) [4].

About 80% of seminal cord tumors are benign and mostly derive from lipomatous tissue. The first case of sarcoma of spermatic cord was reported by Lesauvage in 1845. [5]. Paratesticular malignancies and sarcomas of the seminal cord are in general rare. Most paratesticular malignant tumors are sarcomas. Despite the fact, that lipomas are the predominant benign tumors found in the inguinal region. Lipomatous tissue plays a minor role in seminal cord malignancies, comprising only approximately 5%–7% of all spermatic cord sarcomas. Liposarcoma may arise from the cord tissue representing the extension of retroperitoneal fat or as malignant transformation of preexisting lipoma. The mesenchymal origin rather than malignant transformation of lipomatous cells lead to liposarcomas [6, 7]. These tumors occur more frequently in adults rather than children [8], and although cases aging 16–90 years old are reported, the mean age at presentation is 56 years [9]. Liposarcomas usually present as slow-growing masses of the inguinal canal or the scrotum, mimicking testicular or epididymal tumors or inguinal hernias, and they are often diagnosed postoperatively. In the literature, several cases of various histologic subtypes are reported, including myxoid degeneration, sclerosing or inflammatory types, pleomorphic, and even cases with cartilaginous metaplasia [10–15]. Well-differentiated tumors usually have no metastatic potential, although the rate of metastases is high in undifferentiated tumors, usually through hematological route to lungs and bones [10, 16].

The basic presentations in patients are scrotal mass, that is sometimes associated with pain. Increase in size slowly over a period of months or years is the usual presentation. Liposarcoma is a disease of the older age group. No specific diagnostic procedures for evaluating this scrotal mass have

been recommended so far. In contrast to testicular masses, ultrasonography provides little information on paratesticular sarcomas, as some are visualized as homogenous and isoechogenic, others as inhomogeneous and echo density is quite variable. As liposarcomas are of low density and can be well demarcated the use of CT scans no pathognomonic features for the differentiation of benign versus malignant masses are defined [17]. Use of MRI provides good information on the local situation, but an exact evaluation of any masses again cannot be obtained.

Liposarcomas are locally aggressive tumors, thus recurrence is quite common after incomplete excision. Due to this radical surgical excision of any tumor is necessary. Inguinal radical orchiectomy is the standard approach for sarcomas of the seminal cord, in general, with wide resection margins [18]. The treatment of choice for liposarcomas of the spermatic cord is radical orchidectomy with high ligation of the spermatic cord [19], with excellent prognosis, but these tumors seem to have tendency towards local recurrence (≈ 25%) [20]. However the anatomical features of the inguinal region sometimes make it difficult to achieve this goal, and negative resection margins are sometimes close to the tumor. Local recurrence is a major problem, occurring in up to 50% of patients. However it is established for liposarcomas of the extremities, that the level of differentiation and the histological sarcoma type, as well as the tumorsize have little influence on recurrence rate. Due to the radiosensitivity of liposarcomas some authors recommend adjuvant radiation and the radiation field should cover the internal inguinal ring [21, 22].

Due to their relative resistance against chemotherapy, routine adjuvant systemic therapy is not justified in liposarcoma.

No specific outcome – data are available for liposarcoma patients since this disease is rare. A series of 32 seminal cord sarcoma patients reports a 15-year overall survival rate of 52%. A 10-year local control rate for 8 patients with liposarcoma included in this series was 44% [23].

As late recurrence can occur, follow-up examinations exceed 10 years [24].

Conclusion

Liposarcomas of the seminal cord represent a rare type of tumors which are often misdiagnosed preoperatively. Therapy should include radical surgical excision usually performed by radical inguinal orchiectomy, and mandatory second resection and hemiscrotectomy in cases of unclear resection margins are feasible. If the margin status is in doubt, adjuvant radiation should be performed. There is no clear view regarding the physical course and the proper treatment and prognosis of the disease. Local relapse is common and may occur several years after primary therapy. Thus, follow-up period has to be sufficiently long.

REFERENCES

1. *Sogani PC, Grabstald H, Whitmore WF Jr.* Spermatic cord sarcoma in adults. J Urol 1978; 120(3): 301−5.
2. *Biyani CS, Fitzmaurice RJ, Upsdell SM.* Paratesticular Liposarcoma. Int Urol Nephrol 1999; 31(6): 817−20.
3. *Papageorgiou MS, Dadakas G, Doney K.* Liposarcoma of the Spermatic Cord: A Case Report. Case Report Med 2011; 2011: 2.
4. *García Morúa A, Lozano Salinas JF, Valdés Sepúlveda F, Zapata H, Gómez Guerra LS.* Liposarcoma of the espermatic cord: our experience and review of the literature. Actas Urol Esp 2009; 33(7): 811−5. (Spanish)
5. *Hinman F, Gibson TE.* Tumors of the epididymis, spermatic cord and testicular tunics: a review of literature and report of three new cases. Arch Surg 1924; 8: 100.
6. *Schwartz SL, Swierzewski SJ 3rd, Sondak, VK, Grossmann HB.* Liposarcoma of the spermatic cord: report of 6 cases and review of the literature. J Urol 1995, 153(1): 154−7.
7. *Ballo MT, Zagars GK, Pisters PW, Feig BW, Patel SR, von Eschenbach AC.* Spermatic cord sarcoma: outcome, patterns of failure and management. J Urol 2001; 166(4): 1306−10.
8. *Woodward PJ, Schwab CM, Sesterhenn IA.* Extratesticular scrotal masses: radiologic-pathologic correlation. Radiographics 2003; 23(1): 215–40.
9. *Bostwick DG.* Spermatic cord and testicular adnexa. In: *Bostwick DG, Eble JN*, editors. Urologic Surgical Pathology. St. Louis, Mo, USA: Mosby; 1997. pp. 647.
10. *Domșa I, Olinici CD, Crișan D.* Spermatic cord mixed liposarcoma. Case report and review of the literature. Rom J Morphol Embryol 2008; 49(1): 105–9.
11. *Gómez Dorronsoro ML, Pascual Piédrola I, Córdoba IturriagaGoitia A, Valenti Ponsa C, Manrique Celada M, Garrón Aoiz L.* Spermatic cord liposarcoma: differential diagnostic criteria and treatment. Arch Esp Urol 2000; 53(1): 65−7. (Spanish)
12. *Coleman J, Brennan MF, Alektiar K, Russo P.* Adult spermatic cord sarcomas: management and results. Ann Surg Oncol 2003; 10(6): 669–75.
13. *Panagis A, Karydas G, Vasilakakis JE, Chatzipaschalis E, Lambropoulou M, Papadopoulos N.* Myxoid liposarcoma of the spermatic cord: a case report and review of the literature. Int Urol Nephrol 2003; 35(3): 369–72.
14. *Hornick JL, Bosenberg MW, Mentzel T, McMenamin ME, Oliveira AM, Fletcher CD.* Pleomorphic liposarcoma: clinicopothologic analysis of 57 cases. Am J Surg Pathol 2004; 28(10): 1257–67.
15. *Hagiwara N, Nishida Y, Fujimoto Y, Isogai K, Fujihiro S, Deguchi T.* Local recurrence of liposarcoma of the spermatic cord 6 years after orchiectomy: a case report. Hinyokika Kiyo 2002; 48(7): 443–6. (Japanese)
16. *Bhosale PR, Patnana M, Viswanathan C, Szklaruk J.* The inguinal canal: anatomy and imaging features of common and uncommon masses. Radiographics 2008; 28(3): 819−35, quiz 913.
17. *Cardenosa G, Papinicolaou W, Fung CY, Tung GA, Yoder IC, Althausen AF,* et al. Spermatic cord sarcomas: sonographic and CT-features. Urol Radiol 1990, 12(3): 163−7.
18. *Wilson N, Davis A, Bell R, Wilson AN, Davis A, Bell RS,* et al. Local control of soft tissue sarcoma of the extremity: the experience of a multidisciplinary sarcoma group with definitive surgery and radiotherapy. Eur J Cancer 1994; 30(A6): 746−51.
19. *Goodman FR, Staunton MD, Rees HC.* Liposarcoma of the spermatic cord. J R Soc Med 1991; 84(8): 499–500.
20. *Ulbright TM, Amin MB, Young RH.* Miscellaneous primary tumors of the testis, adnexa, and spermatic cord. In: *Rosai J, Sobin LH,* editors. Atlas of Tumor Pathology. Washington,

DC: Armed Forces Institute of Pathology Press; 1999. pp. 235–266.

21. *Fagundes MA, Zietman AL, Althausen AF, Coen JJ, Shipley WU.* The management of spermatic cord sarcoma. Cancer 1996; 77(9): 1873−6.
22. *Catton CN, Cummings BJ, Fornasier V, O'Sullivan B, Quirt I, Warr D.* Adult paratesticular sarcomas: a review of 21 cases. J Urol 1991; 146(2): 342−5.
23. *Ballo MT, Zagars GK, Pisters PW, Feig BW, Patel SR, von Eschenbach AC.* Spermatic cord sarcoma: outcome, patterns of failure and management. J Urol 2001; 166(4): 1306−10.
24. *Fagundes MA, Zietman AL, Althausen AF, Coen JJ, Shipley WU.* The management of spermatic cord sarcoma. Cancer 1996; 77(9): 1873−6.

Received on November 11, 2011.
Revised on December 22, 2011.
Accepted on January 11, 2012.

CASE REPORT

UDC: 618.1-089-06::[616.62-007.4-089:618.15-007.43-089
DOI: 10.2298/VSP1307699S

Paravesical haematoma following placement of an isolated anterior mesh for cystocele repair

Paravezikalni hematom posle umetanja izolovane prednje mrežice radi korekcije cistokele

Radmila Sparić*, Rajka Argirović*†, Snežana Buzadžić*, Milica Berisavac*†

***Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade, Serbia;**
†Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Abstract

Introduction. Pelvic organ prolapse is a substantial health problem for women around the world. Given the limitations of traditional surgery in the reconstruction of normal vaginal anatomy and function in genitourinary prolapse, various synthetic implants have been developed for surgical repair. Mesh procedures are gaining in popularity, encouraged by preliminary data. Although minimally invasive and relatively safe, serious complications following these procedures have been described. **Case report.** We presented a patient who had underwent an isolated anterior mesh procedure and developed postoperative haematoma which required surgical intervention. **Conclusion.** This report suggests that minimally invasive urogynecological procedures could result in significant complications. Thus, surgeons should be familiar with effective interventions in order to manage them.

Key words:
cystocele; surgical mesh; surgical procedures minimally invasive; postoperative complications; hematoma; treatment outcome.

Apstrakt

Uvod. Prolaps pelvičnih organa predstavlja značajan zdravstveni problem žena širom sveta. Imajući u vidu ograničenja tradicionalne hirurgije u rekonstrukciji normalnog anatomskog izgleda vagine i njene funkcije kod prolapsa genitalnih i urinarnih organa, razvijeni su različiti sintetski implantati koji se koriste pri hirurškom lečenju. Postupci s mrežicom se sve češće primenjuju, a preliminarni podaci su ohrabrujući. Iako su ovi postupci minimalno invazivni i relativno bezbedni, opisane su i teške komplikacije nakon njih. **Prikaz bolesnika.** Prikazana je 71-godišnja žena kojoj je ugrađena izolovana prednja mrežica, posle čega je došlo do nastanka postoperativnog hematoma koji je bilo potrebno hirurški zbrinuti. **Zaključak.** Ovaj slučaj ukazuje na činjenicu da minimalno invazivne uroginekološke procedure mogu biti praćene značajnim komplikacijama. Važno je da hirurzi, koji vrše ove operacije, budu obučeni za intervencije kojima se uspešno zbrinjavaju nastale komplikacije.

Ključne reči:
cistokela; hirurška mreža; hirurgija, minimalno-invazivne procedure; postoperativne komplikacije; hematom; lečenje, ishod.

Introduction

Pelvic organ prolapse is a substantial health problem for women around the world. Studies show that 50% of parous women lose pelvic support that results in prolapse [1]. The pathogenesis of genital prolapse is the result of the weakness of any or all of the pelvic support structures, which include levator ani muscle, connective tissue, uterosacral and cardinal ligaments, and rectovaginal fascia. It has a negative impact on these women's quality of life due to the associated urinary, anorectal, as well as coital dysfunction.

The choice of treatment depends on the patient's general health status, symptoms, quality of life impairment and prolapse type and grade. Surgical treatments aim at restoring the physiological anatomy of the vagina, alleviating symptoms and preserving lower urinary tract, bowel and sexual functions.

Varieties of abdominal and vaginal surgical techniques are used to treat pelvic organ prolapse. Some include the use of biological grafts or absorbable and non-absorbable synthetic meshes. Given the limitations of traditional surgery in the reconstruction of normal vaginal anatomy and function in

Correspondence to: Radmila Sparić, Clinic of Gynecology and Obsterics, Clinical Center of Serbia, Višegradska 26, 11 000 Belgrade, Serbia. Phone.: +381 11 366 3583, Fax.: +381 11 361 5603. E-mail: radmila@rcub. bg.ac.rs

genitourinary prolapse, various synthetic implants have been developed for surgical repair [1]. They are used to substitute or augment supportive tissue, thus improving surgical success and increasing the longevity of repairs [2, 3]. In the era of promoting minimally invasive surgery with the aim to decrease morbidity and hospitalization costs, the vaginal approach using synthetic mesh appears to be more attractive than conventional procedures [1].

Prolapse recurrence is most common in the anterior compartment, and traditional repair by anterior colporrhaphy has been associated with up to 32% failure rate [4]. Thus, mesh procedures, such as isolated anterior mesh, are gaining in popularity and preliminary data are encouraging. This procedure is a unique way of placing a prolene mesh between the vaginal mucosa and the prolapsed organ, thus recreating support for weakened pelvic structures. The surgeon approaches the repair vaginally, passing a specially designed trocars through pelvic landmarks. Trocar placement for anterior vaginal wall repair involves traversing the obturator membrane and the arcus tendineus fascia pelvis near the ischial spine.

Although minimally invasive and relatively safe, serious complications following an isolated anterior mesh procedure have been described, such as haemorrhage and the need for blood concentrate, bladder injuries, urinary retention, urinary tract infection, *de novo* urinary incontinence, infection, fever, buttock and groin pain, fistula formation, mesh shrinkage and erosion, and dyspareunia [1, 3, 5–7]. Haematoma formation has been cited as a possible complication of this procedure [3, 5, 6, 8].

Case report

A 71-year-old woman (G7, P2) presented with bearing-down sensation and incomplete urinary bladder emptying. Her history revealed no complaints of urinary incontinence. Vaginal examination in the dorsal lithotomy position revealed descent of the anterior compartment, while medial and posterior compartments were well-supported. The prolapse was classified according to Pelvic Organ Prolapse Quantification (POP-Q) classification. The patient had an isolated cystocele (Ba = +3), and during Valsava maneuver there was no descensus of the uterine cervix in relation to the hymen level (C = -7, D = -8, Bp = -2). Stress test in the supine and standing positions was negative.

The patient underwent an isolated anterior mesh procedure (Prolift® system, Ethicon, Somerville, NJ, USA) under general anaesthesia. It was performed according to the French TVM group technique, without difficulty, by the surgeon experienced in urogynaecological surgery. No cystocopy was performed, and the total operative time was 40 min. Intraoperative blood loss was average for our experience. A Foley catheter and vaginal packing were introduced for 24 h. The patient voided spontaneously and completely after cathether removal. The patient received intravenous antibiotic (ceftriaxon 2 g per day) and low-molecular-weight heparin prophylaxis for 3 days postoperatively. Her postoperative hemoglobin level was 118 g/L, and coagulation profile was normal. The postoperative course was uneventful, allowing the patient to void normally. The patient was stable at discharge and went home on the postoperative day 4. On the day 42 after the surgery the patient felt pain in the right hypogastric region of the abdomen. She was circulatory stable, but an 8-cm mass was palpated to the right of the uterus, indicating paravesical haematoma. The patient did not report any use of aspirin, anticoagulants or risk medications. Ultrasound scan revealed a hypoechogenic mass to the right of the urinary bladder of 80 × 71 mm (Figure 1). Surgical revision

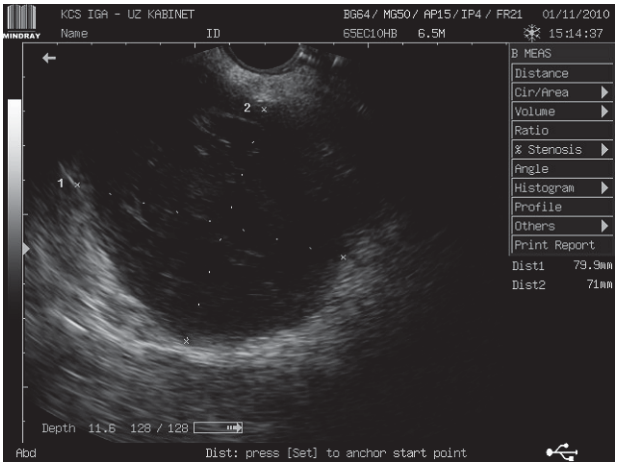


Fig. 1 – Transvaginal ultrasound image showing a hypoechogenic mass (paravesical haemathoma).

was initiated, clotted blood was evacuated from the paravesical space, just above the superior ramus of the right pubic bone, and a drain was inserted. No active bleeding site was found. The mesh could not be seen in the operative field, but was palpable in place. A total hysterectomy and a bilateral salpingoophorectomy were performed. Intravenous antibiotics (ceftriaxon 2 g per day and metronidazole 500 mg/8h) were administered for prophylactic reasons. Preoperative hemoglobin was 126 g/L, and 122 g/L after the procedure. A coagulation profile was repeatedly normal. The drain was removed on the second postoperative day. A repeat ultrasound scan showed no fluid collection in the pelvis, and the clinical examination revealed appropriate position of the mesh without displacement. Further postoperative course was without complications, and the patient was discharged on the postoperative day 7. One year after the surgery the patient was continent, and without complaints. On pelvic exam, the anterior vaginal wall remained well supported with no recurrence of her symptoms.

Discussion

New approaches to pelvic organ prolapse have been evolving rapidly with little data reported on safety issues. As more novel approaches to pelvic organ prolapse are introduced, a new set of complications may evolve. Any new surgical procedure also raises the question of the associated anatomical risks, especially when a part of the procedure is performed blindly.

The Prolift® procedure is a technique that incorporates mesh to compensate for areas of pelvic weakness. Operating in a highly vascularized, confined space, the surgeon may encounter complications that later may be challenging to manage. The placement of troacars near highly vascularized areas creates the possibility of haematoma formation as an operative complication. It is a well-known possibility in pelvic organ prolapse surgery, and therefore, this complication is not unique to the isolated anterior mesh application. Reports from the manufacturer indicate a risk of 1.75% surgery-derived hematomas [6].

If abnormal abdominal pain appears after those procedures, it is necessary to perform both vaginal and ultrasound examination. Patients should also be carefully examined if other complications occur, like buttock or groin pain, signs of shock, brisk vaginal bleeding and urinary retention. Most of the haematomas are asymptomatic or produce only minor symptoms. These are haematomas with small volume and usually no intervention is necessary. In contrast to that, haematomas with a greater volume provoke moderate to severe problems, like abdominal pain, urge symptoms, dysuria or circulatory disturbances. In such cases operative management of the haematoma is indicated [5]. Therefore, the decision if a patient should be treated conservatively or surgically must be made for each patient individually and with their consent.

A possible cause of haematoma formation following the insertion of an isolated anterior mesh is the injury of corona mortis, which refers to vascular connections between the external iliac and obturator systems in the obturator canal. These connections may be arterial, venous or both. It is known to hernia and orthopedic surgeons, but probably less well known to gynaecological surgeons [3]. With the increase in surgery of the anterior pelvic ring, many investigators have started to study the detailed anatomy of the retropubic vascular system. The incidence of communicating vascular channels has been reported to be 83% [3]. The name `crown of death` testifies to the importance of this feature, as significant haemorrhage may occur from its accidental lesion. This bleeding could be either arterial or venous in nature. The slow onset and late presentation of the haematoma in the presented case are not consistent with a corona mortis lesion. The presented patient had no recognized risk factors for postoperative bleeding, except the postoperative use of low-molecular-weight heparin, which might be a factor increasing the likelihood of postoperative haemorrhage. We assume venous source of haematoma. The haematoma was self-tamponaded and resolved after the surgical intervention.

This report illustrates that minimally invasive urogynecological procedures are not without significant complications. Various mesh kits are being heavily marketed, but there is a concern regarding a lack of information on their safety and efficacy [6]. With the number of mesh implants growing, there is always a concern for new complications that may arise whether from the kit itself or the use of the kit by those less experienced than the investigators who publish their data. It is equally important for the patient and the surgeon to be aware of different complications that may occur with these new procedures. Surgeons should counsel women about the complications that may occur when using these procedures, particularly those related to the use of mesh and the possibility that their management might necessitate surgical intervention under general anaesthesia. It is also important for surgeons to be familiar with effective interventions to manage them. Future research should be directed towards well-conducted and adequately powered randomized control trials, comparing vaginal mesh procedures with traditional surgeries with respect to surgical complications rate and how surgeons should manage device-related complications.

Conclusion

The prevalence of corona mortis and its anatomical relation to the pubic bone is important and should be considered when introducing new surgical approaches in pelvic surgery, thus decreasing the incidence of surgical complications and improving the results of operations.

REFERENCES

1. *Argirović R, Gudović A, Babović I, Berisavac M.* Transvaginal repair of genital prolapse with polypropylene mesh using a tension-free technique. Eur J Obstet Gynecol Reprod Biol 2010; 153(1): 104−7.
2. *Fatton B, Ambalard J, Debodinance P, Cosson M, Jacquetin B.* Transvaginal repair of genital prolapse: preliminary results of a new tension-free vaginal mesh (Prolift technique)-a case series multicentric study. Int Urogynecol J Pelvic Floor Dysfunct J 2007; 18(7): 743−52.
3. *Ignjatovic I, Stosic D.* Retrovesical haematoma after anterior Prolift procedure for cystocele correction. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18(12): 1495−7.
4. *LaSala CA, Schimpf MO.* Occurrence of postoperative hematomas after prolapse repair using a mesh augmentation system. Obstet Gynecol 2007; 109(2 Pt2): 569−72.
5. *Darmanis S, Lewis A, Mansoor A, Bircher M.* Corona mortis: an anatomical study with clinical implications in approaches to the pelvis and acetabulum. Clin Anat 2007; 20(4): 433−9.
6. *Hiltunen R, Nieminen K, Takala T, Heiskanen E, Merikari M, Niemi K,* et al. Low-weight polypropylene mesh for anterior vaginal wall prolapse: a randomized controlled trial. Obstet Gynecol 2007; 110(2 Pt2): 455−62.
7. *von Theobald P.* Place of mesh in vaginal surgery, including its removal and revision. Best Pract Res Clin Obstet Gynaecol 2011; 25(2): 197−203.
8. *Simon M, Debodinance P.* Vaginal prolapse using the Prolift™ kit: a registry of 100 successive cases. Eur J Obstet Gynecol Reprod Biol 2011; 158(1): 104−9.

Received on November 28, 2011.
Accepted on December 21, 2011.

CASE REPORT

UDC:616.13/.14-002-02:616-056.43]:[616.5+616.248
DOI: 10.2298/VSP130111022D

Churg-Strauss syndrome: A case report

Čarg-Štrausov sindrom

Miroslav Ž. Dinić*, Lidija Kandolf Sekulović*†, Lidija Zolotarevski†‡, Radoš D. Zečević*†

***Department of Dermatovenerology, ‡Center of Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia; Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Churg-Strauss syndrome (CSS) is an allergic granulomatous angiitis, a rare disease of small and medium arteries and veins, associated with the presence of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA). According to the American College of Rheumatology (ACR), there are four or more criteria out of six for the diagnosis: asthma, eosinophilia (> 10% in peripheral blood), paranasal sinusitis, pulmonary infiltrates, histological evidence of vasculitis with extravascular eosinophils, and mononeuritis multiplex or polyneuropathy. **Case report.** We reported a female patient, aged 80 years, with asthma for many decades and repeatedly verified eosinophilia in peripheral blood, in which CSS was suspected only after the occurrence of skin changes in the form of vesicles, vesiculopustule, purpuric macula, papule and petechiae. Further tests verified pulmonary infiltrates, paranasal sinusitis, extravascular eosinophils on histopathologic sample of skin tissue, and polyneuropathy. The treatment started with methylprednisolone (60 mg/d, with decreasing doses), and continued with pulse doses of cyclophosphamide (800 mg once monthly), also corticosteroid ointment for skin lesions. **Conclusion.** Despite long-standing pulmonary symptoms and laboratory findings of eosinophilia, the appearance of skin changes raised suspicion of possible CSS. Skin changes resolved and the patient was reffered to rheumatologist.

Key words:
churg-strauss syndrome; diagnosis; skin diseases; histological techniques.

Apstrakt

Uvod. Churg-Strauss sindrom (CSS) predstavlja alergijski granulomatozni angiitis. To je retko oboljenje, koje zahvata male i srednje arterije i vene i povezano je sa prisustvom perinuklearnog antineutrofilnog citoplazmatskog antitela (p-ANCA). Prema Američkom koledžu za reumatologiju (ACR), potrebno je četiri ili više kriterijuma od ukupno šest za postavljanje dijagnoze CSS: astma, eozinofilija (> 10% u perifernoj krvi), paranazalni sinuzitis, plućni infiltrati, histološki dokaz vaskulitisa sa ekstravaskularnim eozinofilima i *mononeuritis multiplex* ili polineuropatija. **Prikaz bolesnika.** Prikazali smo bolesnicu staru 80 godina, sa višedecenijskom astmom i eozinofilijom verifikovanom u više navrata, kod koje je postavljena sumnja na ovo reumatološko oboljenje tek posle pojave promena na koži u vidu vezikula, vezikulopustula, purpuričnih makula, papula i petehija. Daljim pretragama verifikovani su i plućni infiltrati, paranazalni sinuzitis, ekstravaskularni eozinofili na histopatološkom uzorku tkiva kože, kao i polineuropatija. Lečenje je započeto metilprednizolonom (60 mg dnevno, sa opadanjem doze), a nastavljeno pulsnim dozama ciklofosfamida (800 mg mesečno), uz kortikosteroidnu mast za lezije na koži. **Zaključak.** Uprkos dugogodišnjim simptomima plućnog oboljenja i eozonofiliji, tek je sa pojavom promena na koži posumnjano na CSS. Promene su se povukle, a bolesnica je nastavila lečenje kod reumatologa.

Ključne reči:
angiitis, alergijski, granulomatozni; dijagnoza; koža, bolesti; histološke tehnike.

Introduction

Churg-Strauss syndrome (CSS) is a systemic vasculitis involving small vessels, affecting several organs. Asthma is usually the first clinical sign of CSS. It is often accompanied by allergic rhinitis and sinusitis. CSS progress to peripheral and tissue eosinophilia, eventualy resulting in necrotizing vasculitis with extravascular granulomas. About 60% of CSS patients have skin lesions in the active phase of the disease, but the skin lesions may appear also in its early stage. Most common are palpable purpura and nodules, usually located on the limbs and scalp. Less usual skin features are livedo reticularis, vesicles, aseptic pustules, ecchymoses and urticarial wheals [1]. Also maculopapular erythematous eruption resembling erythema multiforme has been described [2]. Papular and nodular lesions may turn in necrotic-ulcerative evolution [3]. All of these

Correspondence to: Miroslav Ž. Dinić, Department of Dermatovenerology, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 3608 559. E-mail: miroslavdinic@hotmail.com

features can appear at the same time or in the different stages of CSS. Histopathology examination reveals a leukocytoclastic vasculitis, commonly involving venules; sometimes, the vessel wall reveals fibrinoid changes surrounded by granulomatous inflammation; finding of numerous eosinophils in the infiltrate, in addition to neutrophils, lymphocytes and macrophages, is of diagnostic importance [1]. We presented a female patient with suspected CSS due to cutaneous features which corresponded to lesions described in the literature. It was confirmed by histopathologic report.

Case report

A 80-year-old female was admitted to hospital with few vesicles, vesiculopustules, purpuric maculas, papules and petechiae on the skin of her hands, feet, extremities and trunk (Figures 1, 2 and 3). Skin changes occured a year and a half before, with fatigue and weight loss (about 10 kg of body weight), without neurological and other symptomatology. Data from the personal history revealed repeated eosinophilia in recent decades; diagnosed bronchial asthma was treated with beclomethasone, salbutamol and salmeterol xinafoate inhalation powder; arterial hypertension was not regularly treated.

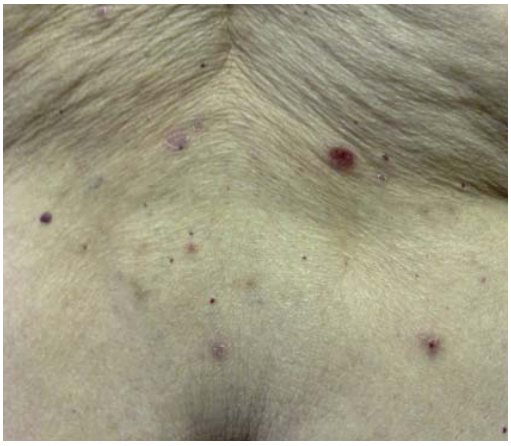


Fig. 1 – Purpuric macules and papules on the lower back.

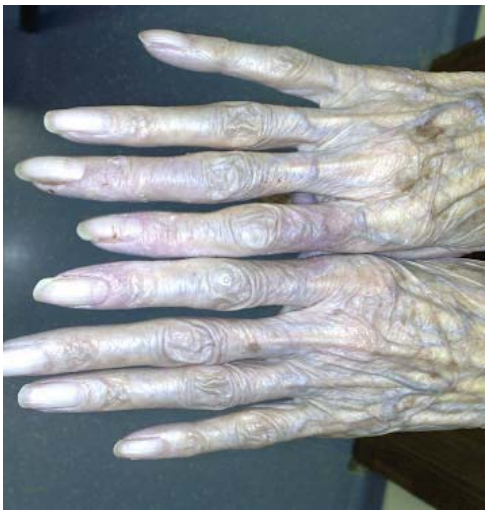


Fig. 2 – Vesicles, vesiculopustules, petechiae and purpuric maculas on the dorsal hand.

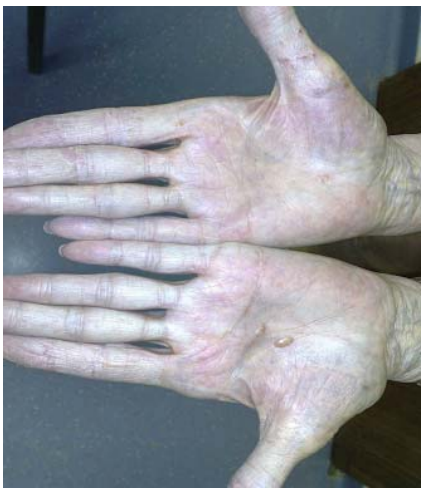


Fig. 3 – Vesicles, vesiculopustules, petechiae and purpuric maculas of the hand.

On admission, laboratory analyses revealed increased erythrocyte sedimentation rate (ESR) (54 mm/h), fibrinogen 6.83 g/L; eosinophilia 45.8% was evident (with normal count of leukocytes 9.57×10^9/L), with relative neutropenia 31.4%, lymphopenia 14.5%, as well as elevated rheumatoid factor of 66.4 U/L and increased immunoglobulin (Ig)E concentration 741 IU/L. Other complete blood count parameters, electrolytes, urea, creatinine, total bilirubine, creatine phosphokinase, protein electrophoresis, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gama-glutamyl transferase (γGT), IgG, IgA, IgM, C3 and C4 were within normal range. Urinalysis was normal. ELISA test for echinococcus, toxocara, HIV, HBsAg and HCV tests were negative. Other infective causes of eosinophilia were also ruled out [stool sample test for intestinal parasites and *Strongyloides stercoralis*, sputum for acido-resistance bacilli (ARB) direct examination and Löwenstein cultivation]. In peripheral blood smear increased number of leukocytes was evident (11.7×10^9/L), as well as elevated percentage of eosinophiles (61%). The concentration of proteins in 24-h urine (Biuret method, in 3 times) was normal; albumin in 24-h urine: normal findings. Antinuclear antibodies (ANA) (tissue-type substrate) +1 : 10; perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA) and anticardiolipin antibodies (ACA) within the physiologic range. Autoantibodies against basement membrane zone (ABMZ) were not detected. Lung function test: normal spirometry finding, transfer factor for carbon monoxide (CO) and transfer coefficient were slightly reduced; oxygen saturation was 96%.

Multislice scanning (MSCT) of the chest revealed changes of partly honeycomb appearance in the lungs bilaterally and in the upper and lower lobe, in favor of fibrosis, in particular mediastinal lymph nodes up to 1 cm in diameter at the level of tracheal carina in the right hilus and below the aortic arch (Figure 4). Bronchoscopy was planned but refused by the patient. Radiologic (X-ray) findings on paranasal sinuses revealed thickened lining of the left frontal and maxillary sinuses (Figure 5). Ultrasound examination of the heart, abdomen and pelvis revealed normal findings.

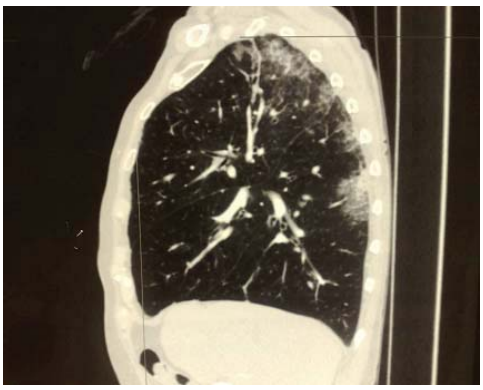


Fig. 4 – Multislice scanning of the chest revealed honeycomb lungs in favor of fibrosis.

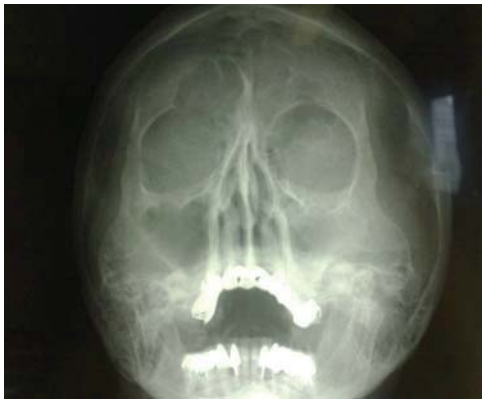


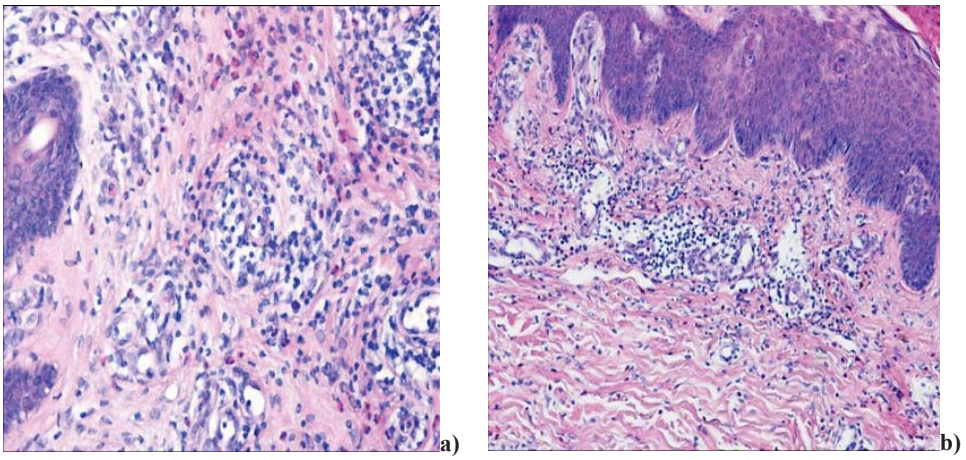
Fig. 5 – X-ray finding of paranasal sinuses showed thickened lining of the left frontal and maxillary sinuses.

Skin biopsy was taken and histopathologic analysis revealed infiltrate of lymphocytes, many neutrophils and eosinophils around capillaries and collagen fibers between the upper dermis, with clustered eosinophils at some places; the presence of cariorexic debri and fresh erythrocytes extravasated; periodic acid schiff (PAS) staining revealed segments of the microvascular wall space with fibrinoid necrosis (Figure 6a and b). Deposits of immunoreactants were not found on direct immunofluorescence (DIF) examination of the skin specimen. Neurologic examination revealed sensorimotor neuropathy.

Considering the changes in the lungs, asthma, hypereosinophilia, thickened mucosa of frontal sinus and sensorimotor neuropathy (consulted specialists were rheumatologist, neurologist, pulmologist, otolaryngologist, hematologist and infectologist) diagnosis of CSS was established and the therapy with methylprednisolone (60 mg daily *iv*, with decreasing doses), pulse dose of cyclophosphamide (800 mg) once monthly, was started, with vitamine B complex and tocopherol recommended by neurologist, bronchodilators recommended by pulmologist and local therapy with momethasone furoat 0.1% ointment once daily until skin changes resolved; then the patient was reffered to the rheumatologist.

Discussion

Churg and Strauss first described this disorder in 1951 when they reviewed 13 autopsy cases that were previously classified as polyarteritis nodosa. These cases were atypical in that asthma and eosinophilia preceded the systemic vasculitis. They named the syndrome "allergic angiitis and allergic granulomatosis", also known as CSS [4]. CSS is an allergic granulomatous angiitis: a very rare disease of small and medium arteries and veins, associated with p-ANCA [5, 6]. p-ANCA directed predominantly against a myeloperoxidase was initially reported in as many as 75–80% of patients with CSS, but in one study showed that only 13% had positive p-ANCA findings. Allergic granulomatosis and angiitis is a disorder characterized by extravascular granulomas, hypereosinophilia, and pulmonary and systemic small-vessel vasculitis. The American College of Rheumatology (ACR) has proposed 6 criteria for the diagnosis of CSS [7]. The presence of 4 or more criteria are enough for diagnosing CSS. These criteria include: asthma, eosinophilia (> 10% in peripheral blood), paranasal sinusitis, pulmonary infiltrates, histological evidence of vasculitis with extravascular eosinophils, and mononeuritis multiplex or polyneuropathy. The differential diagnosis of CSS includes at first place diseases associated with pulmonary infiltrates and eosinophilia (eosinophilic pneumonia, eosinophilic granuloma, infections and Wegener granulomatosis). Corticosteroids are the mainstay of treatment in CSS. The addition of other medications



a) b)

Fig. 6 – Periodic acid schift (PAS) staining showed segments of the microvascular wall space with fibrinoid necrosis [a) HE ×20; b) HE ×5].

(cytotoxic agents) may be necessary in cases of life- or organ-threatening vasculitis. The survival rate of CSS ranges from 68% to 100% at 5 years [8].

Cutaneous findings occur in 60% of CSS patients, and include palpable purpura, subcutaneous nodules (typically on the scalp or extremities), and less often, vesicles, urticaria, livedo reticularis, retiform purpura and papulonecrotic lesions [9]. They usually present on the limb surfaces, but can affect any part of the body. In our patient, few vesicles, vesiculopustules, purpuric macules, papules and petechiae on the skin of her hands, feet, extremities and trunk were found. In differential diagnosis autoimmune bullous dermatosis was observed, but immunopathology test did not aprrove it. Also, in hypereosinophilic syndrome, skin pattern is angioedematous with urticaria and dermographismus, erythematous pruritic papules, plaques and nodules, less common erythroderma and erythema annulare, but skin changes and thickened mucosa of frontal sinus of our patient with the absence of severe cardiac and neurologic manifestations (embolic or thrombotic), which are usual in primary hypereosinophilic syndrome, made clinical distinction. In our patient, skin changes were insufficient for making diagnosis by clinical examination, but histopathologic finding of skin specimen with perivascular eosinophils confirmed diagnosis of CSS cutaneous manifestation. The changes in the lungs, asthma, hypereosinophilia, thickened mucosa of frontal sinus, sensorimotor neuropathy and skin lesions established diagnosis of CSS.

Conclusion

Despite long-standing pulmonary symptoms and laboratory findings of eosinophilia, the appearance of skin changes raised suspicion of possible CSS. Skin changes correspond to changes in this syndrome have been described in the literature.

REFERENCES

1. *Marzano AV, Vezzoli P, Berti E*. Skin involvement in cutaneous and systemic vasculitis. Autoimmun Rev 2013; 12(4): 467−76.
2. *Cox NH, Jorizzo JL, Bourke JF, Savage CO*. Vasculitis *neutrophilic dermatoses and related disorders*. In: *Burns I, Breathnach S, Cox N, Griffiths CC*, editors. Rook's textbook of dermatology. Oxford: Wiley-Blackwell; 2010. p. 2360−454.
3. *Lhote F, Cohen P, Guillevin L*. Polyarteritis nodosa, microscopic polyangiitis and Churg-Strauss syndrome. Lupus 1998; 7(4): 238−58.
4. *Gota CE, Mandell BF*. Systemic necrotizing vasculitis. In: *Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ*, editors. Fitzpatrick's Dermatology in General Medicine. New York: McGraw Hill; 2008. p. 1606−16.
5. *Hellmich B, Ehlers S, Csernok E, Gross WL*. Update on the pathogenesis of Churg-Strauss syndrome. Clin Exp Rheumatol 2003; 21(6 Suppl 32): 69−7.
6. *Grau RG*. Churg-Strauss syndrome: 2005-2008 update. Curr Rheumatol Rep 2008; 10(6): 453−8.
7. *Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP,* et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990; 33(8): 1094−100.
8. *Phillip R, Lugmani R*. Mortality in systemic vasculitis: a systematic review. Clin Exp Rheumatol 2008; 26(5 Suppl 51): S94−104.
9. *Chung L, Kea B, Fiorentino DF*. Cutaneous vasculitis. In: *Bolognia JL, Jorizzo JL, Rapini RP*, editors. Dermatology. Philadelphia: Mosby Elsevier; 2008. p. 361−2.

Received on January 11, 2013.
Accepted on March 13, 2013.
OnLine-First April, 2013.

HISTORY OF MEDICINE

UDC: 61::616.9(091)
DOI: 10.2298/VSP1307706P

History of infectious diseases development in the Old and the Middle Ages with the emphasis on the plague and leprosy

Istorijat infektivnih bolesti u starom i srednjem veku sa osvrtom na kugu i lepru

Ana B. Petruševski

Health Care Center "Studenica", Kraljevo, Serbia

Key words:
history, ancient; history medieval; communicable diseases; plague; leprosy.

Ključne reči:
istorija, drevna; istorija, srednji vek; zarazne bolesti; kuga; lepra.

Introduction

For centuries branded as enigma infectious diseases are as for the common man, and the scientific community, the largest item of interest. Their contagiousness, number, as well as increasing diversity, led to the appearance of enormous challenges in finding the cause for a more effective control. Mass infectious diseases such as leprosy and plague were witnesses and causes of horrific suffering throughout the ages. The valuable data available to us, recorded by the physicians and historians, are the best proof of undisputed reign of illness that marked the old and middle ages.

History of infectious diseases of the Old Ages

For sure, the infectious diseases and outbreaks of the same, existed in prehistoric times, as surely they did not get the image of a mass phenomenon until man has not started reside in larger communities.

Based on skeleton results, drawings and objects originated in prehistoric times, we can see nowadays the level of development of medicine at that time. Disease at curing that period is almost impossible to prove. It is known that primitive tribes attributed the causes of diseases to magic [1, 2].

At ancient times, every disease used to be explained as supernatural, and anger against people in accordance with religious beliefs. Thus, first attempts at combating infectious diseases, and diseases in general, were prayers to God [1]. Babylonians also had a deity-like fly, which by their belief brought the plague, which leads to the assumption that the old people had the idea of insects as vectors of pathogens. Unfortunately, this and many other assumptions about the outbreaks in the Old Century, are difficult to prove because of the insufficient and incomplete number and types of data available to us [1, 2].

It is certain, however, that the emergence of diseases and the increasing number of victims pointed out the urgent need for prevention, finding the causes and efficient treatment procedures. Egyptians defended themselves of infections by garlic, while the later struggle in Rome continued by using cabbage [3].

In ancient Greece, Hippocrates was the first to hold certain dangerous substances, so-called. "miasmas" and although not stated as possible human transmission, it was very close right to the genesis of infection by states that they occur by inhalation of hazardous substances [2].

Other cultures of ancient people before the Greeks, did not come across supernatural causes of infectious disease, but not much progress in the discovery has been made thereof. The ancient Chinese, however, employed a method similar to immunization against measles [1]. Galen (Figure1), was a great Roman physician, whose medicine considered plague as epidemic disease, stressing that "if a disease fall upon many people, it is epidemic". His interpretation of the disease was in the fact that the heart is rapidly freeing enormous heat and "harmful liquid" and hence fever and other symptoms. Asclepius, however, spoke of "big atoms" that clog the pores of the skin. Diodorus and Thucydides, wrote about the mass infections, from "infection Attic" and "Athenian plague" in great detail. Based on old data, it became obvious that some of the prevention, and treatment could be a word [1, 2].

It is certain that the Old Ages already knew a number of infectious diseases as such, but judging by the number and type of infections described, it seems that the most common was the plague [4]. Thucydides describesed as "the Athenian plague" that ruled from 435 to 430 BC, which had its way from Ethiopia, Greece, Rome and finally Dalmatia. It is assumed that plague was in fact typhoid fever, which ended the "golden time" of Athens and destroyed citizenship and sol-

Correspondence to: Ana Petruševski, Health Care Center "Studenica", Kraljevo, Jug Bogdanova 112, 36 000 Kraljevo, Serbia.
E-mail: annaa77@ptt.rs

Fig. 1 – Galen (130–200).

diers of Athens [1, 5]. A terrible plague epidemic struck the Roman Empire next to the eruption of Vesuvius, to a similar repeated in 166 when thousands of people died. Extinguished after 16 years, Galen had this disease himself, so he described it in details in his writings, although there were data that it could be even typhus or smallpox. The disease was brought by the troops from the Middle East and then killed about 5 million people. Next, the so-called "Cyprian Plague" was in a period of 251 to 266 although the above description of this period was probably the smallpox. In addition to plague and smallpox, malaria was one of the most dangerous infectious diseases in the Roman Empire, which was even accused in some writings for the cause of the collapse of the Roman Empire, because for a short period it devastated people and soldiers of Rome [1, 2].

At the turn of the Old in the Middle Ages, the 531 to 580 "Justinian Plague" took a large number of lives, and although no detailed epidemiological data it was probably "bubonic plague" also called "plague of Procopius". Procopius described this plague and stated that during his life of 50 years it destroyed a half the population of the Byzantine Empire [2, 6].

The largest amount of data that tell us about infectious diseases that were prevailing in the Old Century makes an important epidemiological interpretation and notes, but they are based on descriptions of the affected population and the general suffering.

History of infectious diseases in the Middle Ages

Although in ancient Greece and Rome began to pay attention to hygienic conditions and living conditions of the population, this achievement did not come to life in the Middle Ages. Orientation towards religion and church of this period did not leave time for adjustment and sanitation of the cities. Medieval cities were charged, filth, without adequate discharge of waste, no clean drinking water and streets crowded with dead animals [7]. Besides these, for the infectious diseases ideal conditions, were favorable conditions for the use of potable water from impure water wells and streams, and public baths, used by people suffering from various kinds of diseases, along with healthy people.

Sanitary regulations were known by 1104 in Augsburg, as well as concern about cleanliness of streets and houses, and food [2, 7]. Unfortunately, the causes of infections are still called "miasmas", blaming demons and witches, and even as alleged in the statement of the Medical Faculty in Paris in the 14th century and "contrary planet embroidery" [2]. Epidemics are called "pestis" or "pestilentiae", regardless whether it comes out of plague or not, and most historians described them. Infectious diseases were further described by the first Arabian doctors. One of the most common method of treatment in the Middle Ages was the so-called "bloodletting" (Figure 2).

Fig. 2 – Blood drawing.

There were certain rules regarding personal hygiene, maintenance of facilities and waste disposal that came into force in the late 14th century. The Parliament in England dealt with them, and in 1388, adopted the provisions that concerned sanitary waste disposal [7].

Girolamo Fracastoro (Figure 3), Italian physician, pointed to "seminaria contagiosum" – small beings as agents of infectious diseases.

Among the most significant epidemics of the Middle Ages was the epidemic of plague and leprosy.

Fig. 3 – Girolamo Fracastoro (1478–1553).

History of plague development in the Middle Ages

Also called "Black Death" because of massive black bruises on the skin, the plague began his infamous rulein the 40-es of the 14th century in the Northern China and Mongolia and then spreaded throughout Asia, Europe and Africa. In Asia, then, not counting China, killed more than 20 million people [2, 6, 7].

Southeast Asia was an endemic area for the development of plague. Bad living conditions in the Asian region, where people lived together with animals, with many carriers of disease, such as rodents, and insects, were suitable for the beginning of plague [8, 9]. Although they did not know any causes or nature of the disease, in China practiced burning of dead rats at large squares, and escaping into the surrounding forests, until it settled dying rats. It was believed that plague was spread by wind, so people stayed in houses with closed windows [2]. In Egypt and Syria, plague spreaded in 1346. Plague was caused once by the rats population density, from which fleas transmitted the disease, the human approached, the traffic from Asia to Europe developed rapidly, and the hygienic conditions and circumstances worsened [5, 10].

Italy was the first country in Europe where plague appeared in 1347 when in Venice died about 100,000 people [2]. Plague arrived in Sicily, by Italian ships that came from the Black Sea when the crew was found dead, covered with purulent blisters, and the ship was crowded by rats. Then, in Vienna died about 40,000 people. A large "mortality", as they called the plague, took heavy casualties and about 43 million people, of whom 25 million in Europe. In Russia, plague arrived in 1349 carrying death, famine, atrocities, and tremendous changes in the sociocultural and political life. Continuing his deadly rampage, the plague arrived in Dalmatia, and the suffering in Split was best described by archbishop Marin Cutheis who stated that "the disease first appeared in animals" and than in people. He stated that "a man seized by disease, first weakened, and then had on the body red pimples, accompanied by severe fever" [1, 2].

The symptoms of the disease were fever, chills, headache and bloody blisters. Plague could be divided into three types: simple-frequently, septicaemic and pneumonic [8, 9]. In medieval Serbia, people called the plague "cuma" and it was one of the greatest curses that could befall a man. Thanks to the Hilandar code we know that doctors used drugs of plant and animal products, and cupping [11, 12].

All measures undertaken by the doctors were left without success, since squares, houses, streets were full of corpses of patients, which were fed to rodents, birds and animals. For their own protection, doctors had a special suit used in contact with patients, and many were hiding and fleeing from infection [2, 7].

From time to time doctors agreed to examine patients for a high fee when they used sticks to touch them. A special waxed suit was first used to treat fever only during the plague epidemic in Marseilles in 1531. Doctors had a hat and gloves, while the mask in the form of bird face had glass-covered openings for the eyes as well as opening for the nose-shaped beak filled with fragrance believed to purify air. Not knowing anything about the causes, the only salvation people were looking for at churches, prayed to and sacrificed for the Saints. There was a special group called themselves "flagellants", convinced that throwing out the sins, by scourging, would make there able to protect themselves from plague in that way [2, 7].

The treatment of fever was done by onion, garlic, arsenic, even the leaves of tobacco. After 7 years of the rule, abruptly as it began, stifled the epidemic of plague. During the 15th century, along the whole of Europe, emerged as a pandemic of plague, spreading rapidly by the development of traffic, but in a significantly less extent than the 14th century pandemics. It is believed that the bubonic plague during the Middle Ages destroyed a third of the European people. Until 19th century, plague continued with hundreds of outbreaks in Europe, and it is still present in some parts of the world today [4, 6].

As noted, the doctors of the Middle Ages did not know the causes of plague, and so there was no adequate therapy. For the pope`s doctors, "plague is a new and unknown disease" with clinical picture described in details, including enlarged glands and pneumonia. Unfortunately, old drugs such as "mithridate" and "teriyak" and bloodletting yielded no results. A significant progress in curbing infectious diseases, introduced quarantine and enforcement of sanitary measures, and the plague during the 18th century slowly eroded [1, 2, 10].

First measures of isolation were present even in Milan and Venice in 1374. The first official quarantine was open in 1377 in Dubrovnik, where passengers who came to Dubrovnik from infected areas, spent at least 30 days at certain places, such as Cavtat before boarding. Later it increased to 40 days Italian word quaranta means forty, so this type of insulation was called quarantine. The importance of quarantine was high and made all the other outbreaks considerably less pronounced [2, 11, 12].

Much later, in 1894 Kitiyato and Yersin discovered the cause of plague, independently of one another, and the epidemic of plague stopped only during the 19th Century [6].

History of leprosy development in the Middle Ages

Leprosy, which translated from Greek means fish scales, is a chronic infectious disease which clinical symptoms caused skin and mucosal lesions, and peripheral neuropathy [9]. It was mentioned in the 3rd book of Moses stated that people with leprosy must stay closed for 7 days, and in case of no help, they should let go in torn clothing, bareheaded, and some may cover the mouth shouting "Unclean". According to the Gospel of Matthew, Jesus healed leper [13, 14]. Leprosy was mentioned in 1200 BC, and it was, also described by Cicero, who lived in the period from 106 to 43 BC. It was transferred from Asia to Europe in the 3rd century BC by Greek soldiers. John`s Gospel mentioned St. Lazarus as a protector of leprosy patients, so that the order of the Knights "St. Lazarus" took care of lepers from 1120 [13, 14].

It was known in the Middle Ages about the leprosy that it was the contagious and incurable disease. It was present during the period from the 11th to the 14th century. Historians of that time described the illness in detail. They stated that the infected had "nodes" like smallpox all over the body, that they were exhausted and apathetic. Leprosy patients experienced the disintegration of their bodies, loss of fingers, nose, which the nation had not met with understanding, but on the contrary with the conviction, and so they were called "unholy" and burned at the bonefire.

Leprosy was considered as hereditary disease. The patients were identifiable by the clothes they were given to distinguish them from healthy people. As they were forbidden to have contact with their own families and people around, they had to carry sticks to be recognized and to touch objects. They wore a bell on it to alert people of their arrival, even to go to a particular side of the road depending on the direction of wind blowing. They were alive sentenced to death and thus suffered not only physical pain, but also a strong psychological pressure, rejected by the society.

Located in so-called "leprosaria", rooms for isolation, with no rights, stigmatized by condemnation of the society because they were sick, they were shure only in their death. During the 13th century, France had over 2,000 leprosaria and there was a home for leprosy patients in Jerusalem [1, 15, 16].

Very convincing descriptions of leprosy patients could be seen at the paintings of many artists in the Middle Ages, especially during the Crusades when the highest leprosy spreaded [13].

Data on leprosy are in the Chilandar code, and the frescoes from the 14th century of monastery Lesnovo give plenty of motives from the period of leprosy epidemic in that period. So, they show Michael the Archangel who blesses the sick of leprosy, Stefan clerk who gives money to the sick and child his (Figure 4), and quarantine for the treatment of the sick [13, 14].

In medieval Serbia, king Milutin was serving for the leprosy, along with other patients suffering from skin diseases. In London there was a very old hospital for leprosy patients treatment dedicated to St. James [7, 17].

The cause of leprosy was found in 1873 by the Norwegian scientist Gerhard Armauer Hansen (Figure 5), when the disease was called Hansen`s disease.

Fig. 4 – A leprosy patient with a child receiving alms (fresco in the Monastery of Lesnovo, Macedonia)

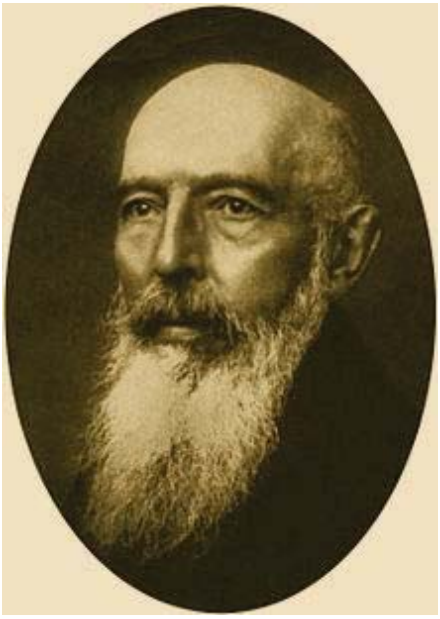


Fig. 5 – Gerhard Armauer Hansen (1841–1912)

Great disaster of the Middle Ages began to wane in the late 14th century. Unfortunately, leprosy as a public health problem exists today because it is still present in some areas of the modern world [9, 18].

Conclusion

Historical records of the Old and Middle Ages contain a significant amount of data on the development and movement of infectious diseases at that time. Capture of physicians and historians are of paramount importance for understanding the way that infectious diseases were passed from then to modern times. Plague and leprosy, as one of the most common diseases and largest of the Old and Middle Ages, and the current date, as well as their impact on lives and historical and social trends, constantly remind us of the enormous power carried by infectious disease.

R E F E R E N C E S

1. *Glesinger L.* The medicine through centuries. Zagreb; Zora; 1954. (Croatian)
2. *Stanojevic V.* History of medicine. Belgrade, Zagreb: Medicinska knjiga; 1962. (Croatian)
3. *Tucakov J.* Healing with plants – phytotherapy. Belgrade: Rad; 1990. (Serbian)
4. *Werner G.*Historical aspects of some infectious diseases: is their gradual eradication utopian? Rev Hist Pharm (Paris) 2009; 57(362): 173−92. (French)
5. *Sabbatanis S, Fiorino S.* The Antonine Plague and the decline of the Roman Empire. Infez Med 2009; 17(4): 261−75. (Italian)
6. *Ristić S.* Important infectious diseases today. Beograd: Ars medica; 1998. (Serbian)
7. Medicine in the Middle Ages. History Learning site. History of Medicine.[cited 2011 May 22]. Available from: http://www.historylearningsite.co.uk/medicine-in-the-middle-ages.htm
8. *Begovac J, Božinović D, Lisić M, Baršić B, Schonwald S.* Infectology. Zagreb: Profil International; 2006. (Croatian)
9. *Božić M, Dokić Lj, Nikolić S, Pavlović M, Šašić M.* The Infective diseases. Belgrade: CIBID; 2004. (Serbian)
10. *JillingS K.* Plague, pox and the physician in Aberdeen,1495-1516. J R Coll Physicians Edinb 2010; 40(1): 70−6.
11. *Katić R.* The origin of Serbian medieval medicine. (Monograph DXXXII). Belgrade: SANU; 1981. (Serbian)
12. *Lalović A.* Serbian Medicine in the Middle Ages. Timočki medicinski glasnik 2004; 29(Suppl 1): 27−30. (Serbian)
13. *Gabelić SD.* Monastery Lesnovo: history and painting. Beograd: Stubovi kulture; 1998. (Serbian)
14. *Rajić A.* Leprosy in our region. III Scientific Conference history of health in Zajecar; 2011. September 29–30. Zaječar: Rajačka škola zdravlja; 2011.
15. *Navon L.* Beggars, metaphors, and stigma: a missing link in the social history of leprosy. Soc Hist Med 1998; 11(1): 89−105.
16. *Makino M.* Learning from the history of leprosy--looking back at one hundred years of medicine at the leprosaria]. Nihon Hansenbyo Gakkai Zasshi 2010; 79(1): 25−36. (Japanese)
17. *Jireček K.* History of the Serbs.Belgrade: Izdavačka knjižarnica G. Kona; 1922. (Serbian)
18. *Grange JM, Lethaby JI.* Leprosy of the past and today. Semin Respir Crit Care Med 2004; 25(3): 271−81.

Received on June 15, 2011.
Revised on July 20, 2011.
Accepted on July 29, 2011.

LETTER TO THE EDITOR

Impact of imaging diagnostics on the budget – Are we spending too much?

Uticaj *imaging* dijagnostike na budžet – Da li previše trošimo?

To the Editor:

Serbia's financial constraints in health care should be regarded typical for a wider Eastern European context. Sustainability of long-term funding is threatened by a number of determinants including population aging, consequences of worldwide economic crisis and current budget deficit issues. The underdeveloped legal framework imposes no mandatory cost-effectiveness evidence submission in approval and reimbursement consideration strategies on new medical technologies and therefore contributes to the issue [1]. Most responsible policies aimed at achieving an optimal value for money in health care focus on prescribing behavior of physicians [2]. The issue of consumption patterns and costs of imaging diagnostic techniques is a particularly underexploited area of research in health economics [3]. We witness an unprecedented contemporary development of novel medical technologies in clinical radiology affecting market supply. A substantial budget impact could be attributed not only to the high tech services such as CT, PET and NMR, but interestingly even to the simple classical X-ray examinations in case of massive utilization [4]. The key long-term obstacle belongs rather to the demand side of the market equilibrium and the growing burden of prosperity diseases within the national health system [5–6]. Getting familiar with determinants of imaging diagnostics utilization patterns and related costs could give us grounds for informed cost saving policy. The amount of avoided unnecessary spending could be essentially allocated to cover current deficits, e.g. in the drug acquisition budget.

In order to give some ground estimates on the extent and structure of radiology related consumption a pioneering local retrospective study was conducted. Electronic registry of 56,007 patient discharge invoices of tertiary university hospital in Kragujevac, Serbia (1,293 beds capacity) was analyzed in 2010. The study provided an in-depth insight into prescription practices of specialty physicians in demanding radiological examination procedures. The observed Serbian tertiary care university hospital is in charge of covering the needs of almost 30% of general population of the central Serbia region. For this reason and due to the paucity of other evidence the authors observed these patterns of care as a likely nationwide state of the art within hospital facilities. In this single year, 16% of patients processed underwent some of nuclear medicine services, while 81% of patients underwent some other imaging diagnostics or emergency radiology services. High tech imaging methods were impressive consumers of hospital budget with CT targeted imaging of particular organs accompanied with the reconstructions on lead (€1,086,895.50). Nevertheless, commonly applied methods of interventional radiology (invasive hemodynamics, followed by selective coronary angiography and cardiac catheterization, PTC revascularisation (without stent implantation) and endovascular treatment of intracranial aneurism exhibited by far the most substantial budget impact (€2,667,510.92). Regardless of monetary value, the authors would like to point out insufficient deployment of interventional radiology methods in Serbia, capable to replace many surgical procedures, greatly shorten the length of hospital stay and reduce the long-term expenses [7]. It should be noted that the average imaging diagnostics costs per patient examined in Serbia vary greatly depending on methods observed: classical radiography €17.2, CT+MR+sonography €77.36, interventional radiology €189.86 and nuclear medicine € 33.23 (see Table 1).

Table 1

Average utilization of imaging diagnostics procedures per 1,000 examined patients and average imaging diagnostics cost per patient (Serbia, 2010)

Examination techniques observed *	Average number of examinations per 1000 patients	Average imaging diagnostics cost per single patient (CSD)
Radiography	370	1,773.00
CT, NMR, Sonography	558	7,996.61
Interventional Radiology	100	19,625.67
Nuclear Medicine	138	3,434.91
	1116 (total)	8,236.02 (average)

***relate to the hospital outpatient, emergency room and inpatient care.**

The golden axiom of health economics claims that policy makers should be focused on population health improvement rather than cost containment. Therefore, we should point out to the high potential value of reliable imaging diagnostics in clinical radiology. A 1998 study by Rao et al.[8] pointed out to the very cost saving CT technology in treating clinically suspected appendicitis. Ultrasound and bone densitometry for osteoporosis screening in postmenopausal women [9] and positron emission tomography for the management of potentially operable non-small cell lung cancer [10] are also among the cost-effective procedures. Some of the proven policy strategies to improve efficiency of radiology diagnostics are quality enforcement strategies in teaching hospitals [11]. Nevertheless, some other approaches such as routine radiology panels were assessed with unsatisfactory cost-effectiveness [12].

University hospital in Kragujevac, Serbia, had a disposable budget in 2011 of approximately €40,000,000. The total expenses of imaging diagnostics services provision amounted to €4,462,368.36 in 2010. Most of the expenses of running the facility were covered by the public domain through contracting with the Republican Health Insurance Fund of Serbia. Hospital budget did not expand substantially from the previous year due to ongoing macroeconomic crisis worldwide. Therefore we can calculate that costs of imaging diagnostics provision account for 11.16% of annual hospital budget. In 2011 Serbia had a total national health care expenditure of €3,604,929,979.2 out of which approximately one half was out-of-pocket and another half public source of funding [13]. It means that the observed institution, Clinical Center Kragujevac actually acquired 1.11% of disposable healthcare budget. The last officially reported total number of hospital beds in Serbia was 38,835 in 200913. This would mean that 42 general hospitals and 7 tertiary care clinics in Serbia consume almost €134,026,353.64 value for imaging diagnostics provision. These services would represent 3.72% of total national health expenditure. Grounded in current methods and field results presented, we are not able to assess outpatient radiology examination costs. Official Australian Ministry of Health and Aging 2010 estimate the reported fraction of 2.6% national health spending attributable to diagnostic imaging in Canada, as the top spending example (nationwide) among OECD countries [14].

The frequency of Serbian imaging diagnostics examinations, in terms of unit utilization is still lagging behind the ones reported in developed countries [15]. We should remain aware that fraction of CT and MR imaging far exceeds classical radiography in Serbian population unlike in mature health systems [15]. A pattern of overutilization of high tech imaging services seems to be present. The authors share the opinion that CT and MR should be considered a prime target for future health policy interventions aimed at more selective screening of patients – candidates for such examinations. This will certainly become hot topic in many similar countries. Many of these methods are exchangeable for radiography or sonography methods with sufficient test sensitivity and specificity to detect many clinical conditions and far more modest budget impact [16–17].

The ongoing regional macroeconomic developments and growing budget deficit in Serbia will likely further constrain resources available to the hospital management [18]. In these conditions, adopting of local guidelines on the recommended frequency of imaging diagnostic examinations in key clinical conditions would be helpful. There is a room for improvement in terms of a wiser resource allocation. Introduction of cost-effectiveness requirements in marketing approvals of the most expensive imaging technologies would be particularly helpful in the future. Frankly speaking, we can observe the landscape of substantial progress in the region through the course of the past decade in terms of service availability for the community [1]. Grounded in this fact we hope that regional clinical radiology provision and management will improve and provide higher value for money in Southeast European health care in the long run.

Acknowledgements

The authors would like to express their gratitude to the Ministry of Science and Education of the Republic of Serbia for the Grant N◦ 175014, out of which this research project was partially financed.

Ana Ranković, Radiology Diagnostic Service, University Clinical Center Kragujevac, Serbia;

Nemanja Rančić, Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia;

Mirjana Jovanović, The Psychiatry Clinic, University Clinical Center Kragujevac, Serbia;

Miloš Ivanović, Department of Mathematics & Informatics, The Faculty of Science, University of Kragujevac;

Olgica Gajović, The Infectious Diseases Clinic, University Clinical Center Kragujevac, Serbia;

Zorica Lazić, Pulmonary Diseases Clinic, Clinical Center Kragujevac, Serbia,

Mihajlo Jakovljević, Pharmacology and Toxicology Department, The Faculty Medical Sciences University of Kragujevac, Serbia (E-mail: jakovljevicm@medf.kg.ac.rs)

REFERENCES

1. *Jakovljevic MB.* Resource allocation strategies in Southeastern European health policy. Eur J Health Econ 2013; 14(2): 153−9.
2. *Medina LS, Altman NR.* Establishing a health outcomes and economics center in radiology: strategies and resources required. Pediatr Radiol 2002; 32(11): 755−64.
3. *Otero HJ, Ondategui-Parra S, Nathanson EM, Erturk SM, Ros PR.* Utilization management in radiology: basic concepts and applications. J Am Coll Radiol 2006; 3(5): 351−7.
4. *Clevert DA, Stickel M, Jung EM, Reiser M, Rupp N.* Cost analysis in interventional radiology--A tool to optimize management costs. Eur J Radiol 2007; 61(1): 144−9.

.

5. *Biorac N, Jakovljević M, Stefanović D, Perović S, Janković S*. Assessment of diabetes mellitus type 2 treatment costs in the Republic of Serbia. Vojnosanit Pregl 2009; 66(4): 271−6. (Serbian)
6. *Radovanovic A, Dagovic A, Jakovljevic M*. Economics of cancer related medical care: estimates worldwide and available domestic evidence. Arch Oncol 2011; 19(3−4): 59−63.
7. *Cannon MA, Beattie C, Speroff T, France D, Mistak B, Drinkwater D*. The economic benefit of organizational restructuring of the cardiothoracic intensive care unit. J Cardiothorac Vasc Anesth 2003; 17(5): 565−70.
8. *Rao PM, Rhea JT, Novelline RA, Mostafavi AA, McCabe CJ*. Effect of computed tomography of the appendix on treatment of patients and use of hospital resources. N Engl J Med 1998; 338(3): 141−6.
9. *Mueller D, Gandjour A*. Cost effectiveness of ultrasound and bone densitometry for osteoporosis screening in postmenopausal women. Appl Health Econ Health Policy 2008; 6(2−3): 113−35.
10. *Nguyen VH, Peloquin S, Lacasse Y*. Cost-effectiveness of positron emission tomography for the management of potentially operable non-small cell lung cancer in Quebec. Can Respir J 2005; 12(1): 19−25.
11. *Patmas MA, Rosenblum R*. Teaching clinic lowers radiology utilization. A look at how to achieve more cost-effective imaging. Physician Exec 2004; 30(1): 16−8.
12. *Tasse JL, Janzen ML, Ahmed NA, Chung RS*. Screening laboratory and radiology panels for trauma patients have low utility and are not cost effective. J Trauma 2008; 65(5): 1114−6
13. *World Health Organization*. Global Health Observatory (GHO). Serbia: country profiles. Available from: www.who.int/.../countries/srb/country [accessed 2013 June 10].
14. *Acil Tasman Economics Policy Strategy*. Final report prepared for Australian Department of Health and Aging. Funding arrangements for diagnostic imaging services. Available from: www.health.gov.au/.../ACIL%20TASMAN%20Fi. [cited 2010 January 21].
15. *Bhargavan M, Sunshine JH*. Utilization of radiology services in the United States: levels and trends in modalities, regions, and populations. Radiology 2005; 234(3): 824−32.
16. *Armao D, Semelka RC, Elias J Jr*. Radiology's ethical responsibility for healthcare reform: tempering the overutilization of medical imaging and trimming down a heavyweight. J Magn Reson Imaging 2012; 35(3): 512−7.
17. *Emery DJ, Shojania KG, Forster AJ, Mojaverian N, Feasby TE*. Overuse of magnetic resonance imaging. JAMA Intern Med 2013;1 73(9): 823−5.
18. *Jakovljevic M, Jovanovic M, Lazic Z, Jakovljevic V, Djukic A, Velickovic R,* et al. Current efforts and proposals to reduce healthcare costs in Serbia. Ser J Exp Clin Res 2011; 12(4): 161−3.

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

Naslov: Primena neurolingvističkih strategija u tretmanu hroničnih somatskih bolesti

Urednik: prof. dr Slobodan Ilić

Izdavač: Univerzitet u Nišu, Medicinski fakultet (Centar za kontinuiranu edukaciju) i Društvo za Liaison psihijatriju

Mesto i godina izdanja: Niš, 2012.

Štampa: „Galaksija“, Niš

Format B5; tvrd povez.

ISBN: 978-86-525-0035-2

Knjiga „Primena neurolingvističkih strategija u tretmanu hroničnih somatskih bolesti“ predstavlja skup radova izloženih na seminaru koji je pod istim nazivom održan 24. juna 2011. u Nišu, u organizaciji Medicinskog fakulteta Univerziteta u Nišu i Udruženja za Liaison psihijatriju. Ova multidisciplinarna publikacija iz oblasti neurofiziologije, psihijatrije, neurolingvistike i kliničke medicine napisana je na 146 strana, sadrži 11 slika i 2 tabele. Autori pojedinih poglavlja u knjizi su: prof. dr Slobodan Ilić (psihijatar i specijalista nuklearne medicine i predsednik Udruženja za Liaison psihijatriju), prof. dr Milkica Nešić (neuropsihijatar), doc. Olivera Žikić, doc. Suzana Golubović i ass. Gordana Nikolić (psihijatri) i Lidija Marković (NLP master).

Knjigu čine tri tematske celine. Prva, pod nazivom „Psihofiziologija i patofiziologija – somatska bolest“ ima dva poglavlja: „Patofiziološke karakteristike i psihičke manifestacije stresa u kontekstu nastanka i evolucije hroničnih somatskih bolesti“ i „Psihofiziološki mehanizmi psihosomatskih bolesti“. Druga celina „Neurolingvističko programiranje – komunikacija – somatska bolest“, takođe ima dva poglavlja: „Integracija kognicije i emocije u konceptu socijalne neuronauke“ i “*Using neuro-linguistic strategies for effective patient – doctor communications*”, dok treća, pod nazivom „Neurolingvističko programiranje – terapija – somatska bolest“ ima tri poglavlja: „Mogućnosti kognitivno-bihevioralne terapije u tretmanu hroničnih somatskih bolesnika“, „Neurolingvistička psihoterapija“ i „Primena neurolingvističkih strategija u tretmanu hroničnih somatskih bolesti“.

Ideja vodilja pri pisanju ove knjige bila je da osvetli u kliničkoj praksi apsolutno zapostavljene psihološke aspekte hroničnih somatskih bolesti, da iste približi kliničkom lekaru i ukaže na praktičan značaj njihovog poznavanja. Poseban akcenat stavljen je na primenu psihoterapijske tehnike u razrešavanju psiholoških problema koji prate ove bolesti i u tom kontekstu je predstavljena neurolingvistička psihoterapija.

O ovome najbolje svedoči želja samih autora „da držeći se navedenog konteksta istražimo mogućnosti, do sada još uvek nedovoljno afirmisane neurolingvističke psihoterapije, fokusirajući njene potencijale, pre svega, opšti doživljaj bolesti“. Urednik knjige, prof. dr Slobodan Ilić ističe da „Bolest nije život, ona je samo deo životne situacije pacijenta, i ispod raznoraznih slojeva koji čine tu životnu situaciju, postoji nešto dublje i sušestvenije – biće pacijenta! Bolest nije neuspeh i ne treba osećati krivicu zbog njenog nastanka, niti optuživati druge ili sudbinu zbog nepravde i predodređenosti. Kad se dogodi – bolest, u njoj je skrivena snažna pouka na koju se u prvom trenutku retko koji pacijent osvrće. Neurolingvistička psihoterapija svojim sadržajnom dinamikom nastoji da

uveri pacijenta u posedovanje sopstvenih izvora za borbu sa bolešću. Za tok i prognozu bolesti to je od suštinske važnosti“. U nastavku navodi „Bolest ništa ne stvara, nego samo oslobađa i reakcija na nju ne zavisi od njene jačine i karaktera, već od premorbidne strukture ličnosti, podrške okoline i kulturoloških obrazaca ponašanja. Ona na najsuroviji, ali i najiskreniji način pokazuje šta je stvarno a šta nestvarno u pacijentovom životu, ili još dublje, šta je u njemu važno, a šta nije“. Zato „Neurolingvistička psihoterapija stvaranjem ambijenta u kome pacijent prepoznaje svoje mogućnosti i dobija nove izbore njihovog korišćenja, može da bude dragocen pomagač na putu trajnog postignuća takvog stanja“.

Autori knjige „Primena neurolingvističkih strategija u tretmanu hroničnih somatskih bolesti“ smatraju da „uloga medicinskog osoblja koje prolazi kroz ovakvu vrstu edukacije, naravno prilagođene specifičnoj profesionalnoj poziciji u tretmanu, je da pacijenta pomeri iz opsesivnog fokusa na somatiku, proširi mu doživljaj sebe i učini ga aktivnim učesnikom u borbi za kvalitetan život uprkos ograničenjima koje bolest donosi“.

Zbog toga knjiga „Primena neurolingvističkih strategija u tretmanu hroničnih somatskih bolesti“ predstavlja, kako njeni autori kažu, „trajni zapis svega izrečenog na seminaru, pledira da svim profesionalnim profilima koji su u neposrednom kontaktu sa hroničnim somatskim bolesnikom, proširi vidike, razvije empatiju i holistički pogled na sve aspekte njihove bolesti“.

dr sc. Rade R. Babić,
Centar za radiologiju Kliničkog centra u Nišu

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 2013. 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 *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2012. 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

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 2013. 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 2013. 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 _____