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ORIGINALNI NAUČNI RADOVI
ORIGINAL STUDIES

University of Novi Sad, Faculty of Medicine, Serbia
Department of Pharmacology, Toxicology and Clinical Pharmacology[1]
Institute for Public Health of Vojvodina, Novi Sad, Serbia
Department of Microbiology[2]

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SUSCEPTIBILITY OF COMMON BACTERIAL RESPIRATORY PATHOGENS TO ANTIMICROBIAL AGENTS IN OUTPATIENTS FROM SOUTH BACKA DISTRICT

OSETLJIVOST NAJČEŠĆIH BAKTERIJSKIH UZROČNIKA INFEKCIJA GORNJIH RESPIRATORNIH PUTEVA NA ANTIBAKTERIJSKE LEKOVE NA TERITORIJI JUŽNOBAČKOG OKRUGA

Olga HORVAT[1], Mira MIHAJLOVIĆ UKROPINA[2], Vesna MIJATOVIĆ[1] and Ana SABO[1]

Summary

Introduction. Acute infections of the upper respiratory tract are the most common reasons why patients visit general practitioners. Overuse of antibiotics in treatment of these conditions is extremely common practice although these infections are most frequently caused by viruses. The aim of this study was to determine the distribution and susceptibility of common pathogens to antimicrobial agents that cause infections of the upper respiratory tract in outpatients and to determine whether the results obtained from the examined sample were in accordance with the recommendations of the current National Guideline. **Material and Methods. .**The study included 945 strains isolated from the throat and nasal swabs from January 1st to March 31st, 2008, as well as from 330 strains isolated from January 1st to March 31st, 2013 in South Backa District, Serbia. Susceptibility tests were performed by the standard disc diffusion method and according to the criteria recommended by the Clinical and Laboratory Standards Institute. **Results.** The most commonly isolated strains were *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Branchamella catarrhalis,* and *Haemophilus influenzae*. Susceptibility of *Streptococcus pyogenes, Branchamella catarrhalis* and *Haemophilus influenzae* to examined antibiotics did not substantially change over the two study periods. None of the isolates of *Staphylococcus aures* demonstrated resistance to methicillin in 2008, while the percentage of resistant strains was 5.93% in 2013. Susceptibility rates of *Staphylococcus pneumoniae* isolates to erythromycin and clindamycin were lower in 2013 than in 2008. **Conclusion.** The investigation results follow the recommendations of the National Guideline for the usage of natural penicillin in the treatment of tonsillopharyngitis. Amoxicillin/clavulanic acid is recommended for the treatment of rhinosinusitis, and second generation cephalosporins are the second choice.

Key words: Respiratory Tract Infections; Bacterial Infections; Microbial Sensitivity Tests; Anti-Bacterial Agents; Practice Guidelines; Drug Resistance, Bacterial; Pharyngitis; Inappropriate Prescribing

Sažetak

Uvod. Akutne infekcije gornjih respiratornih puteva najčešći su razlozi posete pacijenata lekaru opšte prakse. Prekomerna upotreba antibiotika u ovim stanjima izuzetno je rasprostranjena iako su najčešće izazvana virusima. Cilj rada bio je da se ispita zastupljenost i osetljivost na antibakterijske lekove najčešće izolovanih bakterijskih uzročnika infekcija gornjih respiratornih puteva i utvrdi da li su dobijeni rezultati na ispitivanom uzorku u skladu sa preporukama postojećeg nacionalnog vodiča za terapiju infekcija gornjih respiratornih puteva. **Materijal i metode.** Ispitivanjem je obuhvaćeno 945 izolata iz briseva grla i nosa vanbolničkih pacijenata u periodu od 1.1. do 31.3.2008. godine kao i 330 izolata u istom periodu 2013. godine u Južnobačkom okrugu. Izolacija i identifikacija sojeva izolata vršena je primenom standardnih dijagnostičkih postupaka. Osetljivost na antibiotike ispitana je disk-difuzionom metodom prema preporukama *Clinical and Laboratory Standards Institute*. **Rezultati.** Najčešće su izolovani *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae, Branchamella catarrhalis* i *Haemophilus influenzae*. U oba ispitivana perioda osetljivost *Streptococcus pyogenes, Branchamella catarrhalis* i *Haemophilus influenzae* na ispitivane antibiotike nije se bitno menjala. U 2008. godini nisu izolovani sojevi *Staphylococcus aureus* rezistentni na meticilin, dok je u 2013. godini rezistencija zabeležena kod 5,93% izolata. Osetljivost *Staphylococcus pneumoniae* na eritromicin i klindamicin u 2013. godini bila je niža u odnosu na 2008. godinu. **Zaključak.** Rezultati našeg istraživanja slažu se s preporukama nacionalnog vodiča o primeni prirodnih penicilina kao lekova prvog izbora u terapiji tonzilofaringitisa. Za terapiju rinosinuzitisa može se preporučiti primena amoksicilina sa klavulanskom kiselinom, a kao lek drugog izbora cefalosporini druge generacije.

Ključne reči: Infekcije respiratornog trakta; Bakterijske infekcije; Testovi mikrobne osetljivosti; Antibakterijski lekovi; Nacionalni vodiči; Bakterijska otpornost na lekove; Faringitis; Prekomerna upotreba lekova

Corresponding Author: Doc. dr Olga Horvat, Katedra za farmakologiju, toksikologiju i kliničku farmakologiju, Medicinski fakultet Novi Sad, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: olgahorvat15@gmail.com

Abbreviations

CLSI	– Clinical and Laboratory Standards Institute
DDD/1000 inhabitants/day	– The Defined Daily Dose/1000 inhabitants/day
MRSA	– methicillin-resistant Staphylococcus aureus
MIC	– *minimum inhibitory concentration*

Acknowledgements. This work was supported by the Ministry of Science and Technological Development, Republic of Serbia, Project No. 41012.

Introduction

Acute infections of the upper respiratory tract are the most common reasons for seeing general practitioners. These infections account for 30-50% of sick leaves in adults and 60-80% of absence from school in schoolchildren [1].

Overuse of antibiotics in treatment of these conditions is extremely common practice, although these infections are most frequently caused by viruses. Antibiotics treatment is prescribed to 70% of patients with sore throat, 85-98% of patients with rhinosinusitis and 60% of patients with common cold [2,3]. This inadequate administration of antibiotics has resulted in increasing resistance of the most frequent bacterial causes of upper respiratory tract infections to antibiotics throughout the world over the last decades. This especially refers to *Streptococcus pneumoniae* which is becoming resistant to antibiotics most commonly used for treatment of respiratory infections [4,5].

Distribution of respiratory pathogens and degree of their resistance is the information of a great significance for the medical professionals in their everyday practice since it helps them chose the proper antibiotic drugs. Therefore, several national [5,6] and international [4,7,8] surveillance programs were conducted in last two decades. The objective of these programs was to monitor worldwide resistance of the most common respiratory pathogens in outpatients (*Haemphilus influenzae, Streptococcus pneumonia, Moraxella catarrhalis)* and it has been verified as the widespread problem in antimicrobial resistance in many countries in Europe. An increase in antimicrobial resistance has been reported in European countries with high levels of overall antimicrobial use [4].

A systematic surveillance of pathogens that cause upper respiratory tract infections has not been conducted in Serbia, including the region of Vojvodina, so far [9].

Swabs are taken only occasionally; thus, the respiratory infection treatment in outpatients typically involves administration of antibacterial drugs which are expected to be effective in these cases. A surveillance of resistance of the most common pathogens causing upper respiratory infection in outpatients was conducted on the territory of South Backa District in 2000 and 2002 [10,11]. Within the surveillance period, an increase in antimicrobial resistance of these pathogens was reported.

The aim of this study was to determine the distribution and susceptibility to antimicrobial agents of common pathogens causing infections of the upper respiratory tract in outpatients, as well as to determine whether the results obtained from the examined sample were in accordance with the recommendations of the current National Guideline "The choice and use of antibiotics in general practice"of the Ministry of Health of Serbia, in the cases of upper respiratory tract infections - sinusitis and tonsillopharyngitis.

Material and methods

Potentially positive pathogens were isolated in 954 cases and in 330 during the period from January 1st to March 31st, 2008 and from January 1st to March 31st, 2013, respectively.

The study included 1284 isolates from the throat and nasal swabs in outpatients of all ages with the symptoms of upper respiratory tract infections at the Department of Clinical Bacteriology, Center of Microbiology, Institute of Public Health of Vojvodina during the study period.

Identification of bacteria was done on the basis of morphological, cultural, biochemical and serological characteristics using standard methods [12].

Susceptibility tests were performed by the standard disc diffusion method and according to the criteria recommended by the National Committee of Clinical and Laboratory Standards (CLSI) [13].

Susceptibility to the following antimicrobial drugs was determined: penicillin, ampicillin, amoxicillin/clavulanic acid, cefaclor, erythromycin, azithromycin, co-trimoxazole, clindamycin, fusidic acid, ofloxacin, ciprofloxacin, and levofloxacin. Isolates with intermediate susceptibility were classified as resistant.

Susceptibility of *Streptococcus pneumoniae* to penicillin was determined by the oxacillin disk (1 μg). The minimum inhibitory concentration (MIC) for penicillin was tested in 2013 for oxacillin resistant strains.

Cefoxitine discs (30 μg) were used to test staphyloccocal isolates for methicillin-resistance according to the criteria recommended by the CLSI. Strains resistant to cefoxitine were considered resistant to methicillin as well as to all beta-lactam antibiotics and their combination with inhibitors of beta lactamase.

Isolates of *Haemophilus influenzae, Moraxella catarrhalis* and isolates of *Staphylococcus aureus* susceptible to penicillin were tested for the production of beta-lactamase using nitrocefin test.

The discs manufactured by BIO-RAD, France were used in the study.

Results

Bacteria that were most frequently isolated from the outpatients' throat and nasal swabs during the three-month evaluation period in 2008 were *Strepto-*

Table 1. Microorganisms isolated from throat and nasal swabs of outpatients in South Backa District from January 1 - March 31, 2008 and from January 1 - March 31, 2013, expressed in number of isolates and percentage of isolates
Tabela 1. *Bakterije izolovane iz briseva grla i nosa vanbolničkih pacijenata sa teritorije Južnobačkog okruga u periodu od 1. januara do 31. marta 2008. godine i 1. januara do 31. marta 2013. godine izraženo brojem izolata (n) i procentima (%)*

	Year 2008/*Godina 2008.*		Year 2013/*Godina 2013.*	
Microorganisms *Naziv bakterije*	No. of isolates *n (broj izolata)*	% of isolates *% izolata*	No. of isolates *n (broj izolata)*	% of isolates *% izolata*
Streptococcus pyogenes	359	37.63	115	34.85
Staphylococcus aureus	279	29.25	135	40.91
Streptococcus pneumonie	196	20.55	31	9.39
Haemophilus influenzae	75	7.86	17	5.15
Branchamella catarrhalis	23	2.41	32	9.70
Streptococcus β haemolyticus	22	2.30	-	-
Total/*Ukupno*	954	100.00	330	100.00

coccus pyogenes (37.63%) (359/954), *Staphylococcus aureus* (29.25%) (279/954), *Streptococcus pneumoniae* (20.55%) (196/954), *Haemophilus influenzae* (7.86%) (75/954), and *Branchamella catarrhalis* (2.41%) (23/954), whereas *Staphylococcus aureus* (40.91%) (135/330), *Streptococcus pyogenes* (34.85%) (115/330), *Branchamella catarrhalis* (9.70%) (32/330), *Streptococcus pneumoniae* (9.39%) (31/330), and *Haemophilus influenzae* (5.15%) (17/330) were the most frequently isolated bacteria during the investigated period in 2013 (**Table 1**).

Susceptibility of *Streptococcus pyogenes* to antibiotics most commonly used in the treatment of streptococcal infections is shown in **Table 2**. All isolates were susceptible to antibiotics. There was no resistance to penicillin in either period. Only two isolates (0.56%) of *Staphylococcus pyogenes* showed resistance to erythromycin in 2008, while there were three isolates (6.09%) resistant to this antibiotic in 2013. All isolates were susceptible to clindamycin in 2008; however, three isolates showed resistance in 2013.

All isolates of *Staphylococcus aureus* were susceptible to methicillin in 2008, while in 2013 eight isolates were resistant to methicillin. In penicillin-sensitive isolates, the production of beta-lactamases was not proved. Susceptibility to other examined antibiotics was lower in 2013 compared to 2008.

Out of 196 *Streptococcus pneumoniae,* 116 (59.18%) were resistant to oxacillin in 2008. In 2013, 14 (45.16%) out of 31 isolates *Streptococcus pneumoniae* showed resistance to oxacillin. Susceptibility of isolates of *Streptococcus pneumonia* to other antibiotics in 2013 was lower than in 2008, with the exception of co-trimoxazole (67.74% vs. 39.80%).

Isolates of *Haemophilus influenzae* and *Branchamella catarrhalis* did not show a significant change in susceptibility in 2013 compared to 2008.

Discussion

Due to a great number and variety of respiratory infections, monitoring the resistance of their causes is of great significance for both microbiologists and clinical professionals.

Rapid development of bacterial resistance to antibiotics (beta-lactamase positive *Branchamella catarrhalis* as well as *Haemophilus influenzae,* penicillin resistant pneumococcus) and appearance of other multiresistant bacteria, make the current empirical treatment of these infections more complicated in many countries and increase the risk of potential complications as well [4,14,15,16].

Isolates of *Streptococcus pyogenes* in South Backa District have preserved high susceptibility to erythromycin and clindamycin, which is a very good result, considering the fact that resistance to erythromycin is getting higher worldwide [17,18]. In Europe, a high level of resistance to erythromycin has been reported in Italy (30-40%), Portugal (24%), Spain (21%), and France (13%) [19]. Increased resistance of *Streptococcus pyogenes* to erythromycin in 40% of isolates was reported in Finland in late 1980s, which was related to substantially increased prescribing and use of erythromycin in treatment of upper respiratory tract infections. However, a decreased use of erythromycin in this country led to a substantial reduction in the resistance of *Streptococcus pyogenes* isolates [20]. Recent research indicates that the reduced use of long-acting macrolides (claritomycin, roxitromycin, azithromycin) significantly affects decreased bacterial resistance to erythromycin. Thus, in Northern Italy, the reduction of azithromycin use resulted in a considerably decreased resistance of *Streptococcus pyogenes* to erythromycin from 33.3% in 2001 to 0.2% in 2008 [21]. In addition to macrolides use of 3.55 Defined Daily Dose (DDD)/1000 inhabitants/day (which is in accordance with the European countries with medium high consumption), the percentage of *Streptococcus pyogenes* resistance to erythromycin has been extremely low (0.56%) on the territory of South Backa. A similar situation has been reported in Slovakia, where, in addition to macrolides use similar to the one in our district (3 DDD/1000 inhabitants/day), the level of resistance

Table 2. Susceptibility of *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae* and *Branchamella catarrhalis* isolated from nose and throat swabs of outpatients in the South Backa District from January 2013 to March 2013, expressed as the total number of analysed strains and the percent of sensitive strains identified from nose and throat swabs.

Tabela 2. *Osetljivost na antibiotike sojeva Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae izolovanih iz briseva grla i nosa vanbolničkih pacijenata sa teritorije Južnobačkog okruga u periodu od januara do marta 2013. godine, izraženo brojem izolata (n) i procentima (%).*

	Staphylococcus aureus				*Streptococcus pyogenes*				*Branchamella catarrhalis*				*Streptococcus pneumoniae*				*Haemophilus influenzae*			
	year/*godina* 2008		year/*godina* 2013		year/*godina* 2008		year/*godina* 2013		year/*godina* 2008		year/*godina* 2013		year/*godina* 2008		year/*godina* 2013		year/*godina* 2008		year/*godina* 2013	
Antibiotics	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
penicillin	279	2.87	135	6.77	359	100	115	100.0	23	0.00	32	00			31	54.84				
ampicillin									23	0.00	32	0.0					75	93.33	17	82.35
amoxicillin/ clavulanic acid									23	100	32	100					75	100	17	94.12
methicillin	279	100	135	94.07																
cefaclor									23	100	32	100					75	100	17	94.12
erythromycin	279	94.98	135	90.37	359	99.44	115	93.91	23	95.7	32	93.75	196	75	31	51.61				
azithromycin																	75	100	17	100
co-trimoxazole	279	99.64	135	98.51					23	60.9	32	93.75	196	39.80	31	67.74	75	88.0	17	76.47
clindamycin	279	98.56	135	91.91	359	100	115	96.52					196	88.78	31	61.29				
fusidic acid	279	100	135	99.26																
ofloxacin													196	100						
ciprofloxacin											32	100					75	100	17	100
levofloxacin															31	100				

n – total number of analyzed strains; % – percentage of sensitive strains

was the lowest among the countries of Central and Eastern Europe (less than 10%) [19]. The resistance of *Streptococcus pyogene*s to erythromycin was 6.09% in 2013, which is still a low resistance.

According to the National Guideline "Choice and Use of Antibiotics in General Practice" of the Ministry of Health of Serbia in 2004 [22], the use of natural penicillin or first generation cephalosporins has been recommended as an empirical treatment of the first choice in the cases of tonsillopharyngitis caused by *Streptococcus pyogenes*. Namely, despite the 70-year-long, widespread and very frequently uncontrolled use of penicillin, *Streptococcus pyogenes* has still remained 100 per cent susceptive to this antibiotic worldwide [6,7,8], which has also been confirmed in our research. Testing the resistance of the most common cause of tonsillopharyngitis, *Streptococcus pyogenes,* on the territory of South Backa has shown high susceptibility to natural penicillin, which means that the National Guideline is fully applicable in South Backa District.

Fast development of *Streptococcus pneumoniae* resistance to penicillin, macrolides and cephalosporines substantially affected the efficiency of treatment of streptococcus respiratory infections in recent decades. The results of analysis of *Streptococcus pneumoniae* resistance to antibacterial drugs in fifteen European countries indicate that there are significant differences among the countries - North European countries (Norway, Sweden, Denmark and the Netherlands) report a much lower degree of resistance than South and East European countries (Greece, Italy, France and the Slovak Republic). The rate of resistance to penicillin varied from 0% in Denmark to 57.1% in Greece. The rate of resistance to macrolides ranged from 6.9% in Norway to 57.1% in Greece, and the percentage of multiresistant isolates was again highest in Greece, 42.9% [4]. Furthermore, a moderate increase of resistance to penicillin, macroli-

des and fluoroquinolones was reported only in Greece, Italy, Slovenia and Slovakia, as compared to the previous similar research conducted two years earlier (2003-2004); however, it did not have any statistic significance [15].

In 2008, 59.18% isolates of *Streptococcus pneumoniae* were resistant to oxacillin (59.18%). However, since our study did not include determination of the MIC of penicillin for oxacillin resistant isolates in 2008, the resistance of these isolates to penicillin cannot be specified reliably. In 2013, the resistance of *Streptococcus pneumoniae* to penicillin was 45.16%.

Isolates of *Streptococcus pneumoniae* exhibited lower susceptibility to erythromycin in both periods in comparison to the previous research (the resistance was only 10.1% in 2002). Susceptibility to co-trimoxazole was lower not only than the one found in the previous research but also in comparison with the resistance of pneumococcus in Poland, the country with the highest rate of pneumococcal resistance to this antibiotic (48.2%) in Europe, whereas susceptibility was higher in 2013 (67.74%) [4]. Susceptibility of pneumococcus to fluoroquinolones has been maintained for years in South Backa District, as well as in most European countries [4,16].

Furthermore, the results of the study in fifteen European countries indicated a significant association between the levels of antimicrobial use and the rates of antimicrobial resistance in *Streptococcus pneumoniae* [4]. Thus, for example, in France, the resistance of *Streptococcus pneumoniae* to erythromycin was 50.1%, while the overall use of macrolides was 5 DDD/1000 inhabitants/day, whereas the resistance of the same bacteria to erythromycin was 11.3% in the Netherlands, while the use of macrolides was 2 DDD/1000 inhabitants/day.

The results of our research match these results. The resistance of *Streptococcus pneumoniae* to erythromycin in South Backa District was 25% in 2008, while the overall use of macrolides in our area was not especially high (3.55 DDD/1000 inhabitants/day) [23], which is similar to the European countries with medium high consumption, according to annual report of European Surveillance of Antibiotics Consumption - ESAC [24].

A high resistance of *Streptococcus pneumoniae* to co-trimoxazole (60.2%) in 2008 can be explained in terms of higher co-trimoxazole consumption in South Backa District (1.86 DDD/1000 inhabitants/day) than the one in Finland (1.43 DDD/1000 inhabitants/day). In fact, Finland is the country with the highest outpatient consumption of this antibiotic in Europe, the rate of *Streptococcus pneumoniae* resistance to co-trimoxazole being 22% there. Higher susceptibility of *Streptococcus pneumoniae* to co-trimoxazole was found in 2013 in South Backa District compared to 2008, but because of the small number of isolates a valid conclusion cannot be reached. High susceptibility of pneumococcus isolate to fluoroquinones has been reported despite a relatively high use of these antibiotics in our District (1.57 DDD/1000 inhabitants/day), which is in accordance with the countries in Europe with medium high consumption of fluoroquinones – Croatia (1.44 DDD/1000 inhabitants/day), Hungary (1.75 DDD/1000 inhabitants/day) [24].

Since all the isolates were susceptive to ofloxacin (as a part of fluoroquinone screening) in our investigation conducted in 2008, fluoroquinones, recommended for the treatment of respiratory infections such as moxifloxacin and levofloxacin, are expected to be effective in treatment of these infections. In 2013, isolates of *Streptococcus pneumoniae* were susceptible to levofloxacine (100%).

All isolates of *Staphylococcus aureus* obtained from the outpatients' nose swabs in South Backa District were susceptible to methicillin. According to the available reports, there are no specific data on prevalence of methicillin resistant staphylococcus isolated from the nose swabs of outpatients in Serbia. In Cuprija, where *Staphylococcus aureus* was isolated from different swabs (nose, wound, eye, ear, and skin) in outpatients, the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) isolates was 17.7% [25]. In Nis, the prevalence of clinical MRSA isolates (isolates were also obtained from different swabs) was even higher - 35.31% [26].

However, not even in clinical isolates is MRSA present in such a high percentage in South Backa District as it is in other parts of Serbia, which explains their high susceptibility to methicillin. Thus, according to data from 2005, MRSA was present in 41% clinical isolates at Orthopedic Hospital in Banjica, Belgrade; in 44% at Clinical Centre Kragujevac; 49% at Military Medical Academy, Belgrade; 55% at Clinical Centre Nis; up to 81% at Clinical Centre of Serbia, in Belgrade [27]. The frequency of MRSA strains in clinical specimens obtained in hospitalized patients in 2007 at Clinical Centre of Vojvodina was only 7.5% [28].

Isolates of *Haemophilus influenzae* proved to be highly susceptible to all the antibiotics tested in both investigated periods. Higher susceptibility to ampicillin, amoxicillin/clavulanic acid, and cephaclor was reported in comparison to the previous research (2002) in South Backa District.

High prevalence of susceptible isolates of *Haemophilus influenzae* is also reported in Italy (ampicillin 87%, amoxicillin/clavulanic acid 99.6%, cephaclor 97.9% [29], whereas in England the percentage of ampicillin resistant isolates was 17.4% [30] and in the USA it was as high as 29% [13]. High susceptibility to ciprofloxacin and azithromycin is in accordance with the previous research in our District, as well as with the results of other studies [29].

All *Branchamella catharralis* isolates produced beta-lactamases which were detected using nitrocefin test, whereas they showed high susceptibility to other antibiotics in either of the periods.

According to the National Guideline, *Haemophilus influenzae*, *Streptococcus pneumoniae* and

Branchamella catharralis are listed as the most common causes of acute sinusitis, and amoxicillin is recommended to be used as empirical treatment of primary choice. In South Backa District, the most common pathogens isolated from nose swabs were *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Since we do not have any data on susceptibility of *Streptococcus pneumoniae* to amoxicillin, what we can conclude based on susceptibility of *Staphylococcus aureus* and *Haemophilus influenzae* is that amoxicillin cannot be recommended as an antibiotic of first choice in South Backa District due to the high resistance of *Staphylococcus aureus*. This drug can be recommended for treatment of acute rhinosinusitis because of high susceptibility of *Staphylococcus aureus* to methicillin as well as of susceptibility of *Staphylococcus aureus* to methicillin. A second-generation cephalosporine can be recommended as its substitute.

Conclusion

The results show that infections of the upper respiratory tract are most frequently caused by *Streptococcus pyogenes, Staphylococcus aureus*, *Streptococcus pneumoniae, Branchamella catharralis,* and *Haemophilus influenzae* in South Backa District. The investigation results are in accordance with the recommendations of the National Guideline for the usage of natural penicillin in the treatment of tonsillopharyngitis.

Amoxicillin/clavulanic acid can be recommended to treat rhinosinusitis and second generation cephalosporins can be the second choice treatment.

A regular surveillance of the antimicrobial resistance patterns is very valuable not only at the international but also at national levels since these data are of great importance for the empirical use of antibiotics in areas where resistance testing is performed.

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University of Novi Sad, Faculty of Medicine
Clinical Center of Vojvodina
Center for Laboratory Medicine

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THE EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION ON EFFECTIVE RENAL PLASMA FLOW IN PATIENTS WITH DIFFUSE RENAL PARENCHYMAL DISEASES AND HYPERTENSION

UTICAJ INHIBICIJE ANGIOTENZIN-KONVERTUJUĆEG ENZIMA NA EFEKTIVNI BUBREŽNI PROTOK PLAZME KOD BOLESNIKA SA DIFUZNIM PARENHIMSKIM BOLESTIMA BUBREGA I HIPERTENZIJOM

Radmila ŽERAVICA, Zoran STOŠIĆ, Branislava ILINČIĆ, Veljko CRNOBRNJA, Ana JAKOVLJEVIĆ[1] and Romana MIJOVIĆ

Summary

Introduction. Angiotensin converting enzyme inhibitors are commonly used to treat various hypertensive conditions and in addition to lowering blood pressure these drugs affect the local renal hemodynamic status, thereby influencing the glomerular filtration rate and effective renal plasma flow. The study was aimed at determining whether angiotensin converting enzyme inhibitors can produce significant changes in effective renal plasma flow in patients with parenchymal renal disease and to assess whether the changes depend on the pre-existing functional status of the kidney. **Material and Methods.** The study included 80 subjects, 40 subjects with hypertension associated with diffuse renal parenchymal disease and 40 subjects with essential hypertension. All study subjects underwent the baseline effective renal plasma flow measurement and the repeated effective renal plasma flow measurement after administration of captopril. Effective renal plasma flow was determined by 131 I-hippuran clearance in blood samples taken at 20 and 30 minutes. **Results.** Angiotensin converting enzyme inhibitors caused significant effective renal plasma flow changes in 55% of subjects with diffuse renal parenchymal disease and in 75% of subjects with essential hypertension. The effective renal plasma flow changes were more significant in subjects with preserved renal function (normal baseline effective renal plasma flow) compared to subjects with reduced baseline effective renal plasma flow. **Conclusion.** The application of angiotensin converting enzyme inhibitors in patients with diffuse renal parenchymal disease and in individuals with essential hypertension may result in significant hemodynamic changes in the kidney, accompanied by changes in effective renal plasma flow. The extent of the changes caused by angiotensin converting enzyme inhibitors depends on the preexisting functional status of the kidney.

Key words: Angiotensin-Converting Enzyme Inhibitors; Peptidyl-Dipeptidase A; Renal Plasma Flow, Effective; Kidney Diseases; Hypertension; Renin-Angiotensin System

Sažetak

Uvod. Lekovi iz grupe inhibitora angiotenzin-konvertujućeg enzima često se koriste u lečenju svih hipertenzivnih stanja, a ovi lekovi, pored toga što snižavaju krvni pritisak, utiču i na lokalni hemodinamički status bubrega utičući na vrednosti jačine glomerulske filtracije i efektivnog bubrežnog protoka plazme. Cilj ovog istraživanja bio je da se utvrdi da li kod bolesnika sa parenhimskim bolestima bubrega i hipertenzijom, primena inhibitora angiotenzin-konvertujućeg enzima može izazvati značajnije promene efektivnog bubrežnog protoka plazme i da li nastale promene zavise od postojećeg funkcionalnog statusa bubrega. **Materijal i metode.** Istraživanje je sprovedeno kod 80 ispitanika, 40 ispitanika sa udruženim difuznim parenhimskim bolestima bubrega i hipertenzijom i 40 ispitanika sa esencijalnom hipertenzijom. Kod svih ispitanika izvršeno je merenje efektivnog bubrežnog protoka plazme u bazalnim uslovima i ponovljeno merenje nakon premedikacije kaptoprilom. Za merenje efektivnog bubrežnog protoka plazme korišćena je metoda određivanja klirensa 131J-hipurana uz vađenje dva uzorka krvi u 20. i 30. minuti. **Rezultati**. U grupi ispitanika sa difuznim parenhimskim bolestima bubrega i hipertenzijom, primena inhibitora angiotenzin-konvertujućeg enzima u 55% slučajeva rezultirala je značajnim promenama protoka, dok u grupi ispitanika sa esencijalnom hipertenzijom primena inhibitora rezultirala je značajnijim promenama protoka u 75% slučajeva. Promene efektivnog bubrežnog protoka plazme bile su izraženije kod pacijenata sa očuvanom funkcijom bubrega (normalne bazalne vrednost protoka) u odnosu na ispitanike sa izrazito redukovanim bazalnim vrednostima protoka. **Zaključak**. Primena inhibitora angiotenzin-konvertujućeg enzima kod bolesnika sa difuznim parenhimskim bolestima bubrega i hipertenzijom, kao i kod bolesnika sa esencijalnom hipertenzijom, može rezultirati značajnim promenama hemodinamičkih uslova u bubregu, praćenih promenama vrednosti efektivnog bubrežnog protoka plazme, pri čemu promene zavise i od funkcionalnog statusa bubrega.

Ključne reči: ACE inhibitori; Angiotenzin konvertujući enzim; Efektivni bubrežni protok plazme; Bolesti bubrega; Hipertenzija; Renin-Angiotenzin sistem

Corresponding Author: Asist. mr sc. med. Radmila Žeravica, Klinički centar Vojvodine, Centar za laboratorijsku medicinu, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: radmila.zeravica@gmail.com

Abbreviations
ACE – angiotensin converting enzyme
GFR – glomerular filtration rate
ERPF – effective renal plasma flow
CrCl – creatinine celarance

Introduction

Regulation of renal blood flow is very complex and involves the nervous and humoral factors as well as the autoregulatory mechanism. It is predominantly influenced by the activity of three regulatory systems: the renin-angiotensin-aldosterone system, the prostaglandin system, and the kallikrein-kinin system. The latter two are a part of the renal vasodilator system, whereas the renin-angiotensin system represents the renal vasoconstrictor, with the vasodilator systems modulating vasoconstrictive effects of angiotensin II on one hand, and angiotensin II stimulating the secretion of vasodilatory components on the other, which prevents substantial increase in the renal vascular resistance. The relationships and dynamic balance of these interdependent systems determine the renal blood flow and blood redistribution in the kidney. Angiotensin II is biologically the most active factor of the renin–angiotensin system. In the kidney, it causes strong vasoconstriction of the efferent arteriole and moderate vasoconstriction of the afferent arteriole, directly stimulates the reabsorption of sodium ions in the proximal tubule, thereby contributing to an increased volume of extracellular fluid and the development of hypertension [1,2]. The inhibitors of angiotensin converting enzyme (ACE) act by modulating the activity of the rennin-angiotensin system. By blocking angiotensin converting enzyme, ACE inhibitors significantly block the conversion of angiotensin I to angiotensin II, thus lowering blood pressure via reducing the production of the strong vasoconstrictor. ACE inhibitors are frequently used to treat various hypertensive conditions, and besides antihypertensive effects, these drugs have local protective effects on the kidney, by reducing intraglomerular pressure and exerting the antiproliferative effect, thereby slowing down the progression of renal failure and preventing the development of more severe forms of renal failure [3]. The antihypertensive effects of ACE inhibitors are associated with the inhibition of local and systemic effects of angiotensin II on the vascular structures, stimulation of the local vascular kinin system with the secondary stimulation of the prostacyclin system, as well as the effects on renal hemodynamics and excretory functions.

Changes in total effective renal plasma flow (ERPF) in the setting of ACE inhibition result from the changes in local hemodynamic conditions in the kidney due to changed ratios and interactions between the components of the regulatory vasoconstrictor and vasodilator systems of the kidney [4,5].

The aim of the study was to determine whether application of ACE inhibitors in patients with diffuse renal parenchymal disease and hypertension can produce significant changes in ERPF and to assess to what extent the changes in ERBF depend on the preexisting functional status of the kidney.

Material and Methods

The study included a total of 80 subjects, 40 patients with diffuse renal parenchymal disease associated with hypertension and 40 patients with essential hypertension. Out of the 40 patients with diffuse renal parenchymal disease, 14 had been diagnosed with glomerulonephritis and 26 with tubulointerstitial disease. The study design was prospective. The study protocol included baseline measurement of ERPF in all subjects, along with determination of glomerular filtration rate (GFR), serum urea and creatinine levels, blood pressure, and repeated ERPF and blood pressure measurements after administration of captopril. Serum urea concentrations were determined using standard methods on an Olympus AU400 biochemical analyzer and commercial sets produced by Olympus. For the inhibition of angiotensin converting enzyme in the kidney, the subjects were administered 25mg captopril one hour

Table 1. Baseline values of 40 patients with diffuse renal parenchymal disease
Tabela 1*. Bazalne vrednosti kod 40 pacijenata sa difuznom bubrežnom parenhimskom bolesti*

	$\bar{X}$	SD	Min	Max
Age (years)/*Godine starosti*	51.25	10.88	23	66
ERPF (ml/min/1.73 m^2)/*EBPP(ml/min/1.73 m^2)*	360	110	120	631
Deviation of ERPF from expected (ml/min) *Odstupanje EBPP od očekivanog (ml/min)*	-195	105	-11	-509
Deviation of ERPF from expected (%) *Odstupanje EBPP od očekivanog (%)*	-35	16	-2	-70
GFR (ml/min/73 m^2)/*JGF(ml/min/73 m^2)*	72	22	22	109
Creatinine (μmol/l)	118	62	69	373.20
Urea (mmol/l)	8.5	3.6	4.6	17.8

ERPF - effective renal plasma flow/*EBPP - efektivni bubrežni protok plazme*
GFR - glomerular filtration rate/*JGF jačina glomerulske filtracije*

prior to blood sampling/measurements. ERPF was determined by the clearance of 131 I-hippuran in two blood samples, which were taken at 20 and 30 minutes according to Blaufox's method [6]. The normal ERPF values were calculated using regression equations by Schernthaner et al. [7] as following:

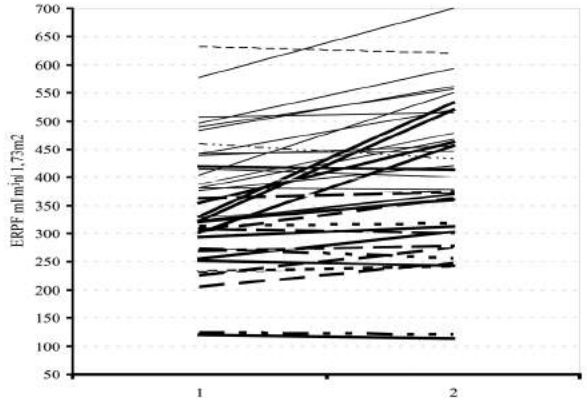
For women : ERPF= 673,3 - (2.92*years of age)

For men: ERPF = 854,2-(5.4*years of age)

The ERPF values were expressed as ml/min/1.73 m^2 and variations in the ERPF values compared to reference values (expected values for the sex and age) were given as ml/min and percentage. A change in the hemodynamic status was regarded significant if ERPF after inhibition was increased by ≥ 10% compared to the baseline values. GFR was estimated by measuring endogenous creatinine clearance (CrCl), by 24h urine collection. The calculated values of CrCl were normalized relative to the body surface of 1.73 m^2. Serum creatinine and the concentrations of creatinine in urine were determined using standard methods (modified Jaffe's method) on an Olympus AU400 biochemical analyzer and commercial sets produced by Olympus. The results were processed using standard statistical analyses (t-test, Spearman's rank correlation).

Results

The baseline values in the subjects with diffuse renal parenchymal disease associated with hypertension and the subjects with essential hypertension are presented in **tables 1, 2**, respectively. A significant change (improvement) in ERPF after administration of ACE inhibitors was recorded in 55% of the subjects with diffuse renal parenchymal disease, whereas ERPF did not change significantly in 45% of them **(Graph 1).** In the group of patients with essential hypertension, ACE inhibition resulted in significant improvements in ERPF compared to the baseline values in 75% of subjects, whereas no significant changes were detected in 25% of them **(Graph 2).** In 76% of the subjects (n=29) with the baseline normal ERPF values, ACE inhibition produced significant ERPF changes. In only 35% of the 20 subjects with the reduced baseline ERPF (defined as >40% of reference values) had significant changes in ERPF after ACE inhibition. Related/dependent samples t-test showed a highly significant decrease in mean systolic and diastolic pressures before and after administration of ACE inhibitors in both groups of subjects (systolic pressure p = 0.000; and diastolic pressure, p = 0.000). The analysis of correlation between the variables using Spearman's rank correlation showed a statistically highly significant correlation between systolic and diastolic pressures (baseline and after ACE inhibition) and ERPF (ρ = -0.450, p = 0.004; ρ = -0.456, p = 0.003 for systolic pressure; and ρ = -0.433, p = 0.005; ρ = -0.378, p = 0.016 for diastolic pressure).



Graph 1. The values of ERPF (ml/min/1.73 m^2) basal (1) and after administration of ACE inhibitors (2) in patients with diffuse parenchymal kidney disease
Grafikon 1*. Vrednosti efektivnog bubrežnog protoka plazme (ml/min/1,73 m^2) bazalno (1) i nakon primene inhibitora angiotenzin-konvertujućeg enzima (2) kod pacijenata sa difuznom parenhimskom bolesti bubrega*

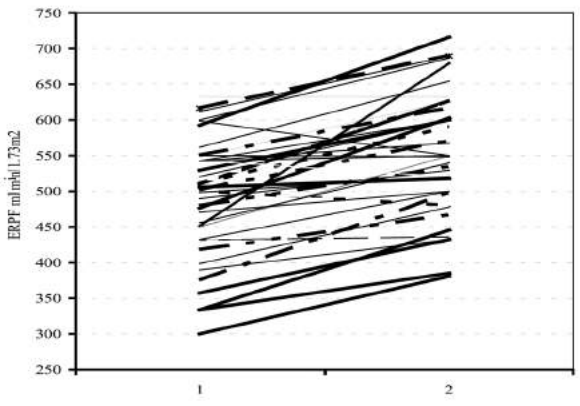
Discussion

ACE inhibitors are widely used to treat various hypertensive conditions and in addition to lowering hypertension, these drugs affect the local renal hemodynamic conditions, thereby affecting GFR and ERPF. A number of clinical studies on the role of ACE inhibitors in decelerating the progression of

Table 2. Baseline values in 40 patients with essential hypertension
Tabela 2*. Bazalne vrednosti 40 pacijenata sa esencijalnom hipertenzijom*

	$\bar{X}$	SD	Min	Max
Age (years)/*Godine starosti*	46.1	15.7	18	65
ERPF (ml/min/1.73 m^2)/*EBPP(ml/min/1.73 m^2)*	466.10	84.03	300	632
Deviation of ERPF from expected/*Odstupanje EBPP od očekivanog (ml/min)*	-30.45	62.74	0	-222
Deviation of ERPF from expected (%)/*Odstupanje EBPP od očekivanog (%)*	-7	8.11	0	-20
GFR (ml/min/73 m^2)/*JGF(ml/min/73 m^2)*	101.1	21.65	70	164
Creatinine (μmol/l)	81.54	14.64	56	105
Urea (mmol/l)	5.57	1.50	2.7	7.9

ERPF - effective renal plasma flow/*EBPP - efektivni bubrežni protok plazme*
GFR - glomerular filtration rate/*JGF jačina glomerulske filtracije*



Graph 2. The values of ERPF (ml/min/1.73 m^2) basal (1) and after administration of ACE inhibitors (2) in patients with essential hypertension

Grafikon 2. *Vrednosti efektivnog bubrežnog protoka plazme (ml/min/1,73 m^2) bazalno (1) i nakon primene inhibitora angiotenzin-konvertujućeg enzima (2) kod pacijenata sa esencijalnom hipertenzijom.*

renal disease have been published, and among the first were those conducted by the Melbourne Diabetic Nephropathy Study Group, the ACE Inhibition in Progressive Renal Insufficiency (AIPRI) Study Group, and the North American Microalbuminuria Group. The results of those studies indicate that ACE inhibitors have a protective role, by decreasing microalbuminuria and slowing down the progression of renal disease [5, 8, 9].

Our results in the subjects with essential hypertension, i.e., significantly increased ERPF and decreased systolic and diastolic pressures, also corroborate the significant role of ACE inhibitors in the protection of renal function in these subjects. The relationship between arterial hypertension and renal function has been long established and well proved. The kidney may initiate arterial hypertension, as well as suffer the consequences of full-blown arterial hypertension. Chronic/constant arterial hypertension is at early stages characterized by increased renal vascular resistance, normal or slightly decreased renal blood flow and increased GFR. The development of renal failure due to arterial hypertension is believed to result both from ischemia due to changes in preglomerular arteries and arterioles and from the effects of increased intraglomerular pressure (hyperperfusion), which inevitably leads to functional and subsequently structural glomerular changes and progressive loss of renal function. Considering the fact that arterial hypertension represents one of the leading causes of end-stage renal failure in our country as well as worldwide, it is clear that timely protection of renal function in patients with essential hypertension is of great importance. Blood pressure regulation, with its maintenance at levels below 130/80 mmHg, and inhibition of the renin-angiotensin system in order to reduce renal vascular resistance and intraglomerular pressure are therefore frequently recommended [10,11].

Different renal diseases that cause damage to individual segments of the nephron (blood vessel, glomerulus, tubule or interstitium) lead to structural and functional changes, as well as to local hemodynamic changes in the kidney. Regardless of the etiological factor involved, the pathogenetic mechanisms underlying the progression of renal disease are the same and include abnormal glomerular hemodynamics (intraglomerular hypertension and glomerular hyperfiltration), hypoxia, proteinuria, and effects of various vasoactive substances (e.g. cytokines, growth factors). Furthermore, a critical role in the pathogenesis of renal impairment is played by angiotensin II, one of its main effects being regulation of renal hemodynamics. The effect of angiotensin II on renal blood flow in the setting of renal parenchymal impairment is determined substantially by its relationship with other vasoactive systems in the kidney. Considering the role of angiotensin II in the progression of renal disease, it is clear that application of ACE inhibitors can be expected to have protective effects on the renal function [12,13]. On the other hand, in the setting of relatively preserved renal function, i.e. when fewer functioning nephrons are affected by pathological processes, the vasoregulatory systems are also relatively intact, so any changes in renal hemodynamics under the influence of ACE inhibition are expected to be more substantial. Likewise, in our study ERPF changes after ACE inhibition differed between the subjects with preserved renal function and those with reduced ERPF. The majority (76%) of subjects with preserved renal function had more significant ERPF changes after ACE inhibition, as opposed to subjects with reduced functional reserve of the kidneys, in whom the majority (65%) did not show any significant changes in renal hemodynamics after ACE inhibition. In the patietns with hypertension and preserved renal function, ACE inhibition is usually associated with increased total ERPF, resulting from decreased resistance to blood flow at the level of glomerular capillaries and efferent arteriole and consequent increase in blood flow at the level of peritubular capillaries. The absence of a significant hemodynamic response to ACE inhibition in our subjects with more severe functional impairment is probably due to the existence of very complex interrelationships between angiotensin II and other regulatory mechanisms involved in the regulation of renal blood flow. Considering the integral parts of all three regulatory systems in renal hemodynamics, the vasoconstrictor activity prevails either due to the stimulation of renin-angiotesin-aldosteron system, or due to the inhibition of prostaglandin and kallikrein-kinin systems that participate in the

maintenance of the optimal hemodynamic conditions in the kidney via their vasodilatory effects. In the setting of significantly reduced functional reserve of the kidney, i.e. with reduced numbers of functioning nephrons, there is a significant decrease in the production of vasodilator prostaglandins. Hence, the significant impairment of renal function entails a significant disturbance of dynamic balance between the vasoregulatory systems, and the renal response to ACE inhibition is indeed determined by a complex interplay of these systems. Another possible explanation is the incomplete inhibition of renin-angiotensin system, since ACE inhibition does not cut off other alternative ways of angiotensin II production. Dragović et al. showed that the individual hemodynamic response in the patients with diabetic nephropathy in the condition of renin-angiotensin system blockade is genetically dependent, as well as focusing on individual therapeutic strategies for the purpose of more effective prevention and prognosis of diabetic nephropathy [14].

Previous research was directed to dual blockade of the renin-angiotensin system, i.e. the simultaneous inhibition of ACE and blockade of angiotensin II receptors. The results obtained suggest that the dual blockade has more significant antiproteinuric and antihypertensive effects compared to monotherapy [15-17]. Finally, the answers should be sought not only within the renal vascular system, but also in the numerous factors outside the kidney that may affect the hemodynamic response.

Conclusion

Angiotensin converting enzyme inhibition by means of angiotensin converting enzyme inhibitors may significantly affect renal hemodynamic conditions and effective renal plasma flow in patients with diffuse renal parenchymal disease and in individuals with essential hypertension, and the extent of the hemodynamic changes depends also on the functional status of the kidney.

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Clinical Center of Vojvodina, Novi Sad, Serbia
Department of Hematology
Faculty of Medicine, University of Novi Sad

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POST-REMISSION THERAPY OF ADULT ACUTE MYELOID LEUKEMIA: HIGH DOSE CYTOSINE-ARABINOSIDE VERSUS OTHER CONSOLIDATION REGIMENS

KONSOLIDACIONA TERAPIJA AKUTNE MIJELOIDNE LEUKEMIJE: VISOKE DOZE CITOZIN-ARABINOZIDA NASPRAM DRUGIH KONSOLIDACIONIH REŽIMA SA CITOZIN-ARABINOZIDOM

Vanja ZEREMSKI and Aleksandar SAVIĆ

Summary

Introduction. Modern therapy makes it possible for 60-80% patients with acute myeloid leukemia to achieve complete remission after induction therapy. However, most of them will relapse within six months to a year without additional cytostatic therapy. The questions regarding post-remission therapy remain unanswered. The objective of this study was to compare the survival and relapse rate among the patients who had received high dose cytosine-arabinoside during consolidation therapy and the patients who had not received high dose cytosine-arabinoside during consolidation therapy. **Material and Methods.** The study included 59 patients aged 18-60 years with *de novo* acute myeloid leukemia (except for *Acute promyelocytic leukemia*, which was excluded according to the French-American-British classification) who achieved complete remission. Thirty-nine patients who received high dose cytosine-arabinoside during consolidation were included in the study group and twenty patients who did not receive high dose cytosine-arabinoside during consolidation were in the control group. **Results.** The results show a statistically significantly longer survival rate (p= 0.003) and a lower relapse rate (p= 0.02) among the study group patients, who received high dose cytosine-arabinoside during consolidation, compared to the controls, who did not receive high dose cytosine-arabinoside. The univariate analysis in the study group suggests that the affiliation to *Acute myeloblastic leukemia with maturation* and *Acute myelomonocytic leukemia* subgroups, as well as achieving complete remission after a single induction therapy has the prognostic significance. In the multivariate analysis, only the affiliation to *Acute myeloblastic leukemia with maturation* and *Acute myelomonocytic leukemia* subgroups retained the independent prognostic significance. **Conclusion.** This study has demonstrated that high dose cytosine-arabinoside used for consolidation therapy results in the higher survival rate and lower relapse rate compared to consolidation therapy without high dose cytosine-arabinoside. Only the patients within *Acute myeloblastic leukemia with maturation* and *Acute myelomonocytic leukemia* subgroups benefited significantly from high dose cytosine-arabinoside.

Key words: Leukemia, Myeloid, Acute; Cytarabine; Consolidation Chemotherapy; Recurrence; Survival Rate; Adult; Middle Aged

Sažetak

Uvod. Savremenom terapijom kod 60−80% obolelih od akutne mijeloidne leukemije postigne se kompletna remisija nakon indukcije. Bez dodatne citostatske terapije, većina bolesnika uđe u relaps u periodu od šest meseci do godinu dana. Pitanje optimalne postremisione terapije je i dalje otvoreno. Cilj studije bio je da se uporedi preživljavanje i učestalost relapsa između bolesnika koji su primili visoke doze citozin-arabinozida u konsolidaciji i bolesnika koji nisu primili visoke doze citozin-arabinozida u konsolidaciji. **Materijal i metode.** Ispitanici starosti 18−60 godina, sa *de novo* akutnom mijeloidnom leukemijom (izuzev *Acute promyelocytic leukemia* po Francusko-američko-britanskoj klasifikaciji), koji su ušli u kompletnu remisiju, podeljeni su u studijsku grupu (39 bolesnika), koja je primila visoke doze citozin-arabinozida i kontrolnu, grupu (20 bolesnika), koja je primila konsolidacionu terapiju bez visokih doza citozin-arabinozida. **Rezultati.** U studijskoj grupi koja je primila visoke doze citozin-arabinozida u konsolidaciji postoji statistički značajno duže preživljavanje (p = 0,003) i niži nivo relapsa (p = 0,02) u poređenju sa kontrolnom grupom, koja nije primila visoke doze citozin-arabinozida. Prognostički značaj za preživljavanje u univarijantnoj analizi u studijskoj grupi pokazuje pripadnost *Acute myeloblastic leukemia with maturation* i *Acute myelomonocytic leukemia* podgrupi, kao i postizanje kompletne remisije nakon prve indukcije. Nezavisni prognostički značaj u multivarijantnoj analizi predstavlja samo pripadnost *Acute myeloblastic leukemia with maturation* i *Acute myelomonocytic leukemia* podgrupama (p = 0,018). **Zaključak.** Visoke doze citozin-arabinozida u konsolidaciji pokazuju statistički značajno duže preživljavanje u odnosu na terapiju bez visokih doza citozin-arabinozida. Povoljan efekat visokih doza citozin-arabinozida ograničen je na *Acute myeloblastic leukemia with maturation* i *Acute myelomonocytic leukemia* podgrupe.

Ključne reči: Akutna mijeloidna leukemija; Citarabin; Konsolidaciona terapija; Relaps; Preživljavanje; Odrasli; Srednje godine

Corresponding Author: Dr Vanja Zeremski, Klinički centar Vojvodine, Klinika za hematologiju, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: zeremski.vanja@gmail.com

Abbreviations

AML – acute myeloid leukemia
CALGB – Cancer and Leukemia Group B
HDAC – high dose cytosine-arabinoside
FAB – French-American-British
CR – complete remission
NPM – nucelophosmin
M_3 – acute promyelocit
FLT3 – FMS-like tyrosine kinase 3

Introduction

Acute myeloid leukemia (AML) is the most common leukemia affecting adults. Approximately 300,000 patients worldwide are diagnosed annually with AML [1]. Modern therapy makes it possible for 60-80% patients with *de novo* AML to achieve complete remission [1-3]. However, most of them will relapse within six months to a year without additional post-remission therapy [4, 5]. Thus, the optimal post-remission therapy is still the major challenge.

A number of studies have suggested that the increased intensity of post-remission therapy prolongs remission duration and leads to superior survival. The Cancer and Leukemia Group B (CALGB) conducted a study in order to assess whether there was a dose-effect correlation of cytosine-arabinoside. The study showed that high dose cytosine-arabinoside (HDAC) (3 g/m^2) given in four courses resulted in the superior survival compared to a lower dose (0.4 g/m^2 and 1 g/m^2), especially among the patients younger than 60 years and the patients with core binding factors (t(8;21)(q22;q22) or inv(16)) [6]. On the other hand, the American Intergroup 1998 study, where HDAC was given as a single course, yielded similar results [7]. Thus, the questions such as the optimal dose and number of courses still remain unanswered. HDAC can cause cerebral and gastrointestinal lesions, dermatitis, conjunctivitis, liver lesion and acute lung failure [4, 8, 9].

The objective of this study was to compare the survival rate and the relapse rate among the patients who received HDAC during consolidation therapy and among the patients who did not receive HDAC during consolidation therapy.

Material and Methods

Patients. The patients were divided into the study and control group. The study group consisted of the patients hospitalized at the Department of Hematology from July 2001 to May 2010. The control group consisted of the patients hospitalized at the Department of Hematology from November 1993 to December 2001. In addition, a gender- and age-matched group was formed of randomly selected patients from the study group in order to achieve greater comparability with the control historical group. The inclusion criteria were as follows: age 18 to 60, *de novo* AML according to French-American-British (FAB) classification [10] **(Table 1)** (excluding acute promyelocytic leukemia M3), achieving complete remission (CR). The patients with incomplete data were excluded **(Table 2).**

Treatment protocol. After being diagnosed with AML, all patients were treated with identical induction treatment. Post-remission therapy differed in the study and the control historical group, as detailed in **Figure 1.**

Assessment of response. The response to treatment (CR, relapse) and outcome (survival) was assessed according to European Leukaemia Net [3].

Statistical analysis. The following descriptive statistical methods were used for statistical analysis: Fisher's exact test and x^2 for categorical variables, the Shapiro-Wilk W test to determine the type of distribution of data and the Mann-Whitney test in the case of continuous data.

Table 1. The French-American-British (FAB) classification of AML
Tabela 1. *Francusko-američko-britanska klasifikacija*

M0	*Acute myeloblastic leukemia, minimally differentiated*
M1	*Acute myeloblastic leukemia without maturation*
M2	*Acute myeloblastic leukemia with maturation*
M3	*Acute promyelocytic leukemia*
M4	*Acute myelomonocytic leukemia*
M4eos	*Acute myelomonocytic leukemia with eosinophilia*
M5	*Acute monocytic leukemia*
M6	*Acute erythroid leukemia*
M7	*Acute megakaryoblastic leukemia*

Table 2. Initial patient characteristics
Tabela 2. *Inicijalne karakteristike bolesnika*

	Study group Studijska grupa	*Control group* Kontrolna grupa	*Matched group* Uporedna grupa
No. of patients/*Broj pacijenata*	39	20	20
Sex/*Pol*			
Men (%)/*Muškarci*	53,18	55	50
Women (%)/*Žene*	46,82	45	50
Age/*Starost*			
Median/*Srednja vrednost*	45	48	46
Range/*Raspon*	18-55	24-60	27-54
FAB *			
M0	5	1	2
M1	3	5	1
M2	11	8	4
M4	15	5	10
M5	4	1	2
M6	1	0	1
M7	0	0	0
WBC † ***Leukociti***			
Median/*Srednja vrednost*	20,25	7,85	11,9
Range/*Raspon*	1,07-197	1,2-50,64	1,43-75,7
Eritrociti/RBC ‡			
Median/*Srednja vrednost*	2,83	2,75	2,94
Range/*Raspon*	1,19-5,65	2,04-3,87	1,19-3,57
Hemoglobin			
Median *Srednja vrednost*	91,5	88,15	92,9
Range *Raspon*	47,6-170	57-127	47,6-117
Hematokrit *Hematocrit*			
Median *Srednja vrednost*	0,26	0,24	0,27
Range *Raspon*	0,13-0,51	0,17-0,38	0,13-0,33
Platelets *Trombociti*			
Median *Srednja vrednost*	70,7	65	67
Range *Raspon*	13-274	20,2-210	13-274
ECOG			
0-1	17	7	9
≥ 2	14	7	8
Unknown/*Nepoznat*	8	6	3
HCTCI			
0	30	10	13
1-2	6	8	4
≥3	3	1	3
Unknown *Nepoznat*	0	1	0

* French-American-British; † White blood cells; ‡ Red blood cells

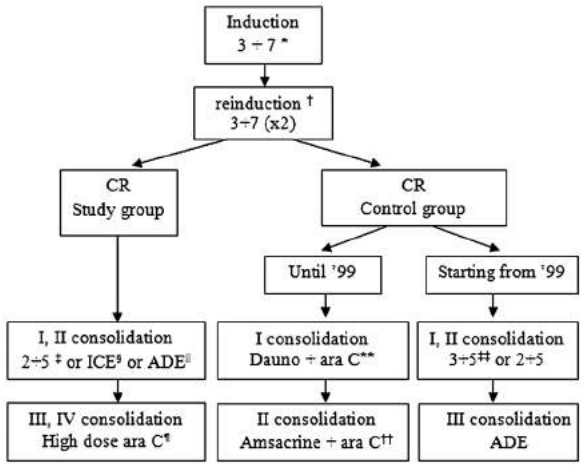

Figure 1. Therapy protocol
Slika 1. *Protokol terapije*

***** daunorubicin 45 mg/m^2 or idarubicin 12 mg/m^2 days 1-3 and ara - C 100 mg/m^2 days 1-7

****** daunorubicin 45 mg/m^2 ili idarubicin 12 mg/m^2 dan 1.-3. i ara - C 100 mg/m^2 dan 1.-7.*

† if not in CR after one course, they received reinduction 3+7 daunorubicin 45 mg/m^2 or idarubicin 12 mg/m^2 days 1-3 and ara - C 200 mg/m^2 days 1-7

† ukoliko KR nije postignuta nakon prve indukcija, aplikovana je reindukcija 3+7 daunorubicin 45 mg/m^2 ili idarubicin 12 mg/m^2 dan 1.-3. i ara - C 200 mg/m^2 dan 1.-7.

‡ daunorubicin 50 mg/m^2 or idarubicin 12 mg/m^2 days 1-2 days and ara - C 500 mg/m^2 days 1-5

‡ daunorubicin 50 mg/m^2 ili idarubicin 12 mg/m^2 dan 1.-2. i ara - C 500 mg/m^2 dan 1.-5

§ idarubicin 12 mg/m^2 days 1-3, etoposide 150 mg/m^2 days 1-3 and ara - C 600 mg/m^2 days 1-5

§ idarubicin 12 mg/m^2 dan 1.-3., etoposide 150 mg/m^2 dan 1.-3. i ara - C 600 mg/m^2 dan 1.-5

‖ daunorubicin 50 mg/m^2 days 1-3, etoposide 150 mg/m^2 days 1-3 and ara - C 600 mg/m^2 days 1-5

‖daunorubicin 50 mg/m^2 dan 1.-3., etoposide 150 mg/m^2 dan 1.-3. i ara - C 600mg/m^2 dan 1.-5

¶ ara - C 3g/m^2 every 12 hours days 1, 3, 5

¶ara - C 3g/m^2 na 12 sati dan 1., 3., 5

****** daunorubicin 45 mg/m^2 days 1- dana and ara - C 500 mg/m^2 every 12 hours days 1-5

******* daunorubicin 45 mg/m^2 dan 1.i ara - C 500 mg/m^2 na 12 sati dan 1.-5*

†† amsacrine 120 mg/m^2 days 1-7 and ara - C 1g/m^2 every 12 hours days 1-7

†† amsacrine 120 mg/m^2 dan 1.-7. i ara - C 1g/m^2 na 12 sati dan 1.-7

‡‡ daunorubicin 50 mg/m^2 or idarubicin 12 mg/m^2 days 1-3 and ara - C 500 mg/every 12 hours days 1-5

‡‡ daunorubicin 50 mg/m^2 ili idarubicin 12 mg/m^2 dan 1.-3. i ara - C 500 mg/na 12 sati dan 1.-5

Survival analyses were done by the Kaplan-Meier method. The survival curves were compared by the log-rank test and the Cox proportional hazards model was used to analyze the effect of prognostic variables on survivals. Statistica 9.1 program was used for the statistical analysis.

Results

Patients' characteristics The study group consisted of 39 patients with median age 45 years (ranging from 18 to 55 years). The control group included 20 patients with median age 48 years (ranging from 24 to 60 years). The age-matched group was formed of 20 patients randomly selected by computer from the study group, their median age being 46 years (ranging from 27 to 54 years). The patients' characteristics on admission are shown in **Table 2.** There were no significant differences in the patients' characteristics either between the study and the control historical group or between the age-matched and control historical group.

Survival, complete remission and relapse. By the time of evaluation, the mean follow up period in the study group was 29 months (ranging from 6.5 to 100 months) and in the control group it was 17 months (ranging from 5 to 209 months). The median survival in the study group was 37.6 months versus 17 months in the control group (the log-rank test, p = 0.003), as showed in **Figure 2A**. The median survival in the matched group was 34.5 months, which differed statistically from the control group. According to the log-rank test p = 0.003, CR after the first induction course was achieved by 54.6% of patients from the study group and 30% of patients from the control group (Yates corrected x^2, p= 0.07). The rate of relapse in the study group was 70.5% versus 90 % in the control group, which resulted in a significant difference according to the x^2 test (p= 0.02).

Factors predictive for survival. The log-rank test revealed a significant difference (p=0.006) when comparing survival for M4 and M1 subgroup, including the study and control historical group; the median survival time being 69.3 months and 11 months for M4 and M1 subgroup, respectively. There was also a significant difference (the log rank, p=0.011) comparing the survival between M4 and M0 subgroup (the median survival time was 16 months), M2 and M4 subgroup (p = 0.02), M4 and M5 subgroup (p = 0.02), but not when comparing the survival between other FAB subgroups. The median survival time in M2 subgroup was 20 months. The median survival for M0, M6 and M7 subgroups was not determined because of a small number of patients. The comparison of survival in M1, M2, M4 and M5 subgroups is shown in **Figure 2B**.

The comparison of survival in M2 and M4 subgroup together with the other FAB subgroups, in the study group alone, resulted in a significant difference in the survival (the log-rank, p = 0.014), the median survival time for M2 and M4 subgroup being 73 months and 28 months for all other FAB subgroups together, as shown in **Fig-**

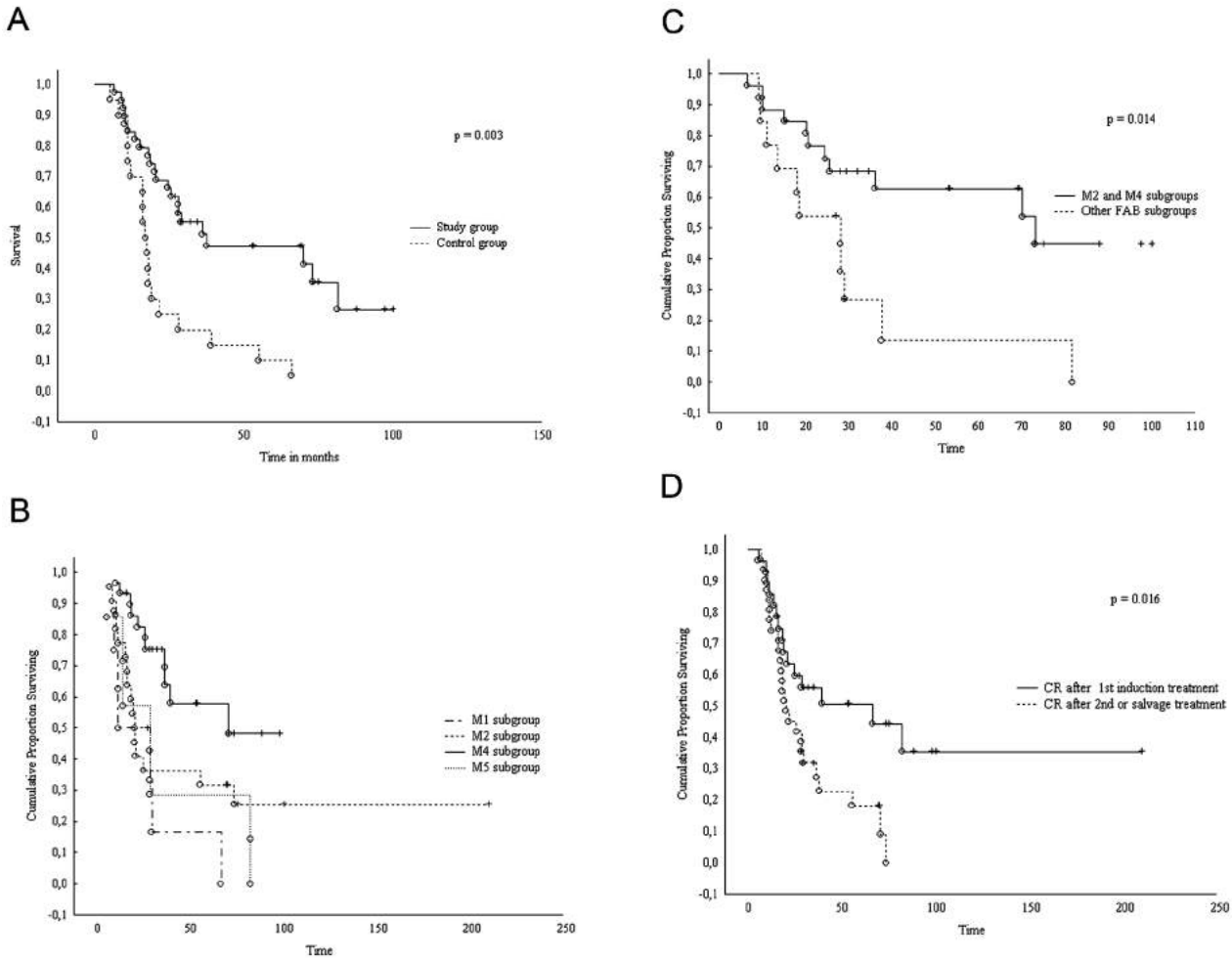

Figure 2. Kaplan-Meier plots of overall survival
Slika 2. *Kaplan-Majer kriva preživljavanja*

A. Cumulative overall survival of study vs control historical group
A. Kumulativno preživljavanje studijske naspram kontrolne grupe
B. Cumulative overall survival of M1, M2, M4 and M5 subgroups
B. Kumulativno preživljavanje M1, M2, M4 i M5 podgrupa
C. Cumulative overall survival within sudy group - M2 and M4 vs other FAB subgroups
C. Kumulativno preživljavanje unutar studijske grupe - M2 i M4 naspram drugih FAB podgrupa
D. Cumulative overall survival according to number of induction courses to CR
D. Kumulativno preživljavanje prema broju terapija do postizanja KR

ure 2C. The same comparison in the control historical group did not show a significant difference.

The median survival rate resulting from the comparison of the study and control group together was significantly higher (the log-rank test, p = 0.016) in the patients who achieved CR after the first induction course (39 months) than in the patients who achieved CR after two or more induction courses (20 months), which is shown in **Figure 2D**. If the same comparison was made within the groups themselves, no significant difference would be found.

Baseline hematological characteristics - median white blood cells, median platelets and median hemoglobin were not predictive of survival (the log-rank test). By using Cox logistic regression model for all patients and by examining consolidation treatment (HDAC versus other treatment), the affiliation to M2 and M4 subgroup versus other FAB subgroups and the number of induction courses to CR, all parameters emerged as significant variables affecting the survival, consolidation treatment (p=0.004), affiliation to M2 and M4 subgroup (p=0.017) and number of induction courses to CR (p=0.031). The application of Cox logistic regression model within the

study group among the parameters tested (the affiliation to M2 and M4 subgroup versus other FAB subgroups and the number of induction courses to CR) resulted in a significant impact on the survival (p=0.02), whereas only the affiliation to M2 and M4 subgroups (p=0.018) seemed to be an independent prognostic factor.

Discussion

AML responses to therapy are still unsatisfactory, the impediments most frequently being disease resistance, prolonged and recurrent infections and bleeding, as well as morbidity and mortality caused by the treatment itself [11, 12]. The need for any form of post-remission therapy was established a long time ago because patients without one would relapse within a year [4, 5]. Consolidation therapy is a standard treatment, whereas alternative treatments include allogeneic bone marrow transplantation, autologous bone marrow transplantation and maintenance therapy. A number of studies [6, 13-15] suggest that the increased intensity of post-remission chemotherapy has a positive effect - both on the survival and prolongation of CR in adults younger than 60 years. A particularly attractive candidate to be intensified is cytosine-arabinoside.

The results of our study have shown that HDAC can indeed prolong the survival and reduce the incidence of relapse compared to the patients who received another form of consolidation therapy. Our results are consistent with the results of previously published randomized and nonrandomized studies [4, 6, 13-15]. However, it should be mentioned that the longest follow-up of patients in the control group was 209 months, as opposed to the longest follow-up in the study group of 100 months. Also, it should not be ignored that the study group patients received one or two more cycles of consolidation more than the patients in the control group. They received two cycles of HDAC, which is in accordance with the recommendations of the National Cancer Center Network [16].

The most favorable prognostic subgroups of AML according to the literature [17] are M2, M3 and M4; however, it has not been confirmed so far [11,18,19]. According to the results of our study on the study and control group patients together, as well as on the patients form the study group itself, the superior survival was found within M4 and M2 subgroup. Therefore, it can be concluded that only the patients from M2 and M4 subgroup benefit from treatment with HDAC.

The karyotype is the strongest predictive factor for the response to induction therapy and for the survival [1, 3, 5]. A favorable outcome, primarily in the patients from M4 and M2 subgroup, can be explained by the fact that these subgroups commonly experienced cytogenetic changes - t (8; 21) in M2 and inv (16) in M4 Eo - which carry favorable prognosis [16, 20]. The results of CALGB 8461 study demonstrated that the median survival was 7.6 years in the patients with favorable karyotype compared to the median survival in the patients with intermediate (1.3 years) and adverse (0.5 years) cytogenetic abnormalities [21]. In our study we did not have data on karyotype for all the patients, thus this parameter was not analyzed.

However, the largest subgroup of AML patients (~40%) has no identifiable cytogenetic abnormalities [3,5,19,21]. In this subgroup, molecular markers are the most important prognostic factors - mutations in the nucleophosmin (NPM1), CCAAT/enhancer binding protein (encoded by the CEBPA gene) and FMS-like tyrosine kinase 3 (FLT3) genes [3]. Mutations of NPM1 and CEBPA gene are associated with a higher CR rate and the better overall survival, if not combined with FLT3 gene mutation [3,5,22]. On the other hand FLT3 gene mutations, alone or combined, lead to inferior outcome [3,5,21]. C-Kit mutation in patients with good cytogenetic disorders also carries the adverse prognostic significance [5]. NPM mutations mostly occur in M4 subgroup [22], CEPBA mutations are mostly found in M1 and M2 subgroup [23], whereas FLT3 mutations are most frequent in M0 and M5 subgroup [24]. We assumed that the favorable outcome of consolidation therapy with HDAC in the M2 and M4 subgroup in our study was partly achieved in the patients with favorable genetic and molecular mutations.

In our study, as in the study performed by Zittuon et al. [18], and by Popović [25], the initial factor with the prognostic value was the number of induction courses to CR. However, in the multivariate analysis of the study group where we observed one versus two or more induction courses and the affiliation to M2 and M4 subgroup versus other FAB subgroups, only the affiliation to M2 and M4 FAB subgroup retained the independent prognostic significance. Therefore, we suppose that the patients from M2 and M4 subgroup achieve a high CR rate after one induction course as a result of associated favorable cytogenetic and molecular aberrations.

AML is a heterogeneous group of disorders where post-remission treatment should be based on the prognostic factors, primarily on karyotype. A reasonable choice for the patients with favorable cytogenetic disorders is consolidation therapy with repetitive cycles of HDAC. For the patients with adverse cytogenetic disorders, a superior outcome is achieved with transplantation [5]. The approach to treatment with intermediate prognosis is not unique, but there is clinical evidence suggesting that transplantation is indicated in the first remission in case of FLT3-ITD mutation, and in case of relapse with isolated NPM1 or CEBPA mutations [3].

Conclusion

This study demonstrates that high dose cytosine-arabinoside used for consolidation therapy results in a higher survival rate and a lower rate of relapse compared to consolidation therapy without high dose cytosine-arabinoside. However, high dose cytosine-arabinoside showed a significant benefit only to the patients in M2 and M4 French-American-British subgroup. In addition to the cytogenetic analysis, which is necessary during the diagnostic evaluation, the molecular genetic analysis is to be done in case of normal karyotype and then, on the basis of results, to opt for post-remission therapy in accordance with conventional prognostic factors. Further investigations on a large group of patients, which would include cytogenetic and molecular analyses, especially FMS-like tyrosine kinase 3 gene and nucleophosmin mutations, are needed in order to evaluate favorable effects of high-dose cytosin-arabinoside on the survival.

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University of Novi Sad, Faculty of Medicine[1]
Clinical Centre of Vojvodina, Department of Ear Throat and Nose Diseases Phoniatrics Ward[2]

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VOICE THERAPY AND ASSISTIVE TECHNIQUES IN VOICE DISORDERS CAUSED BY UNILATERAL VOCAL CORD PARESES

FONOTERAPIJSKE I ASISTIVNE TEHNIKE KOD POREMEĆAJA GLASA UZROKOVANIH JEDNOSTRANIM PAREZAMA GLASNICA

Bojana KAŠTEROVIĆ[1], Mila VESELINOVIĆ[1,2] and Slobodan M. MITROVIĆ[1,2]

Summary

Introduction. Dysphonias due to primary neurogenic disorders are a group of voice disorders that can be caused by both central and peripheral disorders of the larynx innervation. There are numerous causes leading to paralysis of superior and/or inferior laryngeal nerves, particularly of the inferior laryngeal or recurrent nerve. **Voice Therapy in Unilateral Vocal Cord Paresis.** Vocal therapy is an integral part of the conservative treatment. Specific methods are applied to individual vocal problems, while the non-specific ones are applied to a number of dysphonias. Non-specific methods are further divided into integrated and focused vocal methods. Integrated methods treat the voice and speech disorders as a unified entity of all quality and segments of voice and speech. Focused non-specific methods treat the segments and the quality of voice and speech individually. **Assistive Techniques in Voice Disorders Caused by Unilateral Vocal Cord Paresis.** *Digital compression of the larynx by Seeman* includes the treatment of voice with compression of the thyroid cartilage, thus moving the paralyzed and healthy vocal cord medially and upwards, and medially and downwards, respectively. This leads to the proper occlusion of vocal cords because in these conditions the paralyzed vocal cord is lower than the healthy one. According to the theoretical assumption, when the head anc neck are rotated to one or the other side, the anatomic relations in the neck are change and thus the vocal cords are brought into contact with the resulting reduction of the gap between them and the reduced air flow. **Conclusion.** Studies assessing the efficiency of different methods of vocal therapy are scarce bearing in mind the importance of vocal therapy and the fact that many patients refuse surgical treatment. Research on the efficiency of assistive techniques in phoniatric rehabilitation of patients with unilateral vocal cord paresis yields conflicting results. However, assistive techniques are useful practical methods in vocal rehabilitation of these patients.

Key words: Voice Disorders; Voice Training; Vocal Cord Paralysis; Dysphonia

Sažetak

Uvod. Disfonije zbog primarnih neurogenih poremećaja jesu grupa poremećaja glasa koji mogu biti izazvani i centralnim i perifernim poremećajima inervacije larinksa. Postoje brojni uzroci koji dovode do oduzetnosti larinksnih nerava, gornjeg i/ili donjeg, naročito donjeg larinksnog ili rekurentnog nerva. **Vokalna terapija kod jednostranih pareza glasnica.** Vokalna terapija je sastavni deo konzervativnog tretmana. *Specifične metode* se primenjuju za pojedine vokalne poremećaje, dok se *nespecifične* primenjuju na veći broj disfonija. *Nespecifične metode* se dalje dele na integralne vokalne metode i usmerene vokalne metode. Integralne metode tretiraju poremećaje glasa i govora kao jedinstvenu celinu svih kvaliteta i segmenata glasa i govora. Usmerene nespecifične metode tretiraju segmente i kvalitete glasa i govora ponaosob. **Asistivne tehnike kod jednostranih pareza glasnica.** *Digitalna kompresija larinksa po Semanu (Seeman)* podrazumeva vokalni tretman uz kompresiju na tiroidnu hrskavicu tako da se paretična glasnica pomera medijalno i naviše, a zdrava medijalno i naniže. Time se dobija pravilna okluzija glasnica, s obzirom da je kod ovakvih stanja, paretična glasnica postavljena niže u odnosu na zdravu. Teorijska je pretpostavka da se rotacijom glave i vrata u jednu ili drugu stranu, menjaju anatomski odnosi u vratu, te se glasnice dovode u kontakt, što smanjuje zjap među njima a smanjuje se i vazdušni protok. **Zaključak.** Postoji mali broj istraživanja koja procenjuju efikasnost različitih metoda vokalne terapije, s obzirom da je njen značaj neizmeran, posebno ako se uzme u obzir da dosta pacijenata odbija hiruško lečenje. Istraživanja o efikasnosti asistivnih tehnika u fonijatrijskoj rehabilitaciji pacijenata sa jednostranom parezom glasnica daju oprečne rezultate. Asistivne tehnike su ipak korisne praktične pomoćne metode u vokalnoj rehabilitaciji ovih pacijenata.

Ključne reči: Poremećaji glasa; Trening glasa; Pareza glasnica; Disfonija

Corresponding Author: Prof. dr Slobodan M. Mitrović, Klinički centar Vojvodine, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: mitroslo@eunet.rs

Introduction

Dysphonias due to primary neurogenic disorders are a group of voice disorders that can be caused by both central and peripheral disorders of the larynx innervation [1,2]. There are numerous causes leading to paralysis of superior and/or inferior laryngeal nerves, particularly of the inferior laryngeal or recurrent nerve on its ”long way” [3]. This fact requires a systematic and multidisciplinary approach to diagnosis and treatment. Kotby [4] has divided the causes of laryngeal paralysis into central and peripheral, and the peripheral causes can be further divided into idiopathic, cancerous, surgical, traumatic, post-intubation because of chest diseases and others. Mitrovic [3] found that vocal cord paresis was most often due to a neck surgery (strumectomy) in 38% of patients, and idiopathic causes followed immediately, being found in 24% of patients. Mumović [5] found iatrogenic injuries of the recurrent nerve in 10.36% of patients, most of which were also due to the operations of the thyroid gland. Beljin [6] reported that 38.5% of unilateral vocal cord paresis had iatrogenic cause. According to Jovic et al. [7], bilateral vocal cord paresis was also most common after surgery of the thyroid gland. Mitrovic [1] believes that recurrent laryngeal nerve paralysis is more often reported in women, although Stankovic [8] found that unilateral paresis of the larynx was twice more often in men in his study sample and he considered this fact controversial. The well known fact that the left recurrent nerve is more frequently paralyzed has been confirmed by Mitrovic et al. [3], whereas the right vocal cord paresis is more frequent according to Beljin [6] and Mumović [9]. More frequent paralysis of the right recurrent laryngeal nerve was found by Mumovic [5] after neck surgery and by Hillstrom et al. [10] after injection of drugs into the neck.

Glottal closure is a very complex mechanism; therefore, glottal disclosing by unilateral vocal cord paresis is an important parameter of phonation dysfunction. Besides causing pathological phenomena of the voice, glottal disclosing leads to other difficulties, primarily the feeling of breathlessness. That is why the reduction of glottal disclosing is one of the goals in treatment as well as in the final evaluation of the efficiency of therapy [3].

Voice Therapy in Unilateral Vocal Cord Paresis

Vocal therapy is an integral part of the conservative treatment which also includes administration of neuroprotective drugs, electrotherapy and acupuncture [11]. Kotby [4] states that the methods of voice therapy can be divided into specific and non-specific ones. Specific methods are applied to individual vocal problems, while the non-specific ones are applied to a number of dysphonias. Non-specific methods are further divided into integrated and focused vocal methods. Integrated methods treat the voice and speech disorders as a unified entity of all qualities and segments of voice and speech. Focused non-specific methods treat the segments and the quality of voice and speech (height, intensity, purity and resonance of voice, rhythm, tempo, accent, tone of speech) individually [12]. The primary treatment goals for a patient with unilateral vocal cord paresis are to improve glottal closure, to increase the intrinsic muscle strength and agility (without causing supraglottic hyperfunction) and to develop abdominal support for breathing [13]. Heuer et al. [14] investigated the efficiency of voice therapy. They concluded that 92% of female patients and 71% of male patients with unilateral recurrent laryngeal nerve palsy showed significant improvement after three treatments on average. In a sample of 40 patients, Schindler [15] found that eight patients had the total occlusion of the glottis before voice therapy and 14 after the treatment, the patient’s voice became less rough and symptoms of dysphonia were reduced.

The importance of early voice therapy is also emphasized because it can result in a significant

improvement of voice with the possibility of avoiding surgery [13].

The most frequently used techniques of voice therapy in unilateral paresis are:

Hard glottal attacks and pushing exercises

These exercises consist of having the patient breathe in, build air pressure while posturing the vowel without letting the air out, and then release the vowel. The patients are given the list of vowels and one-syllable vowel-consonant combinations to practice twice a day during the week. After that the patient is asked to produce the hard glottal attack with the addition of stretching the vowel while gliding down to a lower pitch. Gliding to a lower pitch encourages contraction of the thyroarytenoid muscle. If a progress is made, the patient incorporates an isometric push to the exercise. The push may be accomplished by pulling his hands up, pressing the chair next to the body. The isometric push is released when the vowel is released [16,17].

Half-swallow boom

The author of this technique is McFarlane. The patient is asked to take a breath and initiate the first part of a swallow, which reportedly improves glottal closure through muscle movements of the pharynx and larynx. At the peak of the half-swallow, the patient forcefully says ”boom’’. When this technique is mastered, the word ”boom” sounds loud and clear [18,19].

Abdominal breathing

The patient should place one hand on his upper chest and one on the lower ribcage. He should be able to feel a slight outward movement of the lower hand on breath with imperceptible movement of the upper hand. The aim is to notice the continuous

movement of the respiratory cycle. As soon as the effortless breathing has been established, the patient should be instructed to breathe more deeply. Inhalation should be shorter, whereas exhalation is getting longer but not extended beyond the available air supply [18,19].

Vocal function

The aim is to strengthen and balance the laryngeal musculature and to balance the airflow to the muscular effort [19].

The program consists of four steps, which are conducted two times each, twice a day for the first six weeks. All exercises should be produced as softly as possible without being breathy [16].

Head, neck, and shoulder relaxation

Compensatory vocal and laryngeal behaviors are frequently associated with increased tension of the shoulder, neck, and upper back. Relaxation of these areas should be practiced on a daily basis and it can be achieved by massage. General body relaxation can be achieved by increased aerobic exercise, yoga, pilates. In addition, it is extremely important to reduce stress at home and at work [16].

Accent method

The therapeutic procedure consists of respiratory, phonatory and articulatory exercises [20]. Practice drills usually start with a relaxed, soft, low pitched voice and then go up to a variety of accentuated vocalizations and intonational contours. Khidr [21] states that the accent method improves the auditory, perceptual, and aerodynamic parameters of patients with unilateral vocal cord paresis.

Lip and tongue trills

These exercises affect normal laryngeal tension by equalizing myoelastic and aerodynamic forces, and they also enhance the coordination of respiration, phonation and articulation. The tongue or the lips act as a valve, creating a difference in pressure of the outside air and the inside cavity, resulting in some oscillatory changes in the air pressure and the velocity of air flow. Subglottal air pressures during the production of trills may be greater than the normal phonation, thus enhancing the oscillation of vocal cords [16].

Appropriate tone focus

A combination of nasal consonants and vowels, such as ”um-hum” and ”me-me” which place perceptible vibration along the bridge of the nose are used in this treatment. This is useful for the patients with hypofunctional (breathy) and hyperfunctional (rough harsh) voices [16].

Therapy programs, such as Lessac-Madsen Resonant Voice Therapy (LMVRST) [22] and Resonance Therapy [23] are not typically used with patients with vocal cord paralysis or paresis, but they should be taken into consideration if they result in easier production of voice.

Assistive Techniques in Voice Disorders Caused by Unilateral Vocal Cord Paresis

Digital compression of the larynx by Seeman

There are many receptors in the mucosa of supraglottis which are highly sensitive to a vibration of laryngeal mucosa; whereas, tactile receptors are found in the epiglottis, aryepiglottic folds and vocal processes [6]. During phonation, the pressure sensitive receptors within the muscular spindles are activated, they send the impulses about the position of the larynx in the neck and the activities of the external laryngeal muscles [24]. External compression of the larynx improves the occlusion between the vocal cords, resulting in a better proprioceptive sensitivity and better control of voice [25].

In as early as 1910, Gutzman [26] described an improvement of vocal function achieved by digital compression applied on the cartilaginous larynx skeleton in the diagnosis of mutational disorders. He realized that compression on the thyroid cartilage in phonastenic voices causes lowering of pitch, which is retained for a longer period even after the pressure stopped, and in healthy voices the voice returns immediately to normal.

Brodnitz described digital compressive tests in the differential diagnosis of mutational disorders and functional dysphonia. It includes lateral manual compression, cricothyroid approximation, combination of lateral compression and cricothyroid approximation, and anteroposterior compression. These tests are applicable in patients with insufficient glottis occlusion of different etiology. Van den Berg suggests that the pitch can be lowered by the compression on the thyroid cartilage which moves it posteriorly as well as by lower lateral pressure. However, if the lateral pressure is too strong especially near the arytenoid cartilage, it can result in higher voice [11].

Blaugraund [26] has stated that compression exerted on the thyroid and cricothyroid cartilage modifies the position, shape and tension of the vocal cords. The method is simple, non-invasive, requiring no instruments. The objective evaluation with aerodynamic tests significantly confirms beneficial effects of the manual compression on the glottis occlusion, as well as the objective acoustic analyses and the videostroboscopic finding. The lateral compression reduces the laryngeal gap, adverse development of compensatory mechanisms and the amount of high-frequency noise components, heard as breathy voice [27].

As a therapy method, compression of the larynx dates back to 1919 when Seeman described it. This method is most frequently applied in glottal insufficiency caused by the recurrent nerve paralysis. Later, the therapists accepted the method in

the evaluation and treatment of different types of dysphonia [11].

The method includes the treatment of voice with compression of the thyroid cartilage by moving the paralyzed vocal cord medially and upwards and the healthy vocal cord medially and downwards [9]. This leads to the proper occlusion of the vocal cords because in these conditions, the paralyzed vocal cord is lower than the healthy one [28].

The efficiency of method

Mitrovic found the following results in his study on the efficiency of the method by Seeman, conducted on a sample of 50 people [3]:

1. Indirect laryngoscopy showed that none of the patients had the total occlusion of the vocal cords before treatment. The disocclusion of 1-2 mm and 2-3 mm was reported in 54% and 24 % of the patients, respectively. The total occlusion was regained after the treatment in 20% of the patients, while the disocclusion of up to 1 mm, 1-2 mm and 2-3 mm persisted in 36%, 20% and 2% of the patients, respectively.

2. The residual movements, described as minimal and irregular vibrations, with the difference in phase were found by laryngostroboscopy in 60% (30 patients) before treatment. After treatment, the residual movements were present in 45 patients (90%).

3. Subjective acoustic analysis showed that all patients (100%) had dysphonia before treatment. Mild and moderate disphonia was present in 30% and 42% of patients, respectively. After treatment, 48 % of the patients regained a satisfactory peach and purity of their voice and 50% of them still had mild dysphonia. Moderate dysphonia was found in 2%, and none of the patients had severe dysphonia.

Mumovic and Arbutina [9] used the objective acoustic analysis with contemporary acoustic means to assess the effects of method by Seeman in their research conducted in the period 2000-2009. In a sample of 27 patients, aphonia was found in 44.44% of patients before treatment and none of the patients had it after treatment. Hoarseness (hoarse voice) was present in 51.8 % of patients before voice therapy, being mild in 25.9%, moderate in 14.8% and severe in 11.1% of the patients; whereas only 3.7% of the patients were symptom free. After treatment, hoarseness was absent in 44.4% of patients, it was mild in 40.7% and moderate in 14.8% of the patients.

Before therapy, roughness (harsh voice) was mild in 11.1% of patients, and in 14.8% of patients it was moderate and severe, each. After treatment, roughness was absent in 55.6% of patients, it was mild in 14.8% of patients, moderate in 22.2% and severe in 7.4% of the patients.

Before therapy, breathiness (breathy voice) was severe in 44.4% of patients, moderate in 7.4% and mild in 3.7%. Not a single patient was free of breathiness in the voice before treatment, which suggests the presence of vocal insufficiency in all the patients. After treatment, breathiness was absent in 11.1% of patients, it was mild in 51.9% of patients, moderate in 11.1% and severe in 25.9% of patients.

The analysis of numerical acoustic parameters after voice therapy by Seeman has showed a significant reduction of irregularity of frequency vibration and noisy components in the voice probably by reducing the gap between the vocal cords. Voice therapy by Seeman improves the relation between the sound and noisy components by reducing the gap between the vocal cords, and perhaps by increasing the presence of harmonic components of voice, i.e. by better resonant conditions of phonation [9].

Head and neck rotation

The theoretical assumption underlying this method is that the rotation of the head sideways changes the anatomic relations in the neck, thus bringing the vocal cords into contact with the resulting reduction of the gap between them and reduced air flow. This technique was described as one of the three in the study by McFarlane et al. [29], who reported on the reduction of air flow after therapy, which was attributed to the improved occlusion of the glottis. In addition, hereby described method of digital compression of the larynx and half-swallow boom method was also used in this research.

Within the program of early vocal therapy, Mattioli et al. [17] used the exercise of head rotation sideways towards the healthy vocal cord, in which the therapist pressed his hand to the patient's cheek on the same side, and the patient had to resist the pressure. In the second exercise, the patient also rotated the neck towards the healthy side and the therapist, coming from the other side, tried to raise the patient's chin, and the patient had to resist.

The technique of lateral positioning of the head is used at the Department of Ear, Nose and Throat Diseases in Novi Sad. The technique is performed by the therapist, with the hands placed on the top of the patient's head and slowly lowers the patient's chin, thus making the lower jaw and the hyoid bone push the larynx down. The lateral rotation of head (right - left, left - right) is performed to achieve the best voice quality, especially in the production of vowels. This maneuver allows the best occlusion of paretic vocal cord, not only in terms of reducing disocclusion between the vocal cords, but also in terms of their alignment, especially if the vocal cords are in two levels.

The efficiency of method

Paseman et al. [30] measured the air flow in patients with unilateral vocal cord paresis during phonation in different head positions (central, rotated to the left and to the right side), while uttering the vowels I and A. The amount of air flow is seen as a reflection of glottal closure. The results obtained in this study

indicate that the position of the head does not affect the improvement of glottal closure. This is in contrast with the results of McFarlane et al. [18], which show a significant improvement of glottal closure in patients with unilateral paresis after the head rotation. The different results obtained in these studies can be explained by various factors. McFarlane et al. applied three methods on the same sample, so it is possible that there was a cumulative effect. Furthermore, the intensity of voice was not controlled during therapy, and higher intensity can affect the glottis width, regardless of the rotation of the head, and neither the degree of rotation was specified. On the other hand, the respondents in the study of Paseman [30] were tested only once, but not before and after treatment. In any case, the different results obtained in these studies should encourage further assessment of the efficiency of this method.

Cantarella et al. [31] studied the efficiency of voice therapy in relation to the time elapsed from the onset of symptoms. Their study sample consisted of 30 patients who were divided into two groups. The first group included 14 patients with unilateral laryngeal paralysis, the mean duration of symptoms being 1.29 months, and the second group consisted of 16 patients with the average disease duration of 29.8 months. Vocal therapy involved the relaxation, abdominal breathing, pronunciation of the consonant ”s” during exhalation, the resonance exercises, digital compression of the thyroid cartilage, neck massage, coughing and laughing exercise and rotation of the head sideways. The results show that vocal treatment alleviates the symptoms of unilateral paralysis of the larynx, both in patients with recent onset of symptoms, and in patients with unilateral paralysis which has been present for some time.

Conclusion

Unilateral vocal cord paresis is one of the most common causes of voice disorders. This condition can impair the quality of life of the patient severely, and it is necessary to develop more methods that would result in the improved quality of voice and alleviation of other symptoms of this disease.

By reviewing literature data it has been concluded that there are very few studies which evaluate the efficiency of different methods of voice therapy. That should be an incentive for experts in this field bearing in mind the importance of vocal therapy, particularly because many patients refuse surgical treatment.

Studies on the efficiency of assistive techniques in phoniatric rehabilitation of these patients described in this paper, compression of the larynx by Seeman and the rotation of the neck sideways have yielded conflicting results.

Digital compression of the larynx can be assessed as a very successful method according to the results of the studies hereby presented. On the other hand, the efficiency of head and neck rotation method has not been fully clarified because of different results obtained by the above mentioned studies. That suggests the need for further research in this area. Assistive techniques are useful practical methods in vocal rehabilitation of patients with unilateral vocal cord paresis.

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Clinical Center of Serbia, Belgrade, Serbia, Department of Pulmonology[1]
University of Belgrade, Serbia, Faculty of Medicine[2]
Clinical Center of Serbia, Belgrade, Serbia, Department of Neurology[3]
Hospital for Psychiatric Disorders „Dr Laza Lazarević", Belgrade, Serbia[4]

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DIAGNOSIS OF NEUROSARCOIDOSIS - NECESSITY OF BIOPSY

DIJAGNOZA NEUROSARKOIDOZE − NEOPHODNOST BIOPSIJE

Mihailo I. STJEPANOVIĆ[1], Violeta MIHAILOVIĆ VUČINIĆ[1,2], Dragana JOVANOVIĆ[1,2], Milija MIJAJLOVIĆ[3], Vesna ŠKODRIĆ TRIFUNOVIĆ[1,2] and Mirjana M. STJEPANOVIĆ[4]

Summary

Introduction: Sarcoidosis can affect any part of the central nervous system presenting with an extremely diverse clinical picture. Clinical presentations actually depend on the localization of granulomas in the central nervous system. Making diagnosis according to the localization and the clinical variations is often a clinical challenge. **Diagnosis of Neurosarcoidosis**. Diagnosis is based on the clinical picture, clinical and radiological findings (magnetic resonance imaging with contrast endocranium), laboratory findings (angio-tenzin-converting enzyme and chitotriosidase in cerebrospinal fluid); however, it is necessary first to exclude all other possible causes of granulomatous inflammation. Recent studies in patients with neurosarcoidosis show a high value of at least one marker of the disease. The safest way and the gold standard in diagnosing this disease would be histopathological confirmation, which is rarely performed due to its invasiveness. **Conclusion**. New diagnostic methods will contribute to better methods of bypassing invasive procedures, and they will significantly facilitate the diagnosis of neurosarcoidosis, which is a real challenge even for experienced clinicians who deal with this disease.

Key words: Sarcoidosis; Central Nervous System; Diagnosis; Granuloma; Signs and Symptoms; Biopsy; Magnetic Resonance Imaging; Spinal Puncture

Sažetak

Uvod. Sarkoidoza može zahvatiti bilo koji deo centralnog nervnog sistema i dati izuzetno šaroliku klinički sliku. Kliničke prezentacije upravo i zavise od lokalizacije granuloma u centralnom nervnom sistemu. Dijagnostika, shodno lokalizaciji ove bolesti i kliničkim varijacijama, često je veliki klinički izazov. **Dijagnoza neurosarkoidoze**. Dijagnoza se postavlja na osnovu kliničke slike, kliničkog i radiološkog nalaza (magnetna rezonancija endokranijuma sa kontrastom), laboratorijskih nalaza (angiotenzin-konvertujući enzim i hitotriozidaze u likvoru), s tim što je prethodno neophodno isključiti sve druge moguće uzroke granulomatozne inflamacije. Dosadašnja ispitivanja kod obolelih od neurosarkoidoze pokazuju postojanje povišene vrednosti bar jednog od markera aktivnosti ovog oboljenja. Najsigurnijji način i zlatni standard u postavljanju dijagnoze ove bolesti bila bi patohistološka potvrda, koja se zbog svoje invazivnosti retko radi. **Zaključak**. Nove dijagnostičke metode doprineće zaobilaženju invazivnih procedura i značajno olakšati postavljanje dijagnoze neurosarkoidoze, što je pravi izazov i za iskusne kliničare.

Ključne reči: Sarkoidoza; Centralni nervni sistem; Dijagnoza; Granulom; Znaci i simptomi; Magnetna rezonanca; Lumbalna punkcija

Introduction

Sarcoidosis is a systemic granulomatous disease most frequently affecting lungs and hilar lymph nodes. Recent studies have shown that sarcoidosis can affect any organ including the central nervous system (CNS) [1].

It is difficult to determine the precise number of patients with sarcoidosis because of a great number of subclinical cases. It is believed that only half of the patients are diagnosed during their lifetime [2].

Sarcoidosis may affect any part of the CNS and give a particularly diverse clinical picture. Clinical presentations depend on the localization of granulomas in the CNS. The symptoms are most frequently given by cranial nerves [2]. Making diagnosis according to the localization of this disease and clinical variations is often a big clinical challenge. We have tried to clarify some of the dilemmas in this text, including the question whether pathohistological confirmation is the only way to diagnose this disease and which are diagnostic criteria.

Diagnosis of Neurosarcoidosis

In hospital, the diagnosis of sarcoidosis is made on the basis of the clinical picture, clinical and radiological findings (magnetic resonance imaging of

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Corresponding Author: Dr Mihailo Stjepanović, Klinika za pulmologiju, Klinički centar Srbije, 11000 Beograd, Koste Todorovića 26, E-mail: mihailo.stjepanovic@kcs.ac.rs

Abbreviations

CNS	– central nervous system
NMR	– magnetic resonance
ACE	– angiotensin-converting enzyme

endocranium with contrast-NMR), laboratory findings (angio-tenzin-converting enzyme-ACE and chitotriosidase in liquor), but it is necessary first to exclude all other possible causes of granulomatous inflammation. The safest way and the gold standard in making the diagnosis of this disease would be the pathohistological confirmation, when noncaseating granuloma is present on the pathohistology slide. In everyday practice, biopsy of the CNS is very rarely performed due to its invasiveness and serious complications. Biopsy of nerve tissue is reserved only for vitally threatened patients [3,4].

American National Cardiopulmonary and Hematology Institute has suggested the criteria for sarcoidosis diagnostics in cases when the localization is known and when sarcoidosis of another organ has been pathohistologically confirmed [5,6].

Criteria of A Case Control Etiologic Study of Sarcoidosis (ACCESS) group in neurosarcoidosis diagnostics are classified in the following way:

I Definite diagnosis of neurosarcoidosis
II Probable diagnosis of neurosarcoidosis
III Possible diagnosis of neurosarcoidosis

I Definite Diagnosis of Neurosarcoidosis

1. Positive NMR finding with characteristic piling up of contrast in meninges or the brain stem
2. CSF with lymphocytosis and/ or an increase in protein level
3. Diabetes insipidus
4. Bell's palsy
5. Dysfunction of some of cranial nerves
6. Biopsy of peripheral nerve

II Probable Diagnosis of Neurosarcoidosis

1. Other pathological finding on NMR
2. Neuropathy of inexplicable cause
3. Positive electromyogram finding

III Possible Diagnosis of Neurosarcoidosis

1. Persistent headaches of unknown cause
2. Radiculopathy of peripheral nerves

According to this classification, the definite diagnosis includes the pathohistological finding, and excludes any other granulomatosis of CNS.

In practice, the most frequently used criteria for making the diagnosis of neurosarcoidosis are those suggested by Zajcek et al.[7]. They are very simple and applicable for doctors who meet, diagnose, and treat these patients in practice. These criteria are based on levels of safety in diagnostics of neurosarcoidosis. They are classified in three categories and each of them includes the clinical presentation indicating the diagnosis of neurosarcoidosis and excludes any other diagnosis in the CNS. They are divided into definite, probable and possible diagnosis of neurosarcoidosis, but *definite diagnosis* is the only pathohistological confirmation of this disease. The criteria for *possible* neurosarcoidosis include: clinical symptoms and diagnosis suggesting neurosarcoidosis, but infections and malignancy are not excluded or there is the pathohistological confirmation of systemic sarcoidosis.

For diagnosis of *probable* neurosarcoidosis, clinical symptoms and diagnostic evaluation suggest neurosarcoidosis. Alternative diagnoses are excluded and there is also the pathohistological confirmation of systemic sarcoidosis.

Lumbar puncture is recommended in all patients for diagnostic purposes. Nonspecific anomalies of liquor described in patients with neurosarcoidosis include lymphocytic pleocitosis, increased protein level, decreased glucose concentration, and increased intracranial pressure. Some studies revealed increased immunoglobulin, lysozome and concentration of beta 2 microglobulin, as well as the ratio of lymphocytes CD4+:CD8+ higher than 5. It is important to know that one third of patients suffering from neurosarcoidosis have no abnormalities in CSF. It is also important to have in mind that the patients having some diseases, such as multiple sclerosis and lupus, have similar finding in CSF as the patients with neurosarcoidosis [8, 9].

Specificity and susceptibility of ACE in CSF is still the subject of research. High values of ACE are non-specific and may appear in some other diseases such as infections and tumors. In addition, patients with neurosarcoidosis may have the normal level of CSF. A study has shown that 55% of patients with neurosarcoidosis, 5% of patients with systemic sarcoidosis, and 13% of patients with other diseases have increased values of ACE in CSF. In general, ACE values in CSF are not sensitive enough (24%-55%) for diagnosis of sarcoidosis of CNS, although they may be relatively specific (94%-95%). Based on these results, increased ACE value in CSF is not enough for making the diagnosis [10, 11]. In recent research, chitotriosidase is used as a sensitive marker for sarcoidosis, as well as neurosarcoidosis, the values of which are determined in CSF [12,13]. Determination of chitotriosidase enzyme values in CSF is the method being just introduced into clinical practice and it is the subject of further research. Previous investigations of patients with neurosarcoidosis show the existence of increased value of at least one activity marker of this disease.

Conclusion

Pathohistological diagnostics of this disease is very difficult; therefore, it is necessary to develop other diagnostic methods in diagnosing neurosarcoidosis. New clinical research dealing with bi-

omarkers in cerebrospinal fluid, such as chitotriosidase, will be helpful in everyday management of these patients. New diagnostic methods will make it possible to avoid invasive procedures and they should considerably facilitate making the diagnosis of neurosarcoidosis, which is a real challenge even for experienced clinicians dealing with this disease.

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STRUČNI ČLANCI
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General Hospital ”Đorđe Joanović” Zrenjanin
Department of Transfusiology[1]
Department of Orthopedics and Traumatology[2]
Department of Pediatrics[3]

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AUTOLOGOUS BLOOD TRANSFUSION IN PATIENTS UNDERGOING HIP REPLACEMENT SURGERY

PRIMENA AUTOLOGNE KRVI KOD PACIJENATA KOJI SU PODVRGNUTI OPERACIJI UGRADNJE VEŠTAČKOG KUKA

Ivana TEŠIĆ[1], Jovan SEKULIĆ[2], Vladimir ARBUTINOV[2], Dragana POPOV[1] and Dušan VELISAVLJEV[3]

Summary

Introduction. Autologous blood transfusion is a set of procedures done in order to collect a patient's blood and reinfuse it during or after a surgical intervention. The aim is to meet the patient's need for blood products without allogeneic transfusion. By observing the hemoglobin and hematocrit values during blood donation in the pre-operative and post-operative period and by counting transfused blood units, the aim of this article was to detect whether there was any difference between the patients receiving autologous blood and those receiving only allogeneic blood. **Material and Methods.** This prospective study was performed at the General Hospital ”Đorđe Joanović” Zrenjanin from October 24th, 2011 to January 24th, 2013. The study included 60 patients who were divided into the experimental group of 30 patients who had been transfused autologous blood and the control group of 30 patients who had been transfused only allogeneic blood. **Results.** The average values of hemoglobin and hematocrit in the first and the second donation were 148.9 g/l and 44.2%, and 138.7 g/l and 40.8%, respectively. Oral iron preparation was given to 12 patients for two weeks before the first donation. The level of hemoglobin and hematocrit in both groups of patients had approximately the same values in the pre-operative and post-operative period. In the post-operative period, 2.53 units were transfused per patient in the experimental group and 3.73 units were transfused per patient in the control group. **Conclusion.** Administration of pre-operatively donated autogenous blood reduces the number of transfused deplasmatised erythrocytes units in comparison to the number of units transfused to the patients receiving only allogeneic blood products. The pre-operative use of oral iron preparations increases hemoglobin values significantly.

Key words: Blood Transfusion, Autologous; Arthroplasty, Replacement, Hip; Blood Transfusion; Hemoglobins; Hematocrit; Iron

Sažetak

Uvod. Transfuzija autologne krvi podrazumeva skup postupaka za prikupljanje bolesnikove krvi i njena reinfuzija za vreme ili neposredno po završenoj hirurškoj intervenciji. Cilj joj je zadovoljenje bolesnikovih potreba u hemoproduktima bez upotrebe alogene transfuzije. Praćenjem vrednosti hemoglobina i hematokrita prilikom doniranja krvi, u preoperativnom i postoperativnom periodu i praćenjem broja transfuzionih jedinica krvi, želeli smo da utvrdimo da li ima razlike između pacijenata koji su primili autolognu krv i pacijenata koji su primili samo alogenu krv. **Materijal i metode.** Ova prospektivna analitička studija rađena je u Opštoj bolnici „Đorđe Joanović” u Zrenjaninu u periodu od 24. oktobra 2011. do 24. januara 2013. godine. U studiju je uključeno 30 pacijenata koji su primili autolognu krv − ispitivana grupa i 30 pacijenata koji su primili samo alogenu krv − kontrolna grupa. **Rezultati.** Srednja vrednost hemoglobina i hematokrita kod prve donacije iznosila je 148,9 g/l i 44,2%, a kod druge 138,7 g/l i 40,8%. Oralni preparat gvožđa ordiniran je kod 12 pacijenata dve nedelje pre prve donacije. Preoperativno i postoperativno, srednje vrednosti hemoglobina i hematokrita u obe grupe pacijenata bile su približno iste. U postoperativnom periodu ispitivana grupa pacijenata primila je 2,53 jedinice krvi po pacijentu, a pacijenti kontrolne grupe 3,73 jedinice po pacijentu. **Zaključak.** Primenom preoperativno donirane autologne krvi smanjen je broj transfuzionih jedinica deplazmatisanih eritrocita u odnosu na pacijente koji su primali samo alogene krvne produkte. Preoperativna primena oralnih preparata gvožđa bitno utiče na povećanje vrednosti hemoglobina.

Ključne reči: Autologna transfuzija krvi; Ugradnja veštačkog kuka; Transfuzija krvi; Hemoglobin; Hematokrit; Gvožđe

Corresponding Author: Dr Ivana Tešić, Opšta bolnica “Đorđe Joanović”, 23000 Zrenjanin, Dr Vase Savića 5, E-mail: drtesicivana@gmail.com

Introduction

Autologous blood transfusion is a set of procedures performed in order to collect a patient's blood and reinfuse it to the same patient during or after a surgical intervention. Autologous blood transfusion differs from allogeneic transfusion when the patient is transfused the blood received from other persons - blood donors. Its aim is to meet the patient's need for blood products without allogeneic transfusion. The patients help themselves by using autologous blood and make chemotherapy more reliable. Thus, by means of using autologous transfusion, the risk of alloimmunization against erythrocyte antigens, leucocytes, thrombocytes and plasma proteins, as well as the risk of immunosuppression and the risk of transmitting the causes of infectious diseases are all eliminated. Hematopoietic system of the autologous blood "donor" is boosted for endogenous cytopoiesis via autologous blood transfusion. For all the above stated reasons autologous blood transfusion is considered the safest of all treatments with blood products [1]. When transfusion is planned for a surgical intervention, strategies for autologous blood accumulation are applied depending on the estimated blood losses. There are several ways of collecting autologous blood, such as: preoperative autologous blood donation (PAD), intraoperative blood salvation (IBS), postoperative blood salvation (PBS) and acute normovolemic hemodilution (ANH) [2]. Autologous blood donation is indicated in patients who are planned for surgical procedures that are usually followed by the demand for blood and blood products, or accompanied by pre-transfusion tests. Most of the health centers worldwide have protocols for various surgical procedures which are the basis for ordering the required number of blood or blood product units [3]. There is such a protocol in our hospital according to which four units of allogenous deplasmatized erythrocytes are ordered for hip replacement surgery, and this protocol justifies preoperative autologous blood collection [4]. It is crucial to emphasize that blood collected in this way cannot totally eliminate the need for allogeneic blood transfusion; yet, the demand for it is reduced significantly. In those situations when the demand for transfusion is higher than estimated, i.e. the number of autologous blood units does not meet the needs of surgical procedure, it is justifiable to use allogeneic blood as well.

Our aim was to detect whether there was any difference between the patients who had been transfused autologous blood and those who had been transfused only allogeneic blood. This was achieved by following the values of hemoglobin and hematocrit in the preoperative and postoperative period, by recording the number of transfused blood units (autologous and allogeneic), by keeping the track of blood loss via a surgical drain during the postoperative period, by recording the frequency of post-transfusion reactions in patients who were transfused allogeneic blood and the ones who were transfused both autologous and allogeneic blood and blood products, as well as by observing the length of hospitalization.

Allogeneic blood transfusion involves certain risks. It can lead to transmission of infectious causes, development of post-transfusion reactions and occurrence of immunomodulation. Autologous blood transfusion does not entail the above mentioned complications, it is actually believed to diminish the need for allogeneic blood and shorten the patient's recovery period [1,2,5].

Autologous blood donation can significantly reduce the costs of treatment [2,5,6].

Material and Methods

This prospective study was performed at the Department of Transfusiology and the Department of Orthopedic and Traumatology of the General Hospital "Đorđe Joanović" in Zrenjanin from October 24th, 2011 to January 24th, 2013 (15 months of observation). Total hip replacement surgery was performed in 116 patients. This surgery had been planned in fifty-four patients and they were referred to the Department of Transfusiology. The study sample was divided into the experimental group, which included 30 hip replacement surgery patients who were transfused pre-operatively donated autologous blood, either during surgery or after it; and the control group, which consisted of 30 patients who were transfused only allogeneic blood. There were 17 women and 13 men in the experimental group; whereas the control group consisted of 19 women and 11 men. The average age of patients in the experimental and control group was 63.8 and 65, respectively, that being a statistically irrelevant difference ($t= 2.00$, $te = 1.77$, $p> 0.05$). To satisfy the important inclusion criterion the patients from both groups had to be operated on by the two surgeons who took part in this study; while the remaining 56 patients were operated on by other surgeons. Indications for autologous blood collecting were all elective surgical procedures where allogeneic blood transfusion was applied in 10% or more cases. The patients were referred to the Department of Transfusiology from the Department of Orthopedic and Traumatology for blood donation.

The authors of this study obtained the approval of Ethical and Scientific Board of General Hospital "Đorđe Joanović" in Zrenjanin.

The choice of patients for autologous blood donation depended on the patient's general condition, age, level of hemoglobin and hematocrit as well as their weight. Autologous blood donation was not done in patients with one of the following conditions: unstable angina pectoris, cardiac decompensation, cyanosis or congenital heart defect, respiratory insufficiency, occlusions or severe defect of central nervous system (CNS), severe hemodynamic problems, active system infection (bacteremia, viremia, sepsis), blood disease and coag-

ulation disorder, disseminated neo process and body weight lower than 40 kg [1,7].

Each patient was explained the favorable effects of autologous blood. The number of autologous blood units for each patient was planned according to the laboratory analysis (Hgb level not lower than 110 g/l and Hct not lower than 0.34%), voluntary blood donors' form, medical examination, as well as the date of the planned surgery. The first blood collecting was planned two or three weeks before the patient was admitted to hospital, and every next collecting was repeated in seven days' time. Prior to each blood donation, the concentration of hemoglobin and hematocrit was checked on hemoglobin meter Hemo Control. If the level of hemoglobin was lower than 135 g/l, Heferol iron capsules were administered orally along with vitamin C tablets [8]. When 450.0 ml of blood is lost, hemoglobin values falls by 10 g/l [1]. If two units of the whole blood are taken from the patient, the initial hemoglobin value falls by 20 g/l which would be approximately 115 g/l before surgery. According to the World Health Organization (WHO) criteria, anemia is defined as hemoglobin concentration lower than 130 g/l or hematocrit lower than 39% in adult males, i.e. hemoglobin concentration lower than 120 g/l or hematocrit lower than 37% in adult females [9,10]. In order not to bring the patient into the anemic condition before surgery, the value of 135 g/l for hemoglobin was taken as the border value when oral iron preparation was introduced.

The minimal period between the last autologous blood donation and surgery was 72 hours, that being the period needed for the organism to recover intravascular volume. Depending on the body weight of the patient, blood was collected in double layered bags of 350.0 ml and 450.0 ml by MacoPharma. Having been collected, each blood unit was centrifuged. Plasma was separated into a transfer bag and frozen at -80 ^{o}C, while deplasmatised erythrocytes were deposed in the refrigerator at +4 ^{o}C. The deplasmatised erythrocytes and the frozen plasma were marked as autologous blood and deposed separately from allogeneic blood and were supposed to be donated only to the donor. The expiry date for deplasmatised erythrocytes is 35 days and for freshly frozen plasma one year. If the freshly frozen plasma was not used during the hospitalization period, it was discharged after the patient was released from the hospital and due to technical reasons was not kept till its expiry date.

Each unit of autologous blood was tested in the serological laboratory. The blood type was determined by Bio-Rad ID-Card DiaClon ABO/D + Reverse Grouping cards at the first donation and by Bio-Rad ID-Card DiaClon ABD-Confirmation for Donors at the second donation. Each donation underwent antibody screening by Bio-Rad ID-Card Liss/Coombs with Test cell reagents for the ID-System-ID-DiaCell Pool cards. Autologous blood units were tested for communicable diseases – the presence of chronic hepatitis C-associated thrombocytopenia (*HCVaT*), Hepatitis B Surface Antigen (*HBsAg*), human immunodeficiency virus (HIVAg/At), TPAt by Biomerieux tests applying the Elisa technique. According to the decision of the hospital transfusion committee, if the test result for the communicable diseases turned out reactive, the blood unit was discharged and was not donated to the patient.

The control group, consisting of patients who did not donate autologous blood and who received only allogeneic blood, also included the patients who had contraindications for autologous blood donation and the patients who were unable to come and donate blood for some other reasons. The hemoglobin level in the control group patients was aprox. 130 g/l.

The patients from both groups underwent total hip replacement surgery. Both cemented and uncemented Zimmer prostheses were used depending on the patient's age. The cemented prostheses were used in patients older than 70 years of age, while the uncemented ones were used in younger patients. All the surgical procedures were done by anterolateral approach.

During the postoperative period, the number of transfused units of blood and blood products (autologous and allogeneic blood), hemoglobin and hematocrit values, value of blood loss via a medical drain, post-transfusion reactions occurrence and the length of hospitalization were observed and recorded.

Nonparametric, χ^2 tests and parametric, Student's t-test were used for the statistical data processing.

Results

Hip replacement surgery was performed in 116 patient at the Department of Orthopedics and Traumatology of the General Hospital "Đorđe Joanović" in Zrenjanin. Out of fifty-four (46.5%) patients who were referred to the Department of Transfusiology, 46 patients donated blood and eight of them were denied autologous donation because they did not satisfy the criteria; thus, 89 autologous blood units were collected, forty-two patients donated two units, each, and 4 patients donated one unit, each. Of 46 patients who had donated blood, three were not operated and their blood was discharged.

The experimental, autologous group consisted of 30 patients who were randomly chosen among 46 patients who had donated autologous blood before surgery. They donated 58 whole blood units in total.

Prior to autologous blood donation, the level of hemoglobin and hematocrit was measured in all the patients from the experimental group. The average value for hemoglobin and hematocrit in the first and the second donation was 148.9 g/l and 44.2%, and 138.7 g/l and 40.8%, respectively.

Table 1. Mean value of hemoglobin (Hgb) and hematocrit (Hct) before autologous blood donation
Tabela 1. *Srednja vrednost hemoglobina (Hgb) i hematokrita (Hct) pre doniranja autologne krvi*

Mean value of parameters/*Srednja vrednost parametara* Blood donation/*Doniranje krvi*	Hgb (g/l)	Hct (%)
First donation/*Prvo doniranje*	148.9	44.2
Second donation/*Drugo doniranje*	138.7	40.8

Table 2. Mean value of haemoglobin (Hgb) and hematocrit (Hct) in pre-operative period in both groups of patients
Tabela 2. *Srednje vrednosti hemoglobina (Hgb) i hematokrita (Hct) u preoperativnom periodu u obema grupama pacijenata*

Mean value of parameters/*Srednje vrednosti parametara* Group/*Grupa*	Hgb (g/l)	Hct (%)
Autologus/*Autologna*	129.8	39.2
Allogeneic/*Alogena*	130.1	44.1

The hemoglobin value was reduced by 10 g/l after each donation, which is to be expected after blood loss of 450 ml (**Table 1**).

Oral iron preparation had been given to 12 patients for two weeks before the first donation and to 6 more patients after the second donation. The patients had been using the iron preparation before they were admitted to hospital. The average value of hemoglobin had been 127.4 g/l in 12 patients before the oral iron preparation was introduced, and after two weeks, i.e. before the first donation it was 136.9 g/l, that being a statistically significant difference ($t= 3.06$, $te = 3.305$, $p< 0.01$).

The level of hemoglobin and hematocrit was measured in both groups of patients before surgery. The average value of hemoglobin and hematocrit for the experimental group was 129.8g/l and 39.2%, and for control group it was 130.1g/l and 44.1%, which implies that both groups of patients had approximately the same values (**Table 2**). The statistical analysis of the hemoglobin values showed that the difference was not statistically significant ($t= 2.00$, $te = 0.26$, $p>0.05$).

The final hemogram showed that the average values of hemoglobin and hematocrit for the experimental and the control group were 101.2 g/l and 30.9%, and 101.5 g/l and 34.7%, respectively, in the post-operative period when there were no transfusions. The data indicate that both groups of patients were transfused approximately to the same values of hemogram. Transfusion of blood and blood products was indicated when hemoglobin values were lower than 90 g/l in both studied groups; however, when these values were higher, transfusion was not considered justifiable (**Table 3**). Student's t test showed that the difference was not statistically significant ($t= 2.00$, $te = 0.43$, $p>0.05$).

In the post-operative period, all units of autologous deplasmatised erythrocytes (58 units) were transfused to the patients who had donated them. Twenty patients (66.7%) from this group received only their own pre-operatively donated blood, and 10 patients (33.3%) had to receive allogeneic blood in addition to their own. Eighteen units of deplasmatised allogeneic erythrocytes were transfused in total, that being 1.8 allogeneic blood units per

Table 3. Mean value of haemoglobin (Hgb) and hematocrit (Hct) in post-operative period in both groups of patients
Tabela 3. *Srednja vrednost hemoglobina (Hgb) i hematokrita (Hct) u postoperativnom periodu u obema grupama pacijenata*

Mean value of parameters/*Srednja vrednost parametara* Group/*Grupa*	Hgb (g/l)	Hct (%)
Autologus/*Autologna*	101.2	30.9
Allogeneic/*Alogena*	101.5	34.7

Table 4. The number of transfused units of deplasmatised erythrocytes in both groups of patients
Tabela 4. *Broj transfundovanih jedinica deplazmatisanih eritrocita u obema grupama pacijenata*

Group/*Grupa* Deplasmatised erythrocytes/*Deplazmatisani eritrociti*	Autologus *Autologna*	Allogeneic *Alogena*
Autologus erythrocytes/*Autologni eritrociti*	58 (1.93)	0
Allogeneic erythrocytes/*Alogeni eritrociti*	18 (0.6)	112 (3.73)
Total/*Ukupno*	76 (2.53)	112 (3.73)

patient. The patients from the experimental group were transfused 76 units of deplasmatised erythrocytes in total, i.e. 2.53 units per patient.

In the post-operative period, 30 patients from the control group were transfused 112 units of allogeneic deplasmatised erythrocytes, i.e. 3.73 units per patient (**Table 4**).

Statistic data processing showed that there was a significant difference between the experimental and control group regarding the number of transfused blood units ($p<0.005$).

The average value of blood loss via a drain was 602.1 ml and 639.6 ml in the experimental and the control group, respectively, that being statistically insignificant ($t= 2.02$, $te = 0.03$, $p>0.05$).

A post-transfusion reaction occurred only in one patient (3.3%) from the control group, while there were no reactions to donated blood in the experimental group.

The average length of hospitalization in the experimental group was 20.3 days, and in the control group it was 21.5 days, that being statistically significant ($t= 2.00$, $te = 2.45$, $p< 0.05$).

Discussion

Osteoarthritis is a widely spread, chronic process impossible to be cured completely. Practically, any elderly man has medical problems associated with degenerative changes in joints.[11]

According to the frequency of occurrence, osteoarthritis takes the fourth place among diseases in the contemporary world; right after cardiovascular, cerebrovascular and lung diseases [12].

The main symptom of arthritis is the pain that is increased with age and physical strain, which is the reason why the patients demand long-lasting medical and physical therapy [11. The main aim when treating arthritis is to decrease the pain, i.e. to meliorate the quality of life and working ability of the patient [13]. In cases of exceptionally advanced forms of disease, when there are prominent destructive changes in bone-joint system, a surgical treatment is needed. The chosen surgery in these cases is aloarthroplasty, i.e. the prosthetic replacement of the affected joint [14]. Surgeries on larger joints, such as the hip joint, are accompanied by numerous problems and potential complications [13]. One of the most difficult complications is the acute loss of blood in the perioperative period, when it is essential to compensate blood loss timely and adequately. In this study we reported the findings gathered while monitoring blood loss via a drain in the postoperative period. Blood loss was found to be larger in the control group of patients than in the experimental group. However, when statistically processed, these data appear insignificant. In order to determine the exact blood loss, it is necessary to measure the intra-operative loss as well as the coagulation parameter values (partial thromboplastin time, i.e. PT, activated partial thromboplastin time, i.e. aPTT) in addition to the postoperative loss. We were unable to analyze all these parameters while doing this study, thus we failed to obtain these relevant data. Having analyzed autologous blood use in the revision hip replacement surgeries, Award et al. explained a smaller postoperative blood loss by the stimulation of hematopoiesis in the group of patients who were transfused autologous blood because preoperative autologous blood transfusion was combined with preoperative autologous blood donation in their study. They came to the conclusion that autologous blood transfusion completely eliminates the need for allogeneic blood transfusion; however, they also performed perioperative blood salvation in addition to preoperative blood donation in their research [15]. Lisander et al. found out that the administration of autologous blood diminishes the usage of allogeneic blood by 35-40% during the perioperative period in total hip replacement surgery [16].

According to the results of our study, autologous blood transfusion is an efficient method that reduces allogeneic blood usage significantly in our circumstances as well. Our choice was preoperative autologous blood collection because its application does not require any additional financial investment. It is considered that intraoperative and postoperative autologous blood collection is not financially justified in primary prosthesis surgery, it is justifiable only in revision prosthesis surgery [17,18]. In our study, we found that 66.7% of the patients who had donated two blood units before surgery did not need allogeneic blood after their own blood was reinfused. The remaining 33.3% of patients received 18 units of allogeneic erythrocytes, i.e. 1.8 units per patient in addition to their own blood. The patients from the experimental group received 2.53 units of deplazmatized erythrocytes, on average. The results of our study show that the amount of transfused deplazmatizes erythrocytes units was reduced due preoperative autologous blood donation.

Nowadays, various, more complex surgical operations are performed with the resulting higher demands for allogeneic blood; however, this increased demand is not followed by the increased number of voluntary blood donors which results in ever more frequent short supplies of allogeneic blood. The only logical solution is to opt for alternatives in order to preserve allogeneic blood supply. Autologous transfusion (predeposit autologous blood, acute normovolaemic hemodilution, intraoperative and postoperative blood salvation) is an economically justifiable and safe alternative. It neither requires pretransfusion tests nor does it cause immunization to alien antigens; it is indicated in patients with rare blood groups and in those with multiple alloantibodies; and it can be performed even in some sects, such as the Jehovah's Witnesses. Autologous transfusion enhances microcirculation, postoperative tissue perfusion and lowers the risk of thromboembolism [5]. We have neither had patients with rare blood diseases in our

hospital, nor have we had patients with multiple alloantibodies for whom predeposit autologous transfusion is the only option in chemotherapy.

While performing preoperative blood donation there is a risk of postponing the surgery in some patients due to various reasons, and in such cases the units of deplazmatized erythrocytes have to be disposed of due to expiry date. In order to avoid this unwanted situation, we collected two blood units from each of our patients, because the period between the first donation and surgery was shorter (approximately two weeks), thus making the period when the blood could be used longer, in case we had to postpone surgery. The application of such a protocol made it possible to postpone surgery for the period of two weeks maximum, i.e. until the expiry date of autologous erythrocytes in case the patient did not undergo surgery as planned. Billote and his associates have concluded that financial loses are extremely high if all collected autologous blood is not transfused. They believe that it is unjustified to collect autologous blood unless the patient is anemic [17].

In the last decades, the need for diagnosis and treatment of preoperative anemia is emphasized in order to make the patients well prepared for possible complications which may be caused by surgery. The recommendations are given in the NATA (Network for the Advancement of Transfusion Alternatives) guidelines [10,19,20]. When doing his research, Goodnough concluded that 35% of patients undergoing elective orthopedic surgeries had hemoglobin values less than 130 g/l. Most of the patients were women and the most common cause of anemia was iron deficiency [20]. Having considered all the previous results, we opted for the administration of oral preparation of iron, combined with vitamin C in all patients who, prior to autologous blood donation, had hemoglobin values less than 135 g/l. After two weeks' administration of this preparation, the hemoglobin values increased, which resulted in the same or even higher hemoglobin values than those prior to the donation of two blood units. In this way, we managed to make the hemoglobin values in the experimental and control group even.

According to the recommendations given in the national guides, the recommended value of hemoglobin and hematocrit is $\leq$70 g/l and $\leq$21%, respectively, when allogeneic blood is to be transfused. This standing point supports the restrictive approach to allogeneic blood transfusion [21]. In our study, we transfused blood when hemoglobin values were $\leq$90 g/l because the patients were elderly people with chronic diseases. Therefore, the hemoglobin values determined in the last hemogram were approximately the same for both the experimental and control group.

Based on the obtained results, we have concluded that preoperative hemoglobin correction by administering oral iron preparations along with preoperative autologous blood donation can significantly reduce the need for allogeneic blood transfusion.

Our Department does not have protocols or exact recommendations for how long the patient should stay in hospital after certain surgical interventions when the postoperative course is uneventful. In cases of hip replacement surgery, the patients stay in hospital for two weeks at least, until the sutures are removed. Since most patients are referred to a stationary rehabilitation institution for physical treatment immediately after the hospitalization, they frequently stay in hospital for a longer period of time until a place in a rehabilitation institution becomes vacant. Therefore, the data on the length of hospitalization in our study cannot be considered relevant in spite of the statistically significant difference, because they are not the result of the quicker recovery but depend on external factors, which cannot be influenced.

Conclusion

Transfusion of allogeneic blood and blood products is a part of everyday clinical practice, particularly at surgical departments for treating severe blood losses during surgical procedures. Although it has become a routine procedure, it must be done with utter caution not only in order to avoid technical and administrative errors, but also because of constant threat of problems that may occur during and after any transfusion. Blood testing is nowadays done in transfusion services at a much higher level, thus making the process of blood and blood products transfusion much safer. Yet, transfusion related complications may still occur. The only real alternative to allogeneic blood transfusion is autologous donation. Considering the fact that the blood donor and the receiver is actually the same person, the possible complications related to allogeneic blood transfusion are eliminated.

According to the above mentioned results, we have concluded that the administration of pre-operatively donated autologous blood reduces the number of transfused deplasmatised erythrocytes units when compared to the number of units transfused to the patients receiving only allogeneic blood products. Pre-operative administration of oral iron preparations significantly increases the hemoglobin values, thus enabling us to take two blood units from the patient before surgery without causing anemia.

Autologous blood collection should be an integral part of the National Program for Blood Donation of every country, as recommended by the World Health Organization and the International Society of Blood Transfusion. According to the Law on Blood, Blood Donation and Blood Transfusion passed on in 2010, each doctor is obliged to inform the patient about the possibility of autologous donation according to the criteria for autologous donation if planning a surgery which usually requires blood or blood products transfusion. In our

hospital all the patients who are about to undergo hip replacement surgery are informed about autologous donation by their orthopedist and referred to the Department of Transfusiology for further medical examination. In General Hospital "Đorđe Joanović" in Zrenjanin, predeposit of autologous donation has become a standard procedure in patients who undergo hip replacement surgery.

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PRIKAZI SLUČAJEVA
CASE REPORTS

University of Novi Sad, Faculty of Medicine
Institute for Child and Youth Health Care of Vojvodina
Department of Pediatrics

Prikaz slučaja
Case report
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PIEBALDISM IN A 3-MONTH-OLD INFANT – CASE REPORT

PIEBALDIZAM TROMESEČNOG ODOJČETA – PRIKAZ SLUČAJA

Olgica MILANKOV, Radojica SAVIĆ and Anica RADULOVIĆ

Summary
Introduction. Piebaldism is an autosomal dominant disorder characterized by the congenital absence of melanocytes in the affected areas of skin and hair due to mutations of the KIT proto-oncogene, which affects the differentiation and migration of melanoblasts. **Case report.** A 3 ½ month old male infant was admitted to hospital due to depigmentation of skin in the area of forehead, trunk and extremities. On admission, he had multiple, irregularly shaped areas of leucoderma present at the forehead, abdomen, lower legs and left forearm. Based on the characteristic skin features and family history, we diagnosed the boy's leucoderma as piebaldism. **Conclusion.** Vitiligo differs from piebaldism by the presence of unstable hypopigmented lesions that are acquired later in life. Albinism presents with widespread skin involvement and lacks the characteristic hyperpigmented macules within hypopigmented areas.
Key words: Piebaldism; Infant; Genes, Dominant; Hypopigmentation; Diagnosis

Sažetak
Uvod. Piebaldizam je autozomno dominantni poremećaj za koji je karakteristično urođeno odsustvo melanocita u pogođenim područjima kože i kose, zbog mutacija u KIT proto-onkogenu, što utiče na diferencijaciju i migraciju melanoblasta. **Prikaz slučaja.** Muško odojče uzrasta tri i po meseca, primljeno je zbog depigmentacije kože u oblasti čela, trupa i ekstremiteta. Na prijemu u bolnicu imalo je višestruka depigmentovana područja, nepravilnog oblika, prisutna na čelu, trbuhu, potkolenicama i levoj podlaktici. Na osnovu karakterističnog stanja kože i porodične istorije, dijagnostikovan je piebaldizam. **Zaključak**. Vitiligo se razlikuje od piebaldizma po prisustvu nestabilnih hipopigmentisanih lezija koje su stečene kasnije u životu. Albinizam karakteriše široka zahvaćenost kože i nema karakterističnih hiperpigmentisanih makula u hipopigmentisanim oblastima.
Ključne reči: Piebaldizam; Odojče; Dominantni geni; Hipopigmentacija; Dijagnoza

Introduction

Piebaldism is an autosomal dominant disorder that presents with congenital poliosis and leukoderma, often with hyperpigmented macules noted on both depigmented patches and normal skin. It is characterized by the congenital absence of melanocytes in the affected areas of skin and hair, due to mutations of the KIT proto-oncogene, which affects the differentiation and migration of melanoblasts [1]. Piebaldism manifests as a white forelock and patches of depigmentation.

This condition differs from vitiligo by its presence from birth, the static nature, and hyperpigmented macules within lesions of piebaldism and on the normal skin, regardless of the specific KIT mutation. It is usually a benign isolated skin condition. However, it is permanent, it may be socially disabling, and the treatment is a challenge.

Waardenburg syndrome, Ziprkowski-Margolis syndrome and Woolf syndrome present with piebaldism and deafness [2]. Piebaldism may be associated with other disorders such as Hirschsprung disease, neurofibromatosis type I, congenital dyserythropoietic anemia type II, Diamond-Blackfan anemia, Grover disease, or transient acantholytic disease [3].

Case report

A 3 ½ month-old male infant was admitted to our Department due to depigmentation of skin in the area of forehead, trunk and extremities. These depigmented areas had been present since birth, and remained unchanged until admission. His mother, brother, uncle and grandfather also had similar depigmentation, which had been present since birth and were stable.

On admission to hospital he had multiple, irregularly shaped areas of leucoderma present at the forehead, abdomen, lower legs and left forearm (**figures 1 and 2**). The areas of normal skin were observed inside the depigmented fields. No

Corresponding Author: Prof. dr Olgica Milankov, Institut za zdravstvenu zaštitu dece i omladine, 21000 Novi Sad, Hajduk Veljkova 10, E-mail: milankom@eunet.rs

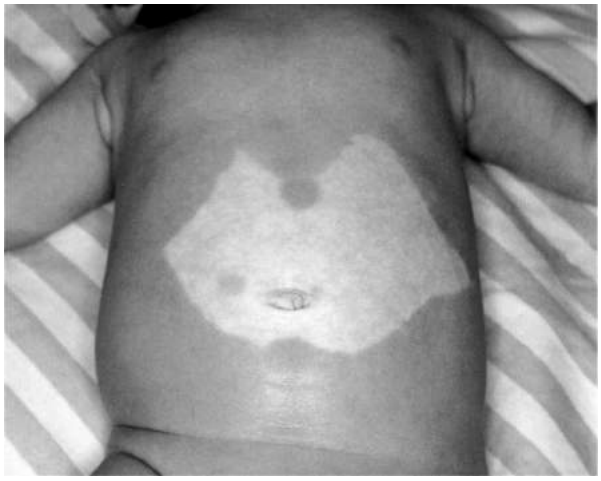

Figure 1. Piebaldism-abdomen
Slika 1. *Piebaldizam - trbuh*

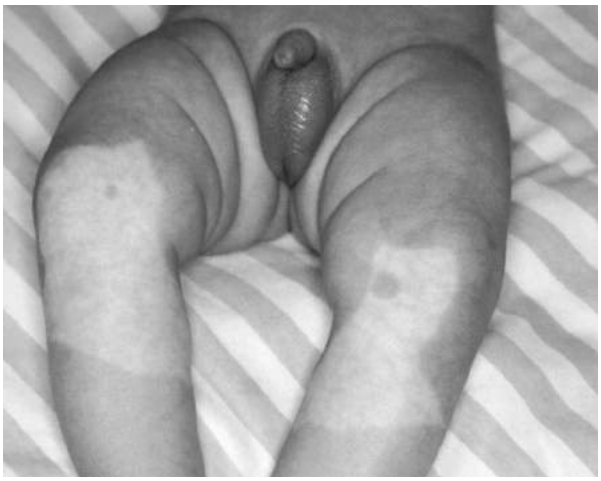

Figure 2. Piebaldism - lower legs
Slika 2. *Piebaldizam - potkolenice*

hyperpigmentation of skin on the edges of depigmented skin were noted.

Based on the characteristic skin features and family history, we diagnosed the boy's leucoderma as piebaldism. Other diagnostic procedures revealed no associated anomalies.

Discussion

Leucoderma in piebaldism is a permanent condition; however, the regression of leucoderma has been described [4]. There are many mutations of the KIT gene reported and the severity of phenotypic expression in piebaldism correlates with the site of the mutation within the gene [4,5]. Although it is an isolated skin condition, it can be socially disabling, and its treatment is a challenge. Topical treatment with make up or artificial pigmenting agents are useful, although temporary [6]. Sunscreens should be recommended to avoid sunburns and to reduce the carcinogenic potential. Several surgical techniques are available for the treatment of stable leucoderma. The use of noncultured epidermal cellular grafting was introduced in 1992 [7]. The patient's satisfaction with treatment in long-term studies was described as 7-9/10 and it improved the quality of patients' life [8] Transplant of autologous melanocytes obtained through the culture of melanocytes or of melanocytes and keratinocytes has been described as a safe and effective treatment for patients with piebaldism. This induced scarless repigmentation using a small donor site [9].

Conclusion

Some other conditions are important in differential diagnosis of piebaldism. Vitiligo is distinguished from piebaldism by the presence of unstable hypopigmented lesions that are acquired later in life. Albinism presents with widespread skin involvement and lacks the characteristic hyperpigmented macules within hypopigmented areas.

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Clinical Center of Serbia, Belgrade, Department of Gynecology and Obstetrics[1]
Clinical Hospital Center Zemun, Department of Internal Medicine, Belgrade[2]
Clinical Hospital Center Priština, Gračanica, Department of Gynecology and Obstetrics[3]
University in Priština, Faculty of Medicine, Kosovska Mitrovica[4]

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OUR EXPERIENCE WITH INTRAOPERATIVE CELL SALVAGE DURING CESAREAN DELIVERY IN WOMEN WITH UTERINE MYOMAS – FOUR CASE REPORTS

NAŠA ISKUSTVA SA INTRAOPERATIVNIM SPASAVANJEM KRVI TOKOM CARSKOG REZA KOD PACIJENTKINJA SA MIOMIMA UTERUSA − PRIKAZ ČETIRI SLUČAJA

Radmila SPARIĆ[1], Biljana LAZOVIĆ[2], Nenad ŠULOVIĆ[3,4] and Snežana BUZADŽIĆ[1]

Summary

Introduction. Cesarean section is more frequent in pregnant women with uterine myomas, and is usually complicated with perioperative hemorrhage. In some cases, cesarean myomectomy represents an inevitable surgery, adding risk of hemorrhage occurrence. Massive obstetric hemorrhage is the most common cause of maternal mortality and morbidity. The aim of this study was to show our experience and results of the implementation of intraoperative blood salvage during cesarean section in the patients with uterine myomas. **Material and Methods.** The study encompassed four patients with uterine myomas who had cesarean delivery at our Department in the period from 2010 to 2011. **Results.** Postoperative transfusion of packed red blood cells was given to one patient. No complications resulting form the intraoperative blood salvage were recorded in our research. **Conclusion.** Intraoperative blood salvage should be applied in patients with uterine myoma, and certainly in those who are planned for cesarean myomectomy and particularly in cases when massive intraoperative hemorrhage is expected.

Key words: Cesarean Section; Myoma; Pregnancy; Uterine Myomectomy; Intraoperative Complications; Hemorrhage; Operative Blood Salvage

Sažetak

Uvod. Carski rez se češće izvodi kod trudnica sa miomima uterusa, a neretko može biti komplikovan i perioperativnim krvarenjem. U nekim slučajevima, miomektomija tokom carskog reza predstavlja neizbežnu operaciju, koja dodatno povećava rizik od pojave krvarenja. Masivno krvarenje u akušerstvu je najčešći razlog maternalnog mortaliteta i morbiditeta. Cilj ove studije je da se prikažu naša iskustva i rezultati primene aparata za intraoperativno spasavanje krvi tokom carskog reza kod pacijentkinja sa miomima uterusa. **Materijal i metode.** Studijom su obuhvaćene četiri pacijentkinje sa miomima uterusa porođene carskim rezom u našoj ustanovi u periodu od 2010. do 2011. godine. Kod dve pacijentkinje tokom carskog reza urađena je i miomektomija. **Rezultati.** Postoperativna transfuzija koncentrovanih eritrocita data je jednoj pacijentkinji. Komplikacije primene intraoperativnog spasavanja krvi nisu registrovane u našem istraživanju. **Zaključak.** Primenu intraoperativnog spasavanja krvi treba razmotriti kod pacijentkinja sa miomima uterusa, a svakako kod onih kod kojih se planira miomektomija tokom carskog reza, naročito u slučajevima kada se očekuje obilno intraoperativno krvarenje.

Ključne reči: Carski rez; Miomi; Trudnoća; Miomektomija; Intraoperativne komplikacije; Krvarenje; Intraoperativno spasavanje krvi

Introduction

Myomas are the most common benign tumor of the genital organs of women of childbearing age. Cesarean section (CS) is more common in women with myomas than in those without them, their incidence being up to 49.1% [1]. The incidence of cesarean section is increased by 26% for every 10 mm increase in the diameter of myoma in the group of women with myomas of 50 mm or more [2]. In some cases, myomectomy during CS is inevitable, which further increases the risk of hemorrhage [1]. Peripartum hemorrhage occurs in 2.5% of women with uterine myomas [3]. Massive obstetric hemorrhage, defined as blood loss greater than 1.5 liter, a reduction in hemoglobin levels greater than 40 g/l, the need for transfusion of more than 4 units of packed red blood cells (RBC) or the occurrence of coagulopathy is the most common cause of maternal mortality and morbidity [4, 5].

The idea of autologous transfusion in cases of obstetric hemorrhage dates back to the nineteenth century, when James Blundell documented the first application of autologous blood in the treatment of postpartum hemorrhage in 1818. The first prototype of cell saver was constructed by Taswell and Wilson, and was used in the Mayo Clinic in 1968 [6, 7].

Intraoperative blood salvage was developed in the 1970s and has been applied in orthopedic, cardiovascular and neurosurgery ever since [8]. The theoretical risks of the amniotic fluid embolism and Rh alloimmunization of the mother slowed down

Corresponding Author: Dr Radmila Sparić, Klinika za ginekologiju i akušerstvo, 11000 Beograd, Višegradska 26, E-mail: radmila@rcub.bg.ac.rs

Abbreviations
CS – cesarean section
No – number
RBC – red blood cells

the application of this method in obstetrics [3, 9]. The first case of cell salvage in obstetrics was published in 1988 and a large number of studies on its use in obstetrics were published during the last decade [10, 11], which resulted from the technological progress in this field and the introduction of leukocyte depletion filter, which was applied in obstetric indications for the first time in 1999, as well as from better understanding of the pathophysiology of amniotic fluid embolism, the risks of administration of allogeneic blood, and increasing complexity of obstetric pathology [5, 11].

The process of intraoperative blood salvage consists of three phases: blood collection (aspiration of blood from the surgical field), blood processing (centrifugation and washing with heparinized saline solution) and reinfusion. The aspiration of blood from the operative field is performed by a dual lumen anticoagulation suction tube. The aspirate passes through the filter and is collected in the reservoir. From the reservoir, blood is pumped into the centrifugation bowl, where it undergoes centrifugation and washing. Following these processes, the supernatant is separated in the waste bag and the washed red cells are collected in the transfer bag. The erythrocytes obtained in this way can be re-infused immediately or within next 6 hours [9, 10].

The aim of this study was to present our experiences and results of applying intraoperative blood salvage during CS in patients with uterine myoma. "*Dideco Electa Essential Concept"* Cell Saver has been used at the Department for Gynecology and Obstetrics, Clinical Center of Serbia since 2009 and is handled by specially trained personnel (**Figure 1**).

Figure 1. Dideco Electa Essential Concept Cell Saver
Slika 1. *Dideco Electa Essential koncept spasavanja krvi*

Material and Methods

1. This retrospective study included patients with intraoperatively confirmed presence of uterine myoma who were hospitalized and delivered by CS at our Department from January 2010 to December 2011.

2. The sources of data were: the operating protocols from the operating room, patients' files, data protocols and histopathology findings of myomas. The criterion for inclusion in the study was the presence of myomas confirmed by visual inspection of the uterus during CS and the application of intraoperative cell salvage device, i.e. cell saver, during CS.

3. The indications for CS were defined on the basis of the primary indication for operation. The duration of operation was assessed on the basis of anesthetic list in the patient's file in minutes from the skin incision to the skin closure. Experience of the surgeon was evaluated on the basis of his years of practice in gynecology and obstetrics.

4. The type, localization and number of myomas was determined based on the operative notes in the patient's file. The size of myoma was defined by the diameter of the largest myoma according to the measurements made by the pathologist on the enucleated myoma, ultrasound measurement before surgery or evaluation given by the surgeon in the operative note. In cases of multiple fibroids, the diameter of the largest fibroid was taken into account. Intraoperative hemorrhage was assessed on the basis of the findings from the operative notes.

5. The hemogram parameters before and after surgery were defined by the values of red blood cells, hemoglobin and hematocrit before operation and on the first postoperative day. Blood transfusion during and after operation was defined as heterologous transfusion and/or autologous trans-

Table 1. Patient characteristics
Tabela 1. *Karakteristike pacijentkinja*

Characteristics/*Karakteristike*	1	2	3	4
Age/*Godine starosti*	33	30	46	39
Parity/*Porođaj po redu*	1	1	1	3
Gestational week/*Nedelja gestacije*	40	39	38	39
Previous myomectomy *Prethodna miomektomija*	No *Ne*	No *Ne*	No *Ne*	Yes *Da*
Indications for cesarean section *Indikacije za carski rez*	Myoma previum *Miom previja*	Fetal indications *Fetalne indikacije*	Fetal indications *Fetalne indikacije*	Previous cesarean section *Prethodni carski rez*
Type of cesarean section *Vrsta carskog reza*	Elective *Elektivan*	Emergency *Hitan*	Elective *Elektivan*	Elective *Elektivan*
No of laparotomy/*Laparotomija po redu*	1	1	1	4
Type of myoma *Tip mioma*	Intramural *Intramuralni*	Pedunclated *Na peteljci*	Multiple *Multipli*	Multiple *Multipli*
Localization of myoma *Lokalizacija mioma*	Posterior wall *Zadnji zid*	Anterior wall *Prednji zid*	Anterior wall *Prednji zid*	Anterior wall *Prednji zid*
Size of myoma/*Dimenzije mioma (mm)*	150	110	45	90
Number of myoma/*Broj mioma*	1	1	2	2
Myomectomy/*Miomektomija*	No/*Ne*	No/*Ne*	Yes/*Da*	Yes/*Da*
Number of incisions on uterus *Broj incizija na uterusu*	1	1	2	2
Duration of surgery (min) *Trajanje operacije (min)*	65	65	105	60
Bacteriological smear of uterus *Mikrobiološki bris uterusa*	Sterile *Sterilan*	*Escherichia coli*	Sterile *Sterilan*	Sterile *Sterilan*
Neonatal weight (gr) *Telesna masa novorođenčeta (gr)*	3 350	3 600	3 200	3 150
Years of specialist service of the surgeon *Godine specijalističkog staža operatora*	27	19	19	27

fusion when the cell saver was applied for intraoperative blood salvage.

6. Febrile morbidity was defined as measured body temperature ≥38 ºC in two consecutive measurements ≥ 6 hours, with the exception of postoperative day zero [3, 9]. Positive culture of the lochia, wound infections, and urinary tract infection after operation were determined according to the microbiological findings of wound swabs, urine and lochia samples. The existence of a hematoma in the uterus and abdominal wall hematoma after operation was determined by visualizing the hematoma during the postoperative ultrasound examination. Uterine subinvolution was defined as the need for providing therapy with uterotonics after the third postoperative day. Postoperative anemia was defined as hemoglobin values at discharge ≤100 g/l [1].

7. The duration of treatment in the intensive care unit and the total duration of postoperative hospitalization were defined by the number of days of treatment in the intensive care unit, or the number of postoperative days from the operation to the discharge from hospital, while the day of operation was taken as day zero.

Results

The patients are presented as the patient number (No) 1, number 2, number 3 and number 4. None of the patients had any comorbidity such as diabetes and pregnancy induced hypertension. Fetus was in cephalic presentation in all four patients. **Table 1** shows the characteristics of patients.

The patients No 1, 2 and 4 were 30-39 years old, while the patient No 3 was 46 years old, and her pregnancy was achieved by in vitro fertilization with an oocyte donation. All patients were delivered by CS in term pregnancy. The patient No 4 was tertipara who had two previous CS and myomectomy before the index pregnancy. The other three patients were nulliparous, without previous surgery on the uterus. The patient No 2 was delivered by emergency CS, while others were delivered by elective CS. Myomectomy was not performed in two women: the patient No 1 had an intramural myoma on the posterior wall of the uterus with a diameter of 150 mm and the patient No 2 who had a pedunculated anterior wall myoma 110 mm in diameter. The operation in both patients lasted 65 minutes. Cesarean myomectomy was performed in two women with multiple fibroids, the largest ones

Table 2. Complications registered in studied patients
Tabela 2. *Registrovane komplikacije kod ispitivanih pacijentkinja*

Complication/*Komplikacija*	1	2	3	4
Number of days of intensive care unit treatment/*Broj dana lečenja u jedinici intenzivne nege*	0	0	7	1
Number of postoperative days in hospital/*Broj dana postoperativnog bolničkog lečenja*	4	4	11	11
Postoperative transfusion (ml)/*Postoperativna transfuzija (ml)*	0	0	0	260
Febrile morbidity after operation/*Febrilno stanje posle operacije*	–	–	–	+
Hemoglobin<100 g/l at discharge/*Hemoglobin<100 g/l pri otpustu*	+	+	+	–
Uterine sub-involution/*Subinvolucija uterusa*	+	–	–	–
Intestinal sub-occlusion/*Crevna subokluzija*	–	–	+	–

being on the anterior uterine wall of the uterus, measuring 45 mm in diameter in one woman and 90 mm in diameter in another. Additional incision on the uterus was therefore required in both cases. Operations on the patient No 3 and No 4 took 105 minutes and 90 minutes, respectively. Microbiological analysis of intraoperative swab of the uterus in the patient No 3 showed the presence of *Escherichia coli*, while in other three patients the finding was sterile. Body weight of all four infants was below 4000 grams. The length of work experience of the surgeon who operated the patient No 1 and No 4 was 27 years and of the surgeon who operated the patient No 2 and No 3 was 19 years. Intraoperative hemorrhage was present in all patients, all patients were given oxytocin intravenously in doses of 10 to 20 IU, and the patient No 2 was given prostaglandin F2α during operation. No injury of the fetus, digestive and urinary organs occurred during operation.

None of the patients had wound infection and/or dehiscence, urinary tract infection, postoperative hemorrhage, hematoma in the uterus and/or in the abdominal wall and bacteremia, nor was it necessary to enlarge the scope of surgery and perform relaparotomy. Data on the postoperative course and reported complications are shown in **Table 2**.

The patients who did not undergo cesarean myomectomy postoperatively were not treated in the intensive care unit; they did not require postoperative transfusion and were discharged on the fourth postoperative day. The patient No 1 had uterine subinvolution. Antibiotic treatment was corrected postoperatively according to the antibiogram in the patient No 2 because of *Escherichia coli* found in the uterine microbiological swab. The postoperative course of the patient No 3 was complicated by intestinal sub-occlusion; she was, therefore, treated in the obstetric intensive care unit for 7 days and her postoperative hospital treatment lasted 11 days in total. The patient No 4 was treated for one day in the intensive care unit. This patient was febrile postoperatively (two days after the application of allogeneic RBC transfusion), and her postoperative hospital treatment lasted 11 days in total. Administration of allogeneic blood was avoided in three patients. All the women studied, except for the patient No 4, who had received RBC after operation, had hemoglobin levels below 100 g/l at discharge.

Hemogram values before and after operation, as well as the volume of intraoperatively salvaged and transfused blood are shown in **Table 3**.

All hemogram parameters were reduced postoperatively in all patients, and the volume of autologous blood transfused was 400-700 ml. The mean preoperative and postoperative hemoglobin and erythrocyte values were 103.80 g/l and 4.00×10^9, and 94.38 g/l and 3.37×10^9, respectively. There was no statistically significant difference between these values (for hemoglobin levels p=0.059; for erythrocyte p=0.132; in both cases, p> 0.05). The lowest postoperative hemogram values were recorded in the patient No 4, and the decrease in hemoglobin levels in this patient was the highest (15.71 g/l), although she received the greatest amount of salvaged blood intraoperatively.

Table 3. Hematological results and volumes of intraoperatively salvaged blood in studied patients
Tabela 3. *Nalazi hemograma i zapremine intraoperativno spašene krvi kod ispitivanih pacijentkinja*

Hematological results and volumes of intraoperatively salvaged blood in studied patients/*Nalazi hemograma i zapremine intraoperativno spasene krvi*	1	2	3	4
Hemoglobin before operation (g/l)/*Hemoglobin pre operacije (g/l)*	104	110	105	96.21
Hemoglobin after operation (g/l)/*Hemoglobin posle operacije (g/l)*	101	96	100	80.5
Erytrocites before operation ($\times10^9$)/*Eritrociti pre operacije ($\times10^9$)*	3.99	3.71	3.91	4.39
Erythrocytes after operation ($\times10^9$)/*Eritrociti posle operacije ($\times10^9$)*	3.55	3.31	3.76	2.86
Hematocrite before operation (%)/*Hematokrit pre operacije (%)*	32.9	34.0	31.6	34.0
Hematocrite after operation (%)/*Hematokrit posle operacije (%)*	30.0	29.2	30.7	29.2
Volume of intraoperatively salvaged and transfused blood (ml) *Zapremina intraoperativno spasene i transfuzijom date krvi (ml)*	670	400	450	700

Discussion

The decision regarding transfusion in young women is complex and should depend on the cause of anemia, its extent and possible chronicity, compensatory abilities of the patient, cardiopulmonary condition and the risk of further blood loss. Potential risks of blood transfusion must be balanced in relation to the expected benefits of its use. Although most of the existing recommendations for administration of blood transfusions are defined on the basis of hemoglobin and hematocrit values, the approach should be individualized, and clinical assessment should be based on the general condition of the patient [12]. However, the decision about transfusion is based on laboratory values of hemoglobin and hematocrit in most cases [13].

The therapeutic goal of transfusion is to improve tissue oxygenation in accordance with the needs of the recipient. Bearing in mind the risks of allogeneic blood use, modern medicine provides three ways to avoid its administration: preoperative autologous donation, acute normovolemic hemodilution and intraoperative blood salvage [5, 12]. Despite many advantages of preoperative autologous donation, it is not the method of choice in obstetric practice for the following reasons: in most cases, the extent of obstetric hemorrhage significantly exceeds the amount of blood that can be taken preoperatively; it can not be used in emergency situations, which are relatively common in obstetrics and it is unacceptable in certain populations for religious reasons [13]. The application of this method in obstetrics is recommended only in exceptional cases such as rare blood groups and the presence of irregular antibodies in pregnant women with a high risk of peripartum hemorrhage, multiple pregnancies, repeat CS (three or more), CS in second stage of labor, low preoperative hemoglobin values, uterine myoma and thrombocytopenia [12, 13]. The relative contraindications for intraoperative blood salvage are the contaminated and septic operative fields, suspected malignancy, β thalassemia, sickle cell anemia and the presence of infectious diseases that are transmitted through the blood [13]. According to the recommendations of the British Committee for Standards in Hematology, the application of the cell saver is indicated in operations with the clear operating field and the expected blood loss of more than 20% of blood volume, in operations that are associated with allogeneic transfusions in more than 10% of cases or where the average number of transfused blood units is greater than one [14]. The recommendation of the American College of Obstetricians and Gynecologists is that intraoperative blood salvage should be used in all patients when profuse bleeding is expected [15]. The Association of Anesthetists of Great Britain and Ireland recommends intraoperative blood salvage during CS in women with large and/or multiple uterine fibroids [12, 13].

The patients presented in this study had other risk factors for perioperative hemorrhage, besides myoma, and the consequent need for blood transfusions, such as age and myomectomy during CS in the patient No 3 and No 4, *Escherichia coli* detected in the uterus in the patient No 2 and prolonged surgery in the patient No 3. Namely, the patient No 4 had had three surgical procedures on the uterus (myomectomy and two CSs) before the index operation as well as the lowest preoperative and postoperative values of hemogram. Thus, despite intraoperative blood salvage, she received a single unit of RBC postoperatively. In the files of this patient neither were the indications for postoperative transfusion precisely defined, nor was the existence of possible clinical symptoms of anemia recorded. This is the only patient who had hemoglobin levels higher than 100 g/l at discharge, and we can not exclude the possibility that she would have recovered successfully without that single unit of RBC she had received. The justification of this transfusion cannot be ascertained, which is not a rare event in obstetric patients. The other three patients had hemoglobin levels below 100 g/l at discharge, but none of them had the value less than 90 g/l.

Uterine subinvolution in the patient No 1 was due to the presence of fibroids. Apart from anemia at discharge, the patient No 2 had no other postoperative complications reported. Intestinal sub-occlusion recorded in the patient No 3 cannot be correlated with intraoperative blood salvage. Fever observed in the patient No 4 could be a consequence of allogeneic transfusion as well as of myomectomy. The fact that fever developed in this patient after she had received the allogeneic transfusion suggests that it could not have been caused by the intraoperative blood retransfusion. Intraoperative blood salvage does not completely exclude the need for the administration of allogeneic blood in cases of profuse intraoperative bleeding.

The efficacy of intraoperative blood salvage defined as the percentage of patients who completely avoided the use of allogeneic blood ranges from 6 to 97.1% in the literature [15]. In our study, the use of allogeneic blood was avoided in three cases. Most authors agree that the use of intraoperative blood salvage reduces the need for allogeneic blood transfusions [16]. Prolonged hospitalization in the patient No 3 and No 4 was not the result of the use of cell salvage.

Complications caused by the use of intraoperative blood salvage described in the literature were not reported in any of the studied patients [12, 13, 16, 17].

Cell savers are unable to distinguish maternal from fetal red blood cells; therefore, Rh alloimmunization of the mother is a real risk of the application of this procedure. Aspiration of fetal red blood cells and their retransfusion in the maternal circulation increases the risk of maternal Rhesus alloimmunization in cases of incompatibility between the mother and the fetus. This can be pre-

vented by the anti-D immunoglobulin. The required dose is calculated by determining the volume of fetal red blood cells transfused to the mother, using the Kleihauer-Betke test [16, 17]. All the women in our study had the positive Rhesus factor. Clinically relevant antibodies can be generated to other erythrocyte antigens as well, which can lead to a hemolytic disease in the newborn, but the risk of such alloimmunization is considered to be similar to the one during vaginal delivery [15, 16]. Adverse events observed after intraoperative blood salvage are: hypotension, disseminated intravascular coagulation, heparin toxicity, adult acute respiratory distress syndrome, hypocalcaemia, hypomagnesaemia and hypoproteinemia [12, 13, 17]. The only fatal outcome after the application of the salvaged blood in obstetrics is the case report published in 2000 by Oei et al, who concluded that the cause of death was amniotic fluid embolism [18]. The cited authors presented a patient with significant obstetric comorbidity, who, due to her religious believes, refused the transfusion of allogeneic blood. She was delivered by an emergency CS due to preeclampsia and HELLP (**H**emolysis, **EL**: elevated liver enzymes, **LP:** a low platelet count) syndrome, with consequent anemia (the hemoglobin level before surgery was 71 g/l and the platelet count was 48×10^9/l) and coagulopathy. The cell saver without leukocyte filter was used during the operation, and a cardiac arrest occurred immediately after the retransfusion of the salvaged blood. The authors of papers published later expressed their disagreement with the presented conclusion, thus it has not been generally accepted in the literature published afterwards that this death could be attributed to the intraoperative blood salvage [4, 9, 10].

So far, only one randomized study on elective application of intraoperative blood salvage during CS has been published, which showed a significant reduction in number of patients who required transfusions in the study group [16, 17]. Multicentre cohort study published by Rebarber et al. has not proved the existence of differences between the groups in terms of infectious complications, the incidence of disseminated intravascular coagulopathy and duration of postoperative hospitalization [19]. In this study, none of the patients developed amniotic fluid embolism or adult acute respiratory distress syndrome.

The application of cell saver reduces the need for allogeneic blood transfusions, thereby avoiding the risk of infection and post transfusion reactions. Most authors agree that the benefits to be gained by the application of cell saver in obstetrics exceed the potential risks [9–11].

Conclusion

Obstetric hemorrhage, which is often associated with operative delivery, is the major cause of maternal morbidity and mortality both in developing and in developed countries. One of the risk factors for peripartum hemorrhage is the myoma, whose incidence in pregnancy is increasing. The leading cause of morbidity after cesarean section in women with myoma is perioperative hemorrhage, which is manifested through the number and scope of perioperative transfusion. Thus, intraoperative blood salvage represents a significant opportunity for a reduction of morbidity in this population.

Intraoperative blood salvage should be considered in patients with uterine myomas, and certainly in those in whom myomectomy during cesarean section is planned, particularly in cases when massive intraoperative hemorrhage is expected.

In the absence of large randomized prospective studies which would confirm the safety of the broad application of intraoperative blood salvage in obstetrics, it is necessary to collect all the data about experiences of its use thoroughly. Although the number of women in our study was relatively small, none of them received a transfusion of allogeneic blood intraoperatively. Recommendations for the use of cell saver during myomectomy have already been given in the publications of authors who have investigated its application during the classical abdominal myomectomy. The use of intraoperative blood salvage in obstetrics could lead to a significant reduction of maternal morbidity and mortality in the future.

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Special Hospital for Rheumatic Diseases Novi Sad[1]
University of Novi Sad, Faculty of Medicine Novi Sad[2]
Clinical Center of Vojvodina, Novi Sad
Department of Physical Medicine and Rehabilitation[3]

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VERIFICATION OF OSTEOPOROTIC VERTEBRAL FRACTURES CAUSED BY GLUCOCORTICOIDS

VERIFIKACIJA VERTEBRALNIH OSTEOPOROTIČNIH PRELOMA UZROKOVANIH GLIKOKORTIKOIDIMA

Tanja JANKOVIĆ[1,2], Jelena ZVEKIĆ SVORCAN[1,2] and Ksenija BOŠKOVIĆ[2,3]

Summary

Introduction. Long-term administration of glucocorticoids leads to rapid osteoporosis, and vertebral fractures are one of its most common complications. The methods used in identification are semi-quantitative ones, based on visual assessment, and quantitative ones, which use morphometric criteria. **Case Report**. A 79-year-old woman, who has suffered from polymyalgia rheumatica since July 2012, was treated with prednisone at a daily dose of 20 mg. Radiography of thoracic and lumbar spine verified the reduction of body height of T 12, L3 and L4 vertebrae. Densitometry findings showed a decrease in bone density at the lumbar segment of the spine and femoral neck. Dual-energy x-ray absorptiometry device was used to perform vertebral morphometry by applying Genant semi-quantitative method, which verified crush fractures of the body of T4 and L3 vertebrae, while the L2 vertebra had a biconcave shape. The spinal deformity index parameter was 8. An intense pain developed in the back after 9 months of glucocorticoids administration. The repeated radiographic findings of thoracic and lumbar spine and vertebral morphometry, which had been done by dual-energy x-ray absorptiometry device, revealed deterioration in the form of serial crush fractures, while fat distribution index parameter increased to 15. Dual-energy x-ray absorptiometry finding showed a decrease in T score at the femoral neck. **Conclusion**. Long-term administration of glucocorticoids is accompanied by a rapid loss of bone mass, and vertebral fractures are one of its most common consequences. Therefore, its prevention, early diagnosis and treatment are required. The combination of qualitative conventional radiography and semi-quantitative dual-energy x-ray absorptiometry vertebral morphometry plays an important role in identifying vertebral fractures.

Key words: Osteoporotic Fractures; Spinal Fractures; Glucocorticoids; Diagnosis; Radiography; Densitometry; Absorptiometry, Photon; Female; Aged

Sažetak

Uvod. Dugotrajna primena glikokortikoida dovodi do brzog nastanka osteoporoze, a vertebralne frakture su jedna od njenih najčešćih komplikacija. Metode koje se koriste u njihovoj verifikaciji su semikvantitativne, koje se zasnivaju na vizuelnoj proceni, i kvantitativne, u kojima se primenjuju morfometrijski kriterijumi. **Prikaz slučaja.** Prikazana je bolesnica stara 79 godina, koja od jula 2012. godine boluje od polimialgije reumatika i na terapiji je pronizonom u dnevnoj dozi od 20 mg. Urađenom radiografijom torakalne i lumbalne kičme verifikovano je smanjenje visine tela T 12, L3 i L4 pršljena. Nalaz osteodenzitometrije pokazivao je umanjenje koštane gustine na lumbalnom segmentu kičme i vratu butne kosti. Sprovedenom vertebralnom morfometrijom za osteodenzinometriju na apartu primenom semikvantitativne Genantove metode verifikovane su kraš frakture tela T4 i L3 pršljena, dok je L2 pršljen imao bikonkavan oblik. Indeks spinalne deformacije bio je 8. Nakon 9 meseci primene glikokortikoida dolazi do pojave intezivnog bola u leđima. Ponovljenim nalazima radiografije torakalne i lumbalne kičme kao i vertebralne morfometrije na aparatu za osteodenzitometriju, konstatovano je pogoršanje u vidu novih serijskih kraš fraktura dok se indeks spinalne deformcije povećao na 15. Nalaz osteodenzitometrije je pokazivao smanjenje T-skora u predelu vrata butne kosti. **Zaključak.** Hronična primena glikokortikoida praćena je brzim gubitkom koštane mase, a vertebralne frakture su jedna od njenih najčešćih posledica. Stoga je neophodna njena prevencija, rana dijagnostika i lečenje. U identifikaciji vertebralnih preloma značajno mesto zauzima kombinacija primene kvalitativne konvencionalne radiografije i semikvantitativne apsorbcione vertebralne morfometrije.

Ključne reči: Osteoporotične frakture; Frakture pršljena; Glikokortikoidi; Dijagnoza; Radiografija; Denzitometrija; DXA apsorpciometrija; Žensko; Stari

Introduction

Long-term application of glucocorticoids (GC) is the most frequent cause of osteoporosis provoked by medicaments. Its effect on bone mineral density (BMD) is manifested through the increase of bone resorption and decrease of bone formation which all lead to the increased risk of the occurrence of fractures [1]. The degree of bone mass loss depends on the drug dose and duration of its administration. It is more pronounced in the trabecular bone area, therefore the fragility is more expressed in the area

Corresponding Author: Dr Tanja Janković, Specijalna bolnica za reumatske bolesti, 21000 Novi Sad, Futoška 68, E-mail: tanjajankovic13@gmail.com

Abbreviations

GC – glucocorticoids
BMDGC – glucocorticoids
BMD – bone mineral density
DXA – dual-energy x-ray absorptiometry
AP – anteroposterior
MRX – morphometric X-ray radiography
MXA – morphometric X-ray absorptiometry
SDI – spinal deformity index
SD – standard deviations
BMI – body mass index
PTH – parathormone bone mineral density

of vertebrae and femur [2]. The decrease of BMD is most expressed in the first 3-6 months, the reduction being up 30% during that period. However, this process is slowed down with further GC administration. The daily dose of more than 7.5 mg of prednisone correlates with the decrease of BMD and the increase of risk of fractures [3,4]. The relative risk of fractures in patients during GC administration is 2.6 for vertebral fractures, 1.6 for hip fractures and 1.3 for all other fractures compared to those patients who do not receive GC. The occurrence of new vertebral fractures is by 69% more frequent than post-menopause osteoporosis which develops in 23% cases [5]. Osteodensitometry finding, i.e. dual-energy X-ray absorptiometry (DXA) represents the golden standard in diagnosing osteoporosis. It measures the lumbar spine and hip BMD which is presented by absolute values (g/cm^2) and in the form of T score presented by standard deviations (SD) [6]. In the patients starting glucocorticoids therapy, BMD is expected to get decreased by 10% or 1 SD during the first year.

Vertebral fractures are most frequently diagnosed by the qualitative conventional anteroposterior (AP) and lateral radiography of thoracic and lumbar spine. According to the multicenter IMPACT study conducted in more than 2000 patients, this method fails to diagnose vertebral fractures in 29-46% patients. In addition, this method is very subjective [7]. Vertebral morphometry is a diagnostic method that enables the identification of vertebral fractures on the basis of measurement of height (vertical dimensions) and/or shapes of vertebrae. It can be performed by using conventional lateral spinal radiographs – morphometric X-ray radiography (MRX), and recently also using the DXA device, performing the lateral scan of the spine (morphometric X-ray absorptiometry - MXA) [8]. The semi-quantitative Genant method, which is considered to be the "golden standard" of vertebral fractures identification, is used to assess the decrease of vertical height of vertebral bodies, as well as the position and shape of their end plates [9]. A vertebral fracture is defined as the decrease of vertebral body height by more than 20%, i.e. 4 mm, as it is standardized [10, 11]. Thus, the fractures are classified into the first (mild), second (moderate) and third (severe) degree characterized by the decrease in the vertebral body height by 20-25%, 25-40% and more than 40%, respectively. According to the position and shape of the end plate of vertebral body, fractures are classified into wedge fractures, when the inferior height of the vertebral body is less than the posterior one. Biconcave fractures occur when the central height of vertebral body is less than the anterior and posterior height and crush fractures occur when the total height of vertebral body is less than the height of the adjacent vertebral body. Genant also suggested the calculation of Spinal Deformity Index (SDI), a parameter that represents the total score of the numbers 0, 1, 2, 3 based on the semi-quantitative analysis of 13 vertebrae from T4 to L1. The increase of SDI parameter can indicate the occurrence of new fractures or deterioration of the old ones. In this way the occurrence of new vertebral fractures can be predicted [12, 13].

Case report

A 79-year-old patient has suffered from polymyalgia rheumatica since July 2012. She has been administered the therapy of prednisone tablets at daily dose of 20 mg, which she had never taken before. As for other illnesses, she has reported the increased blood pressure which is regularly controlled by her cardiologist. The reduction of AP diameter at the level of T4, L3 and L4 vertebral bodies was verified in addition to degenerative changes by AP and lateral radiography of thoracic and lumbar spinal segment performed after the introduction of corticosteroid therapy.

The patient underwent DXA examination on Lunar type device. The value of her body mass index (BMI) was 30.5. The obtained T score of the lumbar part of spine L1-L4 was -1.3 SD, BMD 1.027 g/ cm^2, while in the femur neck it was -1.6 SD, BMD 0.783 g/cm^2.

Some changes on the body of T4 vertebra matching crash fractures were verified by vertebral morphometry (MXA) according to the Genant method. The body of L2 vertebra had a biconcave shape, while the changes in L3 vertebra pointed to the crash fracture. The calculated SDI parameter was 8. The patient had no other risks other than the age as a risk factor of osteoporosis. She was treated by alfacalcidol at daily dose of 0.5 mcg. On the first control examination in September 2012, the pains, as well as the stiffness of the neck muscles, shoulder blade area and pelvis area were reduced. Except for the increased sedimentation, which was 34 mm during the first hour, all the other laboratory parameters were within reference values. The dose of prednisone was reduced to 15 mg per day with prolonged alfacalcidol therapy at the dose of 0.5 mcg per day.

At the beginning of April 2013, she felt intensive back and pelvis pains which spread into both legs resulting in difficult and unstable walking,

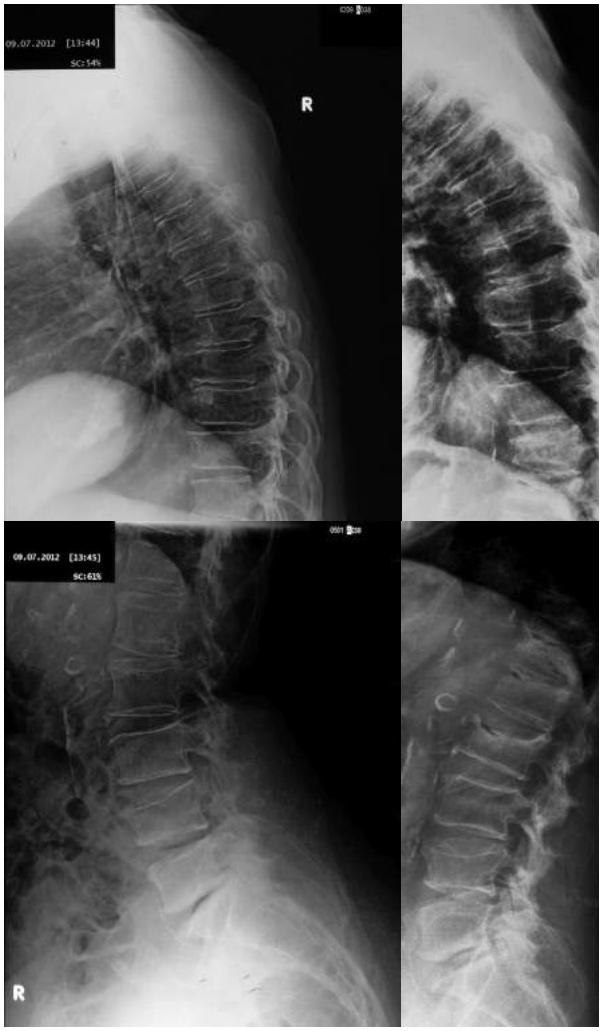

Figure 1. Radiographic findings of the thoracic and lumbar spine at the beginning and after 9 months of glucocorticoid administration
Slika 1*. Radiografski nalaz torakalne i lumbalne kičme na početku i nakon 9 meseci primene glikokortikoida*

but without the neck muscle stiffness or the stiffness of shoulder blade and pelvis area. Except for mild anemia, other laboratory findings were within reference values. Since she had been receiving therapy with higher doses of corticosteroids for several months, the patient underwent another AP and lateral radiography of thoracic and lumbar segment, where new serial fractures of T 4, 6, 7, 8, 10, 11 and 12 bodies, as well as L1, 2 and 3 of vertebrae were verified (**Figure 1**).

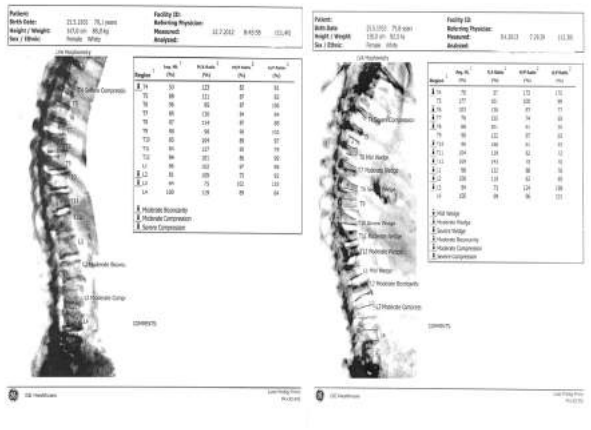

Figure 2. Finding of vertebral morphometry MXA according to the Genant method at the beginning and after 9 months of glucocorticoid administration
Slika 2. *Nalaz vertebralne morfometrije-MXA primenom Genantove metode, na početku i nakon 9 meseci primene glikokortikoida.*

The body height measurements pointed to the fact that the patient's height was decreased by 9 cm, while her BMI was 32.5. The DXA analysis on Lunar type device repeated in April 2013 verified the reduction of T score value and BMD measured in the femur neck (**Table 1**).

Vertebral morphometry (MXA) according to the Genant method verified the changes of the TH 4 vertebral body height and shape which matched the crash fracture. The bodies T 6, 7, 8, 10, 11, 12 had a wedge shape graded from mild (T 6) to moderate (T 7, 11) and severe (T 8, 10). The changes occurring at the level of these vertebrae were not recorded by previously performed vertebral morphometry (MXA). On the lumbar segment, some changes were observed at the level of L1 vertebra which had a wedge shape, L2 was biconcave, while L3 was in the form of the crash fracture. In relation to the previous findings, the changes occurred at the body level of L1 vertebra. SDI was 15, i.e. by 7 higher than the previous one (**Figure 2**).

The biochemical markers were within reference values, except for lower levels of serum 25(OH) D which was 18.4 nmol/l (**Table 2**).

Table 1. DXA values on the first and second measurement
Tabela 1*. Vrednosti DXA nalaza na prvom i drugom merenju*

		T score *T skor* (SD)	BMD (g/cm^2)
Lumbar spine (L1-L4) *Lumbalna kičma*	First measurement–July 2012/*Prvo merenje – juli 2012.*	-1.3	1.027
	Second measurement–April 2013/*Drugo merenje − april 2013.*	-1.4	1.031
Femur neck *Vrat butne kosti*	First measurement– July 2012/*Prvo merenje − juli 2012.*	-1.6	0.783
	Second measurement – April 2013/*Drugo merenje − april 2013.*	-2.1	0.733

Table 2. Values of biochemical markers
Tabela 2*. Vrednosti biohemijskih markera*

Ionized Ca/*Jonizovan Ca*	0.97 mmol/l
Osteocalcin/*Osteokalcin*	31.7 ng/ml
Crosslaps/*Crosslaps*	321pg/ml
25(0H)D	18.4 nmol/l
Parathormone/*Parathormon*	19.4 pg/ml

The patient was administered therapy with bisphosphonate – Alendronate on weekly basis, along with alphacalcidol at the dose of 1 mcg and calcium citrate of 1000 mg on daily basis. The therapy with prednisone at a reduced dose of 10 mg daily was prolonged.

Discussion

The occurrence of osteoporosis is influenced by the following factors, among others: female gender, age, early menopause, the existence of a mild trauma fracture, smoking, insufficient physical activity, family history of osteoporosis, some diseases (rheumatoid arthritis, diabetes mellitus...), as well as the intake of some drugs, especially long-term administration of glucocorticoids [14, 15]. A great number of studies have indicated that osteoporotic fractures can occur in just a few weeks of GC treatment [16, 17]. Their effect to BMD is manifested through inhibition of osteoclast apoptosis and suppression of osteoblasts activity, which leads to increased bone resorption and reduced bone matrix formation, thus leading to the increase of bone resorption. GK also affects the decrease of intestinal absorption of calcium and at the same time increases its renal excretion, thus causing the development of secondary hyperparathyroidism and increased secretion of parathormone (PTH) [18].

Therefore, in order to monitor osteoporosis it is necessary to determine serum PTH, D-vitamin, serum calcium, phosphorus, osteocalcin level, crosslaps and alkaline phosphatase [19]. Doses exceeding 15 mg per day, the cumulative dose being higher than 1 g, significantly increase the risk of the occurrence of osteoporotic fractures which happen in 30-50% of patients [20, 21]. Our case report supports this statement. Except for the age, the presented patient had no other risk factors for the occurrence of osteoporosis before glucocorticoids were introduced. The applied GC dose, which was higher than 15 mg per day over the period of 9 months, led to the occurrence of a series of vertebral fractures, which were verified by AP and lateral radiography. In the repeated DXA finding, the values of T score and BMD in the femur neck were reduced, while in the lumbar spine they were practically unchanged. However, vertebral morphometry (MXA) verified significant changes in the form of decrease of height and shape of thoracic and lumbar vertebral bodies. The obtained SDI parameter value was increased, which can point to the occurrence of new fractures or deterioration of the old ones. Diacinti et al. conducted a study that included 930 women in menopause who underwent the DXA spine examination besides the conventional radiography. This study has showed that the assessment of osteoporotic vertebral fractures by the application of DXA vertebral morphometry can reach a high level of precision when diagnosing vertebral fractures [22]. A study conducted in 344 patients older than 50 years with chronic back pain showed a high prevalence of osteoporosis and undiagnosed vertebral fractures, therefore DXA vertebral morphometry has been recommended as a standard diagnostic method aiming at early fracture identification [23]. The application of DXA vertebral morphometry is clinically useful for diagnosing asymptomatic vertebral fractures, as well as the identification of new fractures, which can influence the selection of adequate therapy [24]. The combination of weekly dose of alendronate and alfacalcidol was introduced into the therapy of our patient. The efficacy of this drug combination has been proven in numerous clinical trials through the increase of bone density and reduction of risk of fractures [25-27].

Conclusion

Long-term administration of glucocorticoids is accompanied by the rapid loss of bone mass, and vertebral fractures are one of its most common consequences. Therefore, its prevention, early diagnosis and treatment are required. The combination of qualitative conventional radiography and semi-quantitative dual-energy x-ray absorptiometry vertebral morphometry takes an important place in identifying vertebral fractures. The occurrence of new vertebral fractures can be predicted by calculating the spinal deformity index parameters.

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University of Novi Sad
Faculty of Medicine, Novi Sad
Clinical Center of Vojvodina, Novi Sad
Department of Infectious Diseases

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THE SUCCESS OF TREATMENT OF CHRONIC HEPATITIS C IN OPIATE ADDICTS IN CLINICAL CENTER OF VOJVODINA

USPEŠNOST LEČENJA HRONIČNOG HEPATITISA C KOD INTRAVENSKIH ZAVISNIKA OD OPIJATA U KLINIČKOM CENTRU VOJVODINE

Author: Milica ODAVIĆ
Mentor: Assist. Prof. Tomislav PREVEDEN, M.D., Ph.D.

Summary
Introduction: Chronic hepatitis C is a disease with a high prevalence in the population of intravenous drug users. Serious clinical course of the disease, which can lead to cirrhosis of the liver with all its complications, has a large epidemiological and clinical significance. This study was aimed at assessing the success of antiviral treatment of chronic hepatitis C in intravenous drug users and defining indicators of successful treatment in this population. **Materials and Methods.** This retrospective study included 316 patients treated with standard therapy for chronic hepatitis C, pegylated interferon and ribavirin, at the Department of Infectious Diseases, Clinical Center of Vojvodina in Novi Sad in the period from January 2007 to December 2012. The patients were divided into a group of intravenous drug users (n = 163) and a group of other modes of transmission of hepatitis C virus (n = 153). The indicators of successful treatment were measured in both groups. **Results.** A total 51.57% of the subjects belonged to the group of intravenous drug users. The therapy was successful in 87.15% of cases, while the success was achieved in only 53.47% of cases in the group of patients infected otherwise. The positive effect of therapy was associated with younger age, shorter duration of infection, low levels of fibrosis and a higher percentage of infected with hepatitis C virus genotypes 2 and 3. **Conclusion.** The population of intravenous drug users can be effectively treated with the standard therapy for chronic hepatitis C, even more successfully than the population infected in some other way.
Key words: Hepatitis C, Chronic; Opioid Related Disorders; Behavior, Addictive; Antiviral Agents; Treatment Outcome; Interferon-alpha; Ribavirin

Sažetak
Uvod. Hronični hepatitis C je oboljenje s visokom prevalencom u populaciji intravenskih zavisnika od opijata. Ozbiljan klinički tok bolesti, koja može dovesti do ciroze jetre sa svim njenim komplikacijama, ima veliki epidemiološki i klinički značaj. Cilj rada bio je da se utvrdi uspeh antivirusnog lečenja hroničnog hepatitisa kod intravenskih zavisnika od opijata i definišu pokazatelji uspešnog lečenja u ovoj populaciji. **Materijal i metode.** Retrospektivna studija je uključila 316 pacijenata lečenih standardnom terapijom za hronični hepatitis C, pegilovanim interferonom i ribavirinom, na Klinici za infektivne bolesti Kliničkog centra Vojvodine u Novom Sadu u periodu od januara 2007. do decembra 2012. godine. Pacijenti su podeljeni na grupu intravenskih zavisnika od opijata (n = 163) i grupu zaraženih drugim načinima transmisije (n = 153) i određivani su pokazatelji uspešnog lečenja u obe ispitivane grupe. **Rezultati.** Ukupno 51,57% ispitanika pripadalo je grupi intravenskih zavisnika od opijata. Terapija se kod njih pokazala uspešnom u 87,15% slučajeva, dok je u grupi ispitanika zaraženih na drugi način uspeh postignut u svega 53,47% slučajeva. Pozitivan efekat terapije bio je povezan sa mlađim životnim dobom, kraćim trajanjem infekcije, nižim stepenom fibroze i većim procentom zaraženih genotipovima 2 i 3 hepatitis C virusa. **Zaključak.** Populacija intravenskih zavisnika od opijata za hronični hepatitis C može se efikasno lečiti standardnom terapijom i to uspešnije nego populacija zaražena na drugi način.
Ključne reči: Hronični hepatitis C; Opijatski zavisnici; Adiktivno ponašanje; Antivirusni lekovi; Ishod lečenja; Interferon alfa; Ribavirin

Introduction

Hepatitis C is an inflammatory disease of the liver caused by the hepatitis C virus (HCV). The genome of this virus was identified in 1989. [1] Serological tests for its identification were introduced in most countries in 1991 [2, 3]. Identifying anti - HCV antibodies in our country began in late 1994 [4].

Corresponding Author: Doc. dr Tomislav Preveden, Klinika za infektivne bolesti,
21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: tomislavpreveden@yahoo.com

Abbreviations

CHC – Chronic hepatitis C
HCV – hepatitis C virus
RNA – ribonucleic acid
IDU – injecting drug users
ALT – alanine aminotransferase
AST – aspartate aminotransferase
PCR – polymerase chain reaction
SVR – sustained viral response
BMI – body mass index

The hepatitis C virus is a small, single-stranded ribonucleic acid (RNA) virus and a member of the genus Hepacivirus of the Flaviviridae family [5]. Six genotypes were defined by analyzing the genomic sequences of HCV, labeled with numbers from 1-6, each with its subtypes and quasispecies [6]. Determining the genotype of the virus has epidemiological significance because certain types are specific to certain parts of the world and also have clinical significance since some genotypes are associated with the development of more severe form of the disease and its faster progression [7].

Pathogenesis of hepatitis C virus infection is still not fully understood. Immune mechanisms and direct cytopathic effect of the virus are reviewed as etiological factors [8, 9].

Epidemiological studies estimate that between 2.2% and 3% of the world population is currently infected with HCV, the total number being between 130 and 170 million people [10]. Data on the number of infected people in Serbia are not available. Since hepatitis C is a blood- transmitted disease, the majority of patients in developed countries belong to the group of injecting drug users (IDUs), the prevalence exceeding 90% [11]. The second most common group is the one that developed post-transfusion hepatitis C, also there is a group with sexual route of transmission, as well as iatrogenic transmission of infection during medical interventions (endoscopy, surgery), through dialysis, transplantation of organs and tissues, during tattooing, piercing and common use objects (scissors, razors, toothbrushes) [12]. Vertical transmission from mother to newborn is possible in 4-7% of cases [13].

Clinical manifestation of acute hepatitis, which develops after the incubation period of 6-7 weeks, runs inconspicuously in 75-80% of cases with nonspecific symptoms such as fatigue, loss of appetite, pain under the right costal margin and jaundice. The laboratory results record ten times more elevated activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), positive HCV RNA polymerase chain reaction (PCR) test which can be detected 1-2 weeks after exposure and anti-HCV antibodies 1-3 months after exposure [14].

Chronic hepatitis C (CHC) develops in 75-85% of cases of hepatitis C viral infection and is characterized by elevated aminotransferase activity for more than six months and the persistent presence of HCV RNA in serum. It is either asymptomatic or has nonspecific symptoms. The chronic stage of disease is characterized by frequent presence of extrahepatic manifestations (arthralgia, myalgia, skin reactions, symptoms of renal impairment, a disorder of the thyroid gland function, and symptoms of diabetes). In the advanced stage of chronic HCV infection, the dominant symptoms and signs of the disease result from liver cirrhosis. Cirrhosis develops in 20-30% of the chronically infected patients within a period of 20-30 years, and the development of hepatocellular carcinoma (HCC) is possible [14-16].

The diagnosis is made according to anamnesis and epidemiological data, clinical features, laboratory findings and liver biopsy. Laboratory tests have significant pathological ALT activity, positive antibodies to hepatitis C virus (anti-HCV) and confirmed presence of HCV RNA by PCR technology [17]. Liver biopsy confirms the diagnosis of chronic liver inflammation and determines the intensity of necro-inflammatory process and the degree of liver fibrosis [18, 19].

Chronic HCV infections therapy is carried out by combining two specific antiviral drug: pegylated interferon α and ribavirin. There are two forms of pegylated interferon available on the market: pegylated interferon α2a, which is applied in a fixed dose of 180 mg and pegylated ınterferon α2b, which is administered at a dose of 1.5 mg per kg of body weight. Ribavirin is an antiviral agent from the group of nucleoside inhibitors, which is applied in the form of tablets of 200 mg and dosed according to the patient's body weight, 800-1200 mg per day. The length of treatment depends on the genotype of HCV, being 24 weeks for genotype 2 and 3 and 48 weeks for the other genotypes [20].

The success of therapy was assessed by measuring the amount of HCV RNA in the serum by PCR method six months after its completion. It is believed that the therapy is successful if it has achieved a sustained viral response (SVR), there are no particles of HCV RNA in the serum of patients six months after completion of antiviral therapy. The patients who achieved SVR are considered cured because such findings are maintained in more than 99% of patients over time [21]. The combined administration of pegylated interferon with ribavirin has yielded positive results in over 60% of patients and in over 80% of selected patients [22].

Studies have shown that as many as 60-90 % IDUs have chronic infection with hepatitis C [23, 24]. Despite great advances in the diagnosis and treatment of these diseases, there is a large number of IDUs with hepatitis C who are not treated [25]. The main problems in the treatment of this population are their inability to maintain abstinence from addictive substances, risk of re-infection, influence of the replacement therapy, alcohol abu-

se and bad adherence. Recent studies have shown that CHC can be effectively and safely treated in IDUs, especially in those receiving replacement therapy with methadone or other drugs [26, 27]. Therefore, treatment of CHC in population of IDUs should become a component of therapeutic guide in all health care systems, because there cannot be a reduction in the prevalence of CHC unless the infection is controlled in this group.

The aims of this study were:

To determine whether intravenous drug users with CHC can be effectively treated with standard dual therapy for this disease.

To compare the results of therapy within IDUs population with the results of treatment of patients infected otherwise.

To define indicators for successful treatment in the population of IDUs.

Material and Methods

The research was conducted as a retrospective study that included 316 patients with CHC, of whom 163 were injecting drug users, examined and treated at the Department for Infectious Diseases, Clinical Center of Vojvodina in Novi Sad from January 2007 to December 2012 year.

The diagnosis of CHC was based on clinical and laboratory findings including aminotransferases ALT and AST activity having at least double values, positive anti-HCV antibodies for more than six months, a positive qualitative and quantitative HCV RNA PCR test to determine the amount of viremia expressed in the number of copies of the virus per milliliter (cop/ml), HCV genotype and histopathological examination of liver biopsy.

Determination of HCV RNA was performed at the Virology Laboratory of the Institute for Infectious and Tropical Diseases, Clinical Center of Serbia in Belgrade. Cobas Amplicor HCV Test, version 2.0 (Roche Diagnostics, Menheim) was used as a qualitative test, with sensitivity of 50 IU/ml (135 cop/ml). The quantitative test was Cobas Amplicor HCV Monitor Test version 2.0 (Roche Diagnostics, Menheim), sensitivity of 600 IU/ml (1620 cop/ml). The genotype was determined by Linear Array HCV genotyping assay (Roche Diagnostics). All patients were tested before the introduction of antiviral therapy, as well as six months after the completion of therapy.

A blind liver biopsy was performed in all patients prior to therapy, except for those in whom this procedure was contraindicated. A sample of liver tissue was treated with standard histological techniques for small biopsies and stained with hematoxylin-eosin method. The degree of fibrosis was expressed by the modified Knodell's numerical score.

The patients were treated with standard therapy for CHC, i.e. the combination of pegylated interferon α2a (Pegasys®) or α2b (Pegintron®) with ribavirin (Copegus® and Rebetol®). The therapy was considered successful when the stable virologic response was achieved (undetectable HCV RNA six months after the treatment). It was considered a failure if HCV RNA values had not been reduced by more than 2 logs compared to the initial ones (non-responders), and if the presence of HCV RNA was proved six months after the completion of treatment which was negative during the treatment (relapsers). The following factors were considered predictive for successful treatment: age, sex, body mass index (BMI), duration of infection, viral genotype and degree of liver fibrosis.

The duration of infection was determined on the basis of data on the presumed date of inoculation, while the duration of infection in patients with unknown mode of transmission of HCV was not taken into consideration.

All intravenous drug addicts were abstinent for at least 12 months prior to treatment, which was confirmed and documented with reports of psychiatrists.

Statistical analysis included descriptive statistics and tests of significance (χ^2 and t-test) while the calculation was done by computer programs Excel and the Statistical Package for the Social Sciences (SPSS).

The statistical significance was determined by p-value, which represents the risk of concluding. The difference was considered statistically significant at $p < 0.05$.

The results are expressed by the absolute numbers, percentages, tables and graphs with the accompanying text.

Results

Out of 316 patients with CHC included in the study, 163 were opiate addicts (51.57%) and 153 patients were infected with hepatitis C virus in some other way (48.43%).

Post-transfusion hepatitis was found in 43/316 (13.61%) patients, infection was sexually transmitted in 8/316 (2.53%) patients, tattoo and piercing caused infection in 11/316 (3.48%) patients, medical procedures (other than hemodialysis) resulted in infection in 8/316 (2.53%) patients, and hemodialysis caused infection in 9/316 (2.85%) patients, while the cause remained unknown in 74/316 (23.42%) patients. Distribution of patients according to the mode of transmission of hepatitis C virus infection is shown in **Graph 1**.

The success of antiviral therapy, estimated by the achievement of a SVR was found in 300/316 (94.94%) patients. The success of therapy remained undetermined in the patients who discontinued treatment themselves (5.06% of the patients, 16/316). The treatment was successful in 213/300 (71.0%) patients and failed in 87/300 (29.0%) patients. Among the unsuccessfully treated patients

Table 1. Indicators of therapy success rate in both groups
Tabela 1. *Prikaz pokazatelja uspešne terapije u obe grupe ispitanika*

		Intravenous opiate addicts *Intravenski zavisnici od opijata*	Others *Ostali*	Statistical significance *Statistička značajnost*
Gender/*Pol*	Men/*Muškarci (%)*	78.53	62.75	p>0,05
	Women/*Žene (%)*	21.47	37.25	
Mean age (years) *Prosečna starost (godine)*		32.61	44.96	p<0.05
BMI *(kg/m²)*		24.11	25.45	p<0.05
Duration of infection (years) *Dužina trajanja infekcije (godine)*		10.09	20.52	p<0.05
Viremia/*Viremija/*(cop/ml*)*		11 517 733,8	9 859 044,95	p>0.05
Genotype *Genotip*	2 and 3 (%)	47.24	24.18	p<0.05
	non 2 and 3 (%)	52.76	75.82	
Fibrosis *Fibroza*	F0, F1 and F2 (%)	90.8	71.85	p<0.05
	F3 and F4 (%)	9.2	28.15	

there were 43/300 (14.33%) non-responders and 44/300 (14.67%) relapsers (**Graph 2**).

The success of therapy was examined in 156 patients from the group of IDUs. Therapy was successful in 136/156 patients (87.18%), while it was unsuccessful in 20/156 (12.82%) cases. In the group of patients infected otherwise the success of therapy was assessed in 144 people and treatment was found to be successful in 77/144 individuals (53.47%) and unsuccessful in 67/144 (46.53%) cases. A statistically significant difference was found in the distribution among the studied groups (p<0.05) **(Graph 3)**.

Furthermore, some indicators of success of therapy were analyzed in both groups, as shown in **Table 1.**

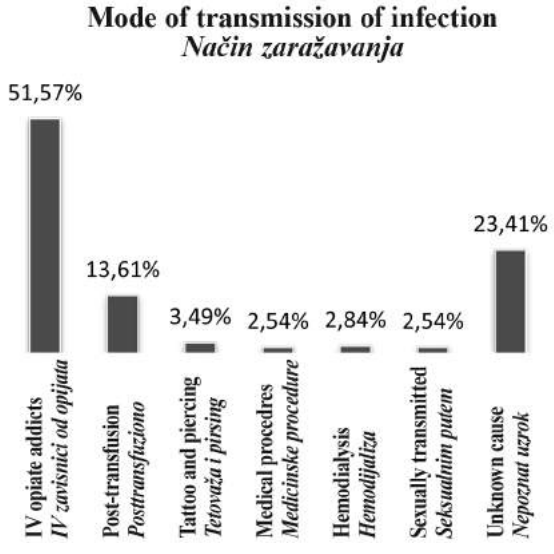
As for gender, there were 128 (78.53%) males and 35 (21.47%) women in the group of IDUs and 96 (62.75%) males and 57 (37.25%) females in the control group. No statistically significant difference was found in the distribution of sex (p>0.05).

The average age of the group of IDU and in the control group was 32.61 (SD± 6.52) years and 44.96 (SD±12.44) years, respectively. The difference was found to be statistically significant (p<0.05).

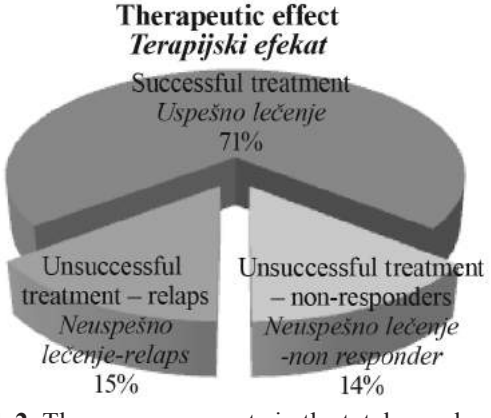
The average BMI in the group of IDUs and in the control group was 24.11 kg/m² and 25.45 kg/m², respectively. Obese patients with BMI above 30 kg/m² were not included in the study. The difference was found to be statistically significant (p<0.05).

The duration of infection in intravenous opiate drug addicts and in the group with the known cause of infection was on average 10.09 (SD± 6.04) years and 20.52 (SD±10.29) year, respectively. The difference was found to be statistically significant (p<0.05).

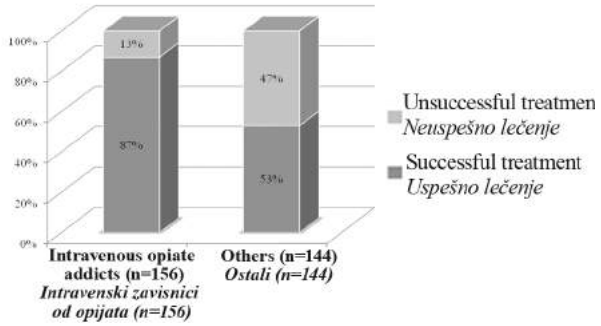
HCV genotyping was done in all patients. The group of opiate addicts had the highest number of genotype 1, 80/163 (49.07%), it was followed by genotype 3 in 75/163 (46.01%), genotype 4 in 4/163 (2.45%), genotype 2 in 2/163 (1.23%) and 2/163



Graph 1. Mode of transmission of HCV infection
Grafikon 1. *Način prenošenja HCV infekcije*



Graph 2. Therapy success rate in the total sample
Grafikon 2*. Uspešnost terapije u ukupnom uzorku*



Graph 3. Therapy success rate intravenous opiate addicts and the controls infected in some other or unknown way

Grafikon 3. *Uspešnost terapije kod intravenskih zavisnika od opijata i kontrolne grupe pacijenata zaražene na neki drugi ili nepoznat način*

(1.23%) patients had two or more genotypes. The control group had 110/153 (71.89%) patients with genotype 1; 30/153 (19.61%) patients had genotype 3; 7/153 (4.57%) patients had genotype 2; 1/153 (0.65%) patients had genotype 4 and 5/153 (3.27%) patients had two or more genotypes. Genotypes 5 and 6 were not identified. A statistically significant difference was found in the distribution of HCV genotypes between the groups ($p<0.05$).

Because of the different therapeutic modalities, the patients were divided into two groups: one group consisted of patients infected with HCV genotypes "2, 3", which are considered easier to treat and achieve better therapy success, and another group of "1, 4" genotypes, classified as other genotypes, whose treatment is difficult and less successful. The number of patients with genotypes "2, 3" was 77/163 (47.24%) in the group of IDUs and 37/153 (24.18%) in the group of patients infected otherwise. The number of patients with "1, 4" genotypes in the group of addicts was 86/163 (52.76%), while in the group of otherwise infected patients it was 116/153 (75.82%). A statistically significant difference was found in distribution between the groups ($p<0.05$) **(Graph 4).**

Viral load was determined in all patients and the group of IDUs was found to have 11 517 733.8 cop/ml (±SD 21 997 317.28), and those infected in some other way had 9 859 044.95 cop/ml (SD± 14 734 036.56). There was no statistically significant difference ($p> 0.05$).

Histopathological examination of liver biopsy specimens revealed liver fibrosis in 298 patients. Liver biopsy was contraindicated in 18/316 (5.7%) of the patients; however, it was not contraindicated in the group of IDUs. Fibrosis was absent in 25/163 (15.34%); low degree, moderate and severe fibrosis was found in 83/163 (50.92%), 40/163 (24.54%), and 14/163 (8.59%), respectively and the signs of liver cirrhosis were present in 1/163 (0.61%). In the group of patients with other or unknown causes of infection, fibrosis was absent in 18/135 (13.33%), low degree, moderate and severe fibrosis was found in 39/135 (28.89%), 40/135 (29.63%), and 27/135 (20.0%), respectively and the signs of liver cirrhosis were present in 11/135 (8.14%) patients. A statistically significant difference was found in the distribution among the studied groups ($p<0.05$).

According to the presence and degree of fibrosis, which are the factors influencing the effectiveness of treatment, the patients were divided into the group without fibrosis or with signs of mild to moderate fibrosis (F0-2) and the group with signs of severe fibrosis and cirrhosis (F3-4). The treatment was successful in the former and less successful in the latter. There were 148/163 (90.8%) F0-2 patients and 15/163 (9.20%) F3-4 patients in the group of addicts. There were 97/135 (71.85%) patients with F0-2, and 28.15% with F3-4 in the group of patients infected in some other way. A statistically significant differences was confirmed in the distribution ($p<0.05$) (**Graph 5**).

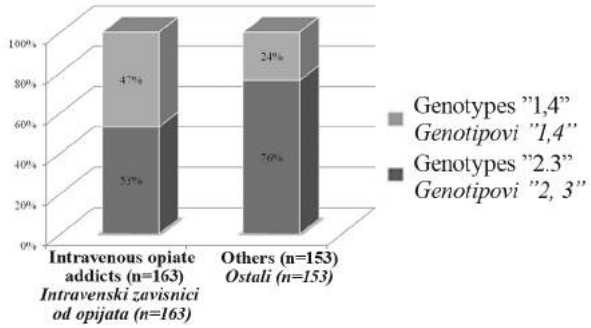
Discussion

Chronic hepatitis C with liver cirrhosis as the final outcome is a major health issue mainly in the working-age population. Correlation between chronic HCV infection and opiate addiction, which is also intensifiying, is becoming an obstacle to be yet overcome by modern medicine.

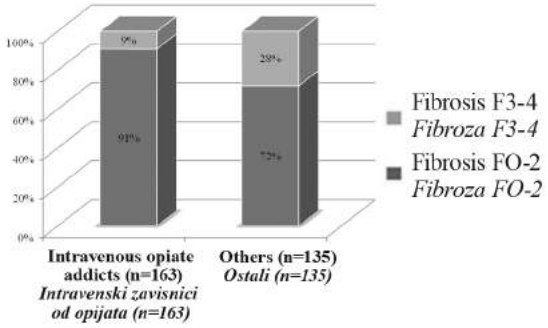
Treatment of CHC is still not efficient enough, although the development of new drugs and better selection of patients increase the treatment success rate. In our study, treatment of CHC was successful in 71.0% of cases from the whole study group, which is in accordance with most published studies, which have reported successful treatment of chronic hepatitis in 50-80% of cases [28, 30]. Worse treatment success than in our study was made in the studies which included previously unsuccessfully treated patients, patients with elevated BMI and patients who were not in stable abstinence from psychoactive substances and alcohol. These are the factors which have negative influence on antiviral treatment.

The report on successful treatment of CHC in our study is consistent with the published study of our colleagues Delic et al. who reported the effectiveness of treatment for CHC in 70.52% of cases [31]. Kuljić-Kapulica et al. reported successful treatment of CHC in 55.5% of cases, which is less than in our study [32]. Their study sample had a smaller number of IDUs, who are known to be more responsive to antiviral therapy, which can explain lower treatment success rate in their study.

Intravenous opiate addicts represent the largest part of the population of patients treated for CHC at the Department of Infectious Diseases, Clinical center of Vojvodina in Novi Sad and they accounted for 51.57% of the subjects in this study. Jovanovic et al. from the Department of Infectious Disea-



Graph 4. HCV genotypes "2, 3" and "1,4" in both groups of patients
Grafikon 4. *Prisustvo HCV genotipova "2,3" u "1, 4"u obe grupe ispitanika*



Graph 5. Presence and degree of liver fibrosis in both groups of patients
Grafikon 5. *Prisustvo i stepen fibroze jetre kod obe grupe ispitanika*

ses in Niš have reported the success of treatment of CHC in 93.5% of IDUs, which is very similar to our result of 87.18% [33]. Although their sample was much smaller (30 patients), the patients were selected for antiviral therapy according to the same principle, and they had very similar demographic and clinical characteristics and were treated by the same therapeutic protocol as ours. Only 30.46% of patients treated with standard viral therapy for CHC were opiate addicts who were included in the study of Kurelac et al. from the Croatian Reference Center for Viral Hepatitis [34]. They reported that successful therapeutic response in their study was achieved in only 57.10% of all patients with CHC, and in 70.75% of intravenous drug addicts. In our study, better therapeutic effects were achieved due to better selection of patients. Due to the current regulations of the Commission for Antiviral Therapy of CHC affiliated to the Republican Health Insurance Fund, our patients undergo more rigorous screening, taking into account predictors of successful treatment, and thus the success of treatment of CHC is higher. In addition, our study sample consisted mainly of patients with CHC virus infection, who had never been treated with antiviral therapy, and it is known that the success of treatment in these patients is greater than in previously unsuccessfully treated patients [35].

Our research has shown that IDUs have a better response to antiviral therapy, which is associated with predictors of successful treatment such as younger age, shorter duration of infection and lower degree of fibrosis.

In relation to the type of genotype in the group of IDUs, there was a significantly higher number of patients with genotypes 2 and 3, (47.24%) than in the group of patients infected with the other genotypes (52.76%), which is consistent with previously published reports in literature [36, 37].

Conclusions

The population of intravenous drug users can be effectively treated by standard dual antiviral therapy for chronic hepatitis C.

Injecting drug users have better therapy results than patients infected otherwise.

Indicators of successful treatment in injecting drug users are: younger age, shorter duration of infection, genotypes 2 and 3, as well as the absence of or a milder form of fibrosis.

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UPUTSTVO AUTORIMA

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1. Uvodnici (editorijali) – do 5 stranica. Sadrže mišljenje ili diskusiju o nekoj temi važnoj za Časopis. Uobičajeno ih piše jedan autor *po pozivu*.

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8. Druge vrste publikacija (feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih ustanova, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novine u medicini, pitanja i odgovori, stručne i staleške vesti i *In memoriam*).

Priprema rukopisa

Propratno pismo

– Mora da sadrži svedočanstvo autora da rad predstavlja originalno delo, kao i da nije objavljivan u drugim časopisima, niti se razmatra za objavljivanje u drugim časopisima.

– Potvrditi da svi autori ispunjavaju kriterijume za autorstvo nad radom, da su potpuno saglasni sa tekstom rada, kao i da ne postoji sukob interesa.

– Navesti u koju kategoriju spada rad koji se šalje (originalni naučni rad, pregledni članak, prethodno saopštenje, stručni članak, prikaz slučaja, istorija medicine).

Rukopis

Za pisanje teksta koristiti *Microsoft Word for Windows*. Tekst treba otkucati koristeći font *Times New Roman*, na stranici formata A4, proredom od 1,5 (i u tabelama), sa marginama od 2,5 cm i veličinom slova od 12 pt. Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana. Naslovna strana treba da sadrži kratak i jasan naslov rada, bez skraćenica, zatim kratki naslov (do 40 karaktera), puna imena i prezimena autora (najviše 6 autora) indeksirana brojkama koje odgovaraju onima kojim se u zaglavlju navode uz pun naziv i mesta ustanova u kojima autori rade. Na dnu ove stranice navesti titulu, punu adresu, e-mail i broj telefona ili faksa autora zaduženog za korespondenciju.

2. Sažetak. Sažetak treba da sadrži do 250 reči, bez skraćenica, sa preciznim prikazom problematike, ciljeva, metodologije, glavnih rezultata i zaključaka. Sažetak treba da ima sledeću strukturu:

– originalni naučni radovi: uvod (sa ciljem rada), materijal i metode, rezultati i zaključak;

– prikaz slučaja: uvod, prikaz slučaja i zaključak;

– pregled rada: uvod, odgovarajući podnaslovi koji odgovaraju onima u tekstu rada i zaključak.

U nastavku navesti do deset ključnih reči iz spiska medicinskih predmetnih naziva (*Medical Subjects Headings, MeSH*) Američke nacionalne medicinske biblioteke.

3. Sažetak na engleskom jeziku. Sažetak na engleskom jeziku treba da bude prevod sažetka na srpskom jeziku, da ima istu strukturu i da sadrži do 250 reči, bez upotrebe skraćenica.

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– Tekst originalnih članaka mora da sadrži sledeće celine:

Uvod (sa jasno definisanim ciljem rada), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su korišćene u tekstu) i eventualna zahvalnost autora onima koji su pomogli u istraživanju i izradi rada.

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– Koristiti mere metričkog sistema prema Internacionalnom sistemu mera (*International System Units – SI*). Temperaturu izražavati u Celzijusovim stepenima (°C), a pritisak u milimetrima živinog stuba (mmHg).

– Ne navoditi imena bolesnika, inicijale ili brojeve istorija bolesti.

Uvod sadrži precizno definisan problem kojim se bavi studija (njegova priroda i značaj), uz navođenje relevantne literature i sa jasno definisanim ciljem istraživanja i hipotezom.

Materijal i metode treba da sadrže podatke o načinu dizajniranja studije (prospektivna/retrospektivna, kriterijumi za uključivanje i isključivanje, trajanje, demografski podaci, dužina praćenja). Statističke metode koje se koriste treba da budu jasne i detaljno opisane.

Rezultati predstavljaju detaljan prikaz podataka dobijenih tokom studije. Sve tabele, grafikoni, sheme i slike moraju da budu citirani u tekstu, a njihova

numeracija treba da odgovara redosledu pominjanja u tekstu.

Diskusija treba da bude koncizna i jasna, sa interpretacijom osnovnih nalaza studije u poređenju sa rezultatima relevantnih studija publikovanim u svetskoj i *domaćoj* literaturi. Navesti da li je hipoteza istraživanja potvrđena ili opovrgnuta. Izneti prednosti i ograničenja studije.

Zaključak u kratkim crtama mora da odbaci ili potvrdi pogled na problem koji je naveden u Uvodu. Zaključci treba da proizilaze samo iz vlastitih rezultata i da ih čvrsto podržavaju. Uzdržati se uopštenih i nepotrebnih zaključivanja. Zaključci u tekstu moraju suštinski odgovarati onima u Sažetku.

5. Literatura. Literatura se u tekstu označava arapskim brojevima u uglastim zagradama, prema redosledu pojavljivanja. Izbegavati veliki broj citata u tekstu. Za naslove koristiti skraćenice prema *Index Medicus*-u (http:// www.nlm.nih.gov/tsd/serials/lji.html). U popisu citirane literature koristiti Vankuverska pravila koja precizno određuju redosled podataka i znake interpunkcije kojima se oni odvajaju, kako je u nastavku dato pojedinim primerima. Navode se svi autori, a ukoliko ih je preko šest, navesti prvih šest i dodati et al.

Članci u časopisima:

∗ *Standardni članak*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *Organizacija kao autor*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *Nisu navedena imena autora*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *Volumen sa suplementom*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *Sveska sa suplementom*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *Sažetak u Časopisu*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije:

∗ *Jedan ili više autora*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Urednik(ci) kao autor*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *Poglavlje u knjizi*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders;1974. p. 457-72.

∗ *Rad u zborniku radova*

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.

∗ *Disertacije i teze*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ *Članak u Časopisu u elektronskoj formi*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monografije u elektronskoj formi*

CDI, clinical dermatology illustrated [monograph on CDROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *Kompjuterski dokument (file)*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computeried Educational Systems; 1993.

6. Prilozi (tabele, grafikoni, sheme i fotografije).

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– Tabele, grafikoni, sheme i fotografije dostavljaju se na kraju teksta rukopisa, kao posebni dokumenti na posebnim stranicama.

– Tabele i grafikone pripremiti u formatu koji je kompatibilan sa programom *Microsoft Word for Windows.*

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– Naslov, tekst u tabelama, grafikonima, shemama i legendama navesti na srpskom i na engleskom jeziku.

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– U legendama mikrofotografija navesti korišćenu vrstu bojenja i uvećanje na mikroskopu. Mikrofotografije treba da sadrže merne skale.

– Ukoliko se koriste tabele, grafikoni, sheme ili fotografije koji su ranije već objavljeni, u naslovu navesti izvor i poslati potpisanu izjavu autora o saglasnosti za objavljivanje.

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Ukoliko autor i svi koautori nisu uplatili članarinu za Medicinski pregled, rad neće biti štampan. Radovi koji nisu napisani u skladu sa pravilima Medicinskog pregleda, neće biti razmatrani. Recenzija će biti obavljena najkasnije u roku od 6 nedelja od prijema rada. Uredništvo zadržava pravo da i pored pozitivne recenzije donese odluku o štampanju rada u skladu sa politikom Medicinskog pregleda. Za sva dodatna obaveštenja obratiti se tehničkom sekretaru:

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Vase Stajića 9
21000 Novi Sad
Tel. 021/521 096; 063/81 33 875
E-mail: dlv@neobee.net

INFORMATION FOR AUTHORS

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2. Summary. The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

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– review papers should have the introduction, subtitles corresponding to those in the paper and conclusion. It is to be followed by up to 10 Key Words from the list of Medical Subject Headings, MeSH of the American National Medical Library.

3. The summary in Serbian language. The summary in Serbian should be the translation of the summary in English, it should be structured in the same way as the English summary, containing up to 250 words, without any abbreviations.

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– All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C). and pressure in mmHg.

– No names, initials or case history numbers should be given.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Material and methods should contain data on design of the study (prospective/retrospective, eligibility

and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the problem mentioned in the introduction. Conclusions must be based solely on the author's own results, corroborating them. Avoid generalised and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

5. References. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by et 'al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organisation as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *Acomputer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

6. Attachments (tables, graphs, schemes and photographs). The maximum number of attachments allowed is six!

– Tables, graphs, schemes and photographs are to be submitted at the end of the manuscript, on separate pages.

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Društvo lekara Vojvodine
Vase Stajića 9
21000 Novi Sad
Tel. 021/521 096; 063/81 33 875
E-mail: dlv@neobee.net