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CONTENTS / SADRŽAJ

The World Cancer Day marks on February 4 each year with the aim to help save millions of lives by raising awareness and education about cancer and its prevention and pressing to governments across the world to take action against the disease.
This day was established by the Union for International Cancer Control, a global consortium of more than 470 cancer-fighting organizations in over 120 countries.

Svetski dan borbe protiv raka obeležava se svake godine 4. februara sa ciljem da se spasu milioni ljudskih života podizanjem svesti i znanja o ovoj bolesti i o mogućnostima njene prevencije, kao i pritiscima na državne organe širom sveta da preduzmu sve potrebne mere.
Ovaj dan je ustanovila Unija za internacionalnu kontrolu raka koja okuplja više od 470 organizacija koje se bore protiv te bolesti, iz preko 120 zemalja sveta.

ORIGINAL ARTICLES

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Nosocomial infections in the Intesive Care Unit, Univerisity Hospital for Infectious and Tropical Diseases, Belgrade, Serbia

Bolničke infekcije u Odeljenju intenzivne nege Univerzitetske klinike za infektivne i tropske bolesti, Beograd

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Abstract

Bacground/Aim. Nosocomial infections (NIs) are an important cause of morbidity, mortality and prolonged hospitalizations. Fifty percent of NIs have been reported in Intensive Care Units. The aim of this study was to determine the frequency and type of NIs among critically ill patients treated in the University Hospital for Infectious and Tropical Diseases, Clinical Centre of Serbia, as well as risk factors for acquiring them. **Methods.** This prospective cohort study included 52 patients treated in the Intensive Care Unit from January to June 2004. The diagnosis of NI was established according to the Centers for Disease Control and Prevention (CDC) definition, based on clinical presentation, radiological and microbiological findings, etc. Statistical data processing was done by using the electronic data base organized in SPSS for Windows version 10.0. The level of statistical significance was defined as $p < 0.\ 05$. **Results.** NIs were found in 33 (63.4%) of 52 inpatients. Urinary tract infections (UTIs), pneumonia, and soft tissue infections, the most common nosocomial infections in our setting, were recorded in 41.0%, 25.6%, and 23.1%, of patients, respectively. Several factors contributed to a high incidence of these infections: chronic comorbidities ($p < 0.01$), the presence of indwelling devices such as urinary tract catheters ($p < 0.01$), endotracheal tubes ($p < 0.05$) along with mechanical ventilation ($p < 0.05$). **Conclusion.** The majority of patients with NIs had chronic underlying comorbidities. All the patients with UTIs had urinary catheters. The most important risk factors for the development of nosocomial pneumonias were endotracheal intubation and mechanical ventilation. The patients with pneumonia had the highest mortality.

Key words:
cross infection; risk factors; comorbidity; urinary catheterization; intubation, intratracheal; respiration, artificial; mortality.

Apstrakt

Uvod/Cilj. Intrahospitalne infekcije su važan uzrok morbiditeta i mortaliteta, kao i produžetka bolničkog lečenja. Polovina svih bolničkih infekcija javlja se u jedinicama intenzivne nege. Cilj ove studije bio je da se utvrde učestalost i vrsta bolničkih infekcija kod bolesnika lečenih u Odeljenju intenzivne nege Klinike za infektivne i tropske bolesti Kliničkog centra Srbije, kao i faktori rizika od obolevanja. **Metode.** Ovom prospektivnom kohortnom studijom bilo je obuhvaćeno 52 bolesnika koji su lečeni u Odeljenju intenzivne nege u Klinici za infektivne i tropske bolesti u periodu od januara do juna 2004. Dijagnoza je postavljana na osnovu definicije bolničkih infekcija Centra za kontrolu i prevenciju bolesti (CDC), kliničke slike, radioloških, mikrobioloških i drugih nalaza. Statistička obrada podataka urađena je pomoću statističkog paketa SPSS za Windows verziju 10.0. Nivo statističke značajnosti bio je $p < 0,05$. **Rezultati.** Intrahospitalne infekcije nađene su kod 33 (63,4%) od ukupno 52 lečena bolesnika. Infekcije urinarnog trakta (41,0%), pneumonije (25,6%) i infekcije mekih tkiva (23,1%) bile su najčešće bolničke infekcije. Nekoliko faktora doprinosilo je visokoj učestalosti ovih infekcija: hronične komorbidne bolesti ($p < 0,01$), prisustvo stalnih pomagala, kao što su urinarni kateter ($p < 0,01$), endotrahealni tubus ($p < 0,05$), kao i mehanička ventilacija ($p < 0,05$). **Zaključak.** Većina bolesnika sa bolničkim infekcijama imala je i neku hroničnu bolest. Svi bolesnici sa infekcijama urinarnog trakta imali su urinarni kateter. Najznačajniji faktori rizika od razvoja intrahospitalne pneumonije su endotrahealna intubacija i mehanička ventilacija. Bolesnici sa pneumonijom imali su i najveću smrtnost.

Ključne reči:
infekcija, intrahospitalna; faktori rizika; komorbiditet; kateterizacija urinarnog trakta; intubacija, endotrahejna; disanje, mehaničko; mortalitet.

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Introduction

Nosocomial infections (NIs) are a global health problem, present in all health systems, irrespective of a health service level. NIs cause a variety of medical, ethical, economical and legal consequences. These infections cause substantial morbidity and mortality, prolong hospitalization, and increase direct patient-care costs and the number of hospital staff [1, 2].

According to the Centers for Disease Control and Prevention (CDC) definition, nosocomial or hospital-acquired infections are manifested 48 hours after hospital admission, during the course of receiving treatment for other conditions within a healthcare setting. They are neither present nor incubating at the time of admission, but are acquired in hospital, and even manifest after discharge. NIs are more common in large university hospitals and intensive care units (ICUs), where critically ill patients are treated. According to the published studies, even in the developed countries, 5–10% of patients treated in hospitals are going to be infected. Although ICUs usually account for less then 10% of hospital capacity, over 25% of NIs develop in these departments [3]. The prevalence rate of ICU-acquired infections reaches up to 20% [1]. Multiresistant bacteria cause most of the infections in ICUs [4].

The aim of this study was to determine the frequency and type of NIs in the ICU in our setting along with risk factors for acquiring them.

Methods

This prospective cohort study was established according to the CDC metodology [5]. We investigated NIs in the patients admitted to the ICU in Infectious and Tropcal Diseases University Hospital in Belgrade, from January to June 2004. The patients admitted to the ICU in our setting suffered mostly severe central nervous system (CNS) infections, septicemia, and/or bacterial endocarditis, and less frequently tetanus and botulism. The patients with several severe non-infectious disesases were occasionaly hospitalized due to differential diagnosis. NIs were considered if diagnosed at least 48 hours after the admission, based on clinical, radiological and microbiological findings. Data from patients' charts and by interviewing hospital staff were collected. Radiological examinations included chest X-ray and chest computed tomography (CT) scan when needed. Specific bacteral NIs were confirmed by using standard microbiological methods for cultivation and identification of microorganisms. Infection rate (IR) was expressed as the total number of NIs *per* 1,000 patient days. We calculated device utilization rates by dividing the total number of devices days by the total number of ICU patients days, and device utilization NI rate by dividing the number of device associated NIs by the total number of device days [6].

All analyses were performed using an electronic database organized in the SPSS (version 10.0) statistical package. Non-parametric variables were analyzed using Chi-square or Fisher's exact test, as appropriate. The same method was used to access association between the appearance of NIs and possible risk factors, while One way ANOVA test was used to compare means. The level of significance was 0.05.

Results

Fifty-two patients, representing 1,116 patients-days, were treated in the ICU during 6-month study period (January – June 2004) (Table 1). The mean patients age was 50.5 ± 18.2 years, and they were predominantly male (44.2%).

The most prevalent diagnoses on admission were various CNS infections, septicemia, and/or endocarditis, as well as fever of unknown origin (Table 1). Other less common diagnoses were: ostemyelitis, cavernous sinus thrombosis, paraparesis, tetanus and stroke, recorded in one patient each, respectively. Thirty-two (61.5%) of the patients had comorbidities.

Table 1

Patients treated in the Intensive Care Unit (ICU) during a 6-month period (January – June 2004)

Parameter	Value
Male, n (%)	29 (55.8)
Female, n (%)	23 (44.2)
Age, (years), $\bar{x} \pm SD$	50.52 ± 18.21
Admission diagnosis	
neuroinfections, n (%)	33 (63.5)
sepsis, n (%)	7 (13.5)
fever of unknown origin, n (%)	5 (9.6)
endocarditis, n (%)	2 (3.8)
other, n (%)	5 (9.6)
Comorbidities n (%)	32 (61.5)
cardiovascular diseases, n (%)	15 (29.0)
diabetes mellitus, n (%)	7 (13.5)
malignancy, n (%)	2 (3.8)
connective tissue diseases, n (%)	2 (3.8)
chronic obstructive pulmonary disease, n (%)	2 (3.8)
hematological disease, n (%)	2 (3.8)
cerebrovascular disease, n (%)	2 (3.8)
Total, n (%)	52 (100)

Out of 52 observed patients, 33 (63.4%) acquired 39 different NIs (Table 2). Of those, 24 (46.2%), and 6 (11.5%) patients had one, and two concomitant infections, respectively, while one (1.9%) patient had even three NIs.

Table 2

Nosocomial infections in ICU patients

Infection	Patients, n (%)
Urinary tract infections	16 (41.0)
Pneumonia	10 (25.6)
Skin and soft tissue infections	9 (23.1)
Stomatitis	2 (5.1)
Enterocolitis	1 (2.6)
Blood stream infection	1 (2.6)
Total	39 (100)

ICU – Intensive Care Unit

The development of NI was not associated with reported admission diagnosis ($p > 0.05$).

There was no statistical difference in the distrubution of NIs in relation to the patients' gender and age. Comorbidities had a significant association with the acquisition of NI ($p < 0.01$). Endotracheal intubation, mechanical ventilation and the presence of urinary catheters were also associated with the development of NIs (Table 3).

Out of 39 NI cases, etiological diagnosis was established in 21 patients (Table 4). In all 16 urinary tract infection (UTI) cases, always associated with indwelled urinary catheters, causative agents were identified, including Gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella-Enterobacter, Escherichia coli* and *Citrobacter freundii*, recorded in 4, 2, 4 and 1 case, respectively. All *Escherichia coli* and *Klebsiella-Enterobacter* isolates were extended spectrum beta-lactamase producing bacteria (ESBL). The remaining five UTIs were caused by *Enterococcus* spp, a Gram-positive bacteria susceptible to vancomycin. The average duration of urinary bladder catheterisation was 19.71 ± 11.32 days (range 5–52 days).

A total of 10 patients developed bilateral intrahospital pneumonia, however without etiological confirmation. Eight out of 10 patients with pneumonia were intubated and mechanically ventilated. The mean length of hospital stay for patients with nosocomial pneumonias was 18.3 ± 8.12 days (range 8–50 days). Even 80% of patients with nosocomial pneumonia died.

Skin and soft tissues infections were recorded in 9 patients. Methicilin resistant *Staphylococcus aureus* (MRSA) was cultured from skin lesions in 2 out of 9 patients.

One of the patients acquired bacteriemia caused by *Enteroccocus* spp*.,* while two patients had oropharyngeal candidiasis. In one enterocolitis case no causative pathogen was cultured from the stool.

The mean overall nosocomial infection rate (IR) was 29.5 infections *per* 1,000 patient-days (Table 5).

The device utilization rate in patients on mechanical ventilation was 0.37 (160 device days, 427 patient days) and 0.65 in the patients with urinary catheters (Table 6) (662 device days, 1,026 patient days).

The device utilization nosocomial IR for nosocomial pneumonia in the patients on mechanical ventilation was 50 *per* 1,000 patient-days. The device utilization nosocomial IR for urinary tract infection in the patients with urinary catheters was 24 *per* 1,000 patient-days.

Out of 52 observed patients, 15 (28.8%) succumbed, while remaining 37 were discharged recovered. Age and gender did not influence the mortality ($p > 0.05$). Comorbid-

Table 3

Factors associated with the development of nosocomial infections (NI)

Parameter	NI	Without NI	*p*
Age (years), $\bar{x} \pm SD$	52.21 ± 12.3	49.18 ± 10.1	0.357
Male (n)	16	13	
Female (n)	11	12	0.336
Comorbidities (n)	22	10	
Without comorbidities (n)	11	9	0.008
Intubation, mechanical ventilation (n)	15	5	
Without intubation (n)	18	14	0.028
Urinary tract catheter (n)	14	6	
Without urinary tract catheter (n)	0	32	0.011

Table 4

Etiology of nosocomial infections in Intensive Care Unit patients

Etiologic agent (confirmed)	Patients, n (%)
Gram-positive infections	8 (38.1)
Enterococcus spp.	6 (28.6)
Staphylococcus aureus	2 (9.5)
Gram-negative infections	11 (52.4)
Pseudomonas aeruginosa	4 (19.1)
Esherichia coli	4 (19.1)
Klebsiella-Enterobacter	2 (9.5)
Citrobacter freundii	1 (4.7)
Candida spp	2 (9.5)
Total	21 (100)

Table 5

Monthly distribution of patients, nosocomial infections (NI) and infection rates

Month	Patients (n)	Patient days (n)	NI (n)	Infection rate
January	7	94	4	42.5
February	17	255	5	19.6
March	18	279	5	17.9
April	10	145	4	27.6
May	10	157	5	31.8
June	13	186	10	53.7
Total	75	1116	33	29.5

ities and duration of treatment were significantly related to the poor outcome (Table 6). No statistical difference was recorded between the subgroups with, and without NI regarding survival ($p > 0.05$). Nosocomial pneumonias, intubation and mechanical ventilation were significantly related to the lethal outcome. Indwelling urinary catheters were also associated with poor outcome (Table 6). None of the causative agents affected survival ($p > 0.05$).

Table 6

Clinical outcome in Intensive Care Unit patients

Parameter	Patients (n)		*p*
	survived	died	
Male	23	6	
Female	14	9	0.218
Age (years), $\bar{x} \pm SD$	48.24 ± 11.95	56.13 ± 15.04	0.159
Previous comorbidity	19	13	
No previous comorbidity	18	2	0.027
Duration of treatment (days), $\bar{x} \pm SD$,	29.84 ± 14.11	15.67 ± 11.95	0.001
NI	21	12	
Without NI	16	3	0.203
Urinary tract infection	8	1	
Pneumonia	0	7	
Skin /soft tissue infections	9	3	
Other	4	1	0.003
Intubation and MV	7	13	
Without intubation	30	2	0.000
UC	23	14	
Without UC	14	1	0.019

NI – nosocomial infections; MV – mechanical ventilation; UC – urinary catheter.

Discussion

We analyzed NIs in 52 patients treated in the ICU in the University Hospital for Infectious and Tropical Diseases in a 6-months period. The patients with various CNS infections and neurointoxications (tetanus, botulism) were included, as well as several patients with severe non-infectious diseases, and indeed even 63.4% of the admitted patients developed NI, without a relationship between initial diagnosis and prevalence of specific NI. Among the observed series of patients, the most prevalent were UTIs, pneumonias and skin and soft tissues infections, with rather high mortality rate among those with pneumonia.

There are many factors that contribute to the high incidence and unfavorable outcome of NIs: ICU patients have more frequent and more difficult comorbidities as well as more profound pathophysiological disturbances than patients in other hospital's departments. In these patients physiological barrieres to infections are often disturbed by using various medical devices, urinary catheters, endotracheal tubes, central vasular catheters, which allow direct breakthrough of microorganisms into the tissues and blood stream. In addition, indwelled catheters demand frequent contacts with hands of health-care workers which can lead to colonisation and infection with hospital pathogens. It is well-known that hospital and especially ICU hygiene and infection control practice are more problematic in developing countries [7], where Serbia belongs. This is why the prevalence of NIs in our setting has reached this high prevalence of over 60%.

Multi-resistant pathogens, such as MRSA, vancomycin-resistant *Enterococci* (VRE), ESBL-producing *Enterobacteriaceae* ect. are more common in ICUs [8]. This is in concordance with our findings.

The patients' gender and age had no influence on the development of NIs, although it had previously been shown that patients' age was important factor in susceptibility to infection, especially for infants, young children, and elderly.

In our series of patients comorbidities were associated with a higher prevalence of NI, which is in concordance with the National Nosocomial Infection Surveillance System (NNIS) report [9].

UTIs were shown to be the most prevalent NI [9]. In addition, it was previously demonstrated that indwelled urinary catheter was the leading risk factor for the acquisition of nosocomial UTIs and bacteremia (70–80%), followed by cystoscopy or other urological procedures [10]. These infecions

were also the most frequent in our series of patients. And indeed, all the patients with UTIs had indwelled urinary catheters. Similarly, Crouzet et al. [11], demonstrated that the duration of urinary catheterization had a great impact on catheter-associated UTI. During the first 3 days of catheterisation, urinary tract infections are rare if closed method of urinary drainage is used, but the risk for acquiring catheter-associated infection increases by additional 5% for every single day of catheterization afterwards. Reported IRs vary widely, ranging from 1–5% after a single brief catheterization to virtually 100% for patients with indwelling urinary catheters draining into an open system for longer than 4 days [11]. The average duration of catheterisation among our patients was even 19 days, so it is not suprising that all of them developed UTIs.

Likely, etiological diagnosis was established in all 16 UTIs. These infections were caused mostly by Gram-negative bacteria, and far less freqent were infections caused by Gram-positive cocci, such as *Enterococcus* spp, that were susceptible to vancomycin. One patient also had *Enterococcus* spp. bacteriemia/septicemia. Fortunately, all the patients from our series experienced favourable response to antibiotic therapy.

Pneumonia was reported to be the second most common NI in the USA, associated with the high morbidity and mortality rates among all hospital-acquired infections [12]. This is also in concordance with our data. Both intubation and mechanical ventilation were significantly associated with NI. Safdar et al. [13] showed the incidence of 1.3% nosocomial pneumonias per day, counting from the time of endotracheal intubation, with the highest incidence from the day 5 to 15. Similarly, most of our patients developed pneumonia about 2 weeks after admission to the hospital. No causative agents were demonstrated in our patients with pnumonia. Bronch aspiration and cannula swab cultures were performed in two patients, without results. However, these specimens are not recommended for etiological diagnosis of nosocomial pneumonia [14], since these techniqes are not capable of distinguishing "colonisations" from infection. Recommendations for the diagnosis of pneumonia include: the protected specimen brush (PSB) with quantitative cultures, bronchoalveolar lavage (BAL), and protected BAL (pBAL) [14]. Since these techniques were not available in our ICU, no etiological diagnosis for patients with pneumonia were established.

Skin and soft tissue infections were the third most common NIs in our series of patients. Most of them were localized around vascular access lines. MRSA was isolated from two swab cultures of skin lesions. MRSA has been common in our ICU. According to European Prevalence of Infection in Intensive Care (EPIC) study data, MRSA was present in 86% of isolated *Staphyloccoci* [3]. Pittet et al. [15], showed that prevention strategy for reducing colonisation and MRSA transmission, such as introducing mandatory handwashing in hospital personnel, was followed by dramatic reduction of NIs at the University Hospital in Geneva.

The mean overall NI rate was approximate or even lower but the device utilization NI rate for ventilator associated pneumonia and catheter-associated UTI was higher compared to other studies [16, 17].

It is very important to understand the significance of NIs in order do decrease their rate in hospitals. The mortality rate in our group of patients treated in the ICU was 28.8%. An EPIC study demonstrated quite similar results in Mediterranean countries [3]. Even though it was not possible to demonstrate with a small number of patients that ICU-acquired infections increased mortality rate in critically ill, we did find that certain NIs (nosocomial pneumonias) were associated with an increased risk of death in the ICU. The comorbidities, duration of hospitalization, the acquisition of nosocomial pneumonia, endotracheal intubation and mechanical ventilation along with prolonged urinary bladder catheterisation had significant influence on clinical ouctome of patients treated in the ICU.

Conclusion

In a series of 52 patients treated in the ICU, Serbian University Hospital for Infectious and Tropical Diseases, UTIs were the most common NIs, and easy to treat. On the contrary, among those with pneumonia, associted with endotracheal intubation and mechanical ventilation, the mortality rate was as high as 80%.

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REFERENCES

1. *Harbarth S, Pittet D.* Excess mortality and impact of intensive care unit-acquired infections. Curr Opin Anaesth 1996; 9:139–45.
2. *Hugonnet S, Chevrolet JC, Pittet D.* The effect of workload on infection risk in critically ill patients. Crit Care Med 2007; 35(1): 76−81.
3. *Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH*, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA 1995; 274(8): 639−44.
4. *Brinsley K, Srinivasan A, Sinkowitz-Cochran R, Lawton R, McIntyre R, Kravitz G,* et al. Implementation of the Campaign to Prevent Antimicrobial Resistance in Healthcare Settings: 12 Steps to Prevent Antimicrobial Resistance Among Hospitalized Adults-experiences from 3 institutions. Am J Infect Control 2005; 33(1): 53−4.
5. *Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM.* CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988; 16(3): 128−40.
6. *Emori TG, Culver DH, Horan TC, Jarvis WR, White JW, Olson DR, et al.* National nosocomial infections surveillance system

(NNIS): description of surveillance methods. Am J Infect Control 1991; 19(1): 19−35.

7. *Rosenthal VD, Jarvis WR, Jamulitrat S, Silva CP, Ramachandran B, Dueñas L., Gurskis V*, et al. Socioeconomic impact on device-associated infections in pediatric intensive care units of 16 limited-resource countries: international Nosocomial Infection Control Consortium findings. Pediatr Crit Care Med 2012; 13(4): 399−406.
8. *Gopal Katherason S, Naing L., Jaalam K, Kamarul Iman Musa K, Nik Abdullah NM,Aiyar S,* et al. Prospective surveillance of nosocomial device-associated bacteremia in three adult intensive units in Malaysia. Trop Biomed 2010; 27(2): 308−16.
9. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992-April 2000, issued June 2000. Am J Infect Control 2000; 28(6): 429−48.
10. *Saint S, Meddings JA, Calfee D, Kowalski CP, Krein SL.* Catheter-associated urinary tract infection and the Medicare rule changes. Ann Intern Med 2009; 150(12): 877−84.
11. *Crouzet J, Bertrand X, Venier AG, Badoz M, Husson C, Talon D.* Control of the duration of urinary catheterization: impact on catheter-associated urinary tract infection. J Hosp Infect 2007; 67(3): 253−7.
12. *Freeman MK.* Nosocomial Pneumonia. US Pharm 2010; 35(7): HS2−HS4.
13. *Safdar N, Crnich CJ, Maki DG.* The pathogenesis of ventilator-associated pneumonia: its relevance to developing effective strategeis for prevention. Respir Care 2005, 50(6): 725−39; discussion 739−41.
14. *Mayhall CG.* Ventilator-Associated Pneumonia or Not? Contemporary Diagnosis. Emerg Infect Dis 2001; 7(2): 200−4.
15. *Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S,* et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. Lancet 2000; 356(9238): 1307−12.
16. *Inan D, Saba R, Yalcin AN, Yilmaz M, Ongut G, Ramazanoglu A*, et al. Device-associated nosocomial infection rates in Turkish medical-surgical intensive care units. Infect Control Hosp Epidemiol 2006; 27(4): 343−8.
17. *Moreno CA, Rosenthal VD, Olarte N, Gomez WV, Sussmann O, Agudelo JG,* et al. Device-associated infection rate and mortality in intensive care units of 9 Colombian hospitals: findings of the International Nosocomial Infection Control Consortium. Infect Control Hosp Epidemiol 2006; 27(4): 349−56.

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Economic burden of cardiovascular diseases in Serbia

Kardiovaskularne bolesti u Srbiji – ekonomski teret

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Abstract

Background/Aim. Cardiovascular disease imposes a burden to society in terms of mortality, morbidity and economic losses. The aim of this study was to estimate the economic burden of cardiovascular disease in Serbia in 2009 from the perspective of the society. **Methods**. For the purpose of the study cardiovascular disease was defined by the International Classification of Diseases, 10th revision, as the following diagnosis: hypertension, coronary heart disease, cardiomyopathy, heart failure and cerebrovascular disease. The prevalence, top-down method was used to quantify the annual cardiovascular costs. Productivity losses were estimated using the human capital approach and the friction cost method. A discount rate of 5% was used to convert all future lifetime earnings into the present value. **Results.** The total direct costs of cardiovascular disease in 2009 were € 400 million. The results showed that more than half a million working days were lost due to incapacity resulting from cardiovascular diseases, yielding the € 113.9 millon. The majority of total costs (€ 514.3 million) were for: medication (29.94%), hospital days (28.97%) and hospital inpatient care – surgical and diagnostic interventions (17.84%). The results were robust to a change in 20% of volume or the unit price of all direct and indirect cost and to discount rate 2% and 10%. **Conclusions.** The total cardiovascular disease costs in 2009 represented approximately 1.8% of the Serbian gross domestic product. The results of the study would be valuable to health policy makers to bridge the gap between invested resources and needs, in order to improve cardiovascular disease outcomes.

Key words:
cardiovascular diseases; health care costs; serbia.

Apstrakt

Uvod/Cilj. Kardiovaskularne bolesti predstavljaju teret za društvo u smislu mortaliteta, morbiditeta i ekonomskih gubitaka. Cilj ove studije bio je procena ekonomskog značaja kardiovaskularnih bolesti u Srbiji u 2009. godini iz perspektive društva. **Metode.** Za potrebe istraživanja, kardiovaskularne bolesti su definisane pomoću Međunarodne klasifikacije bolesti, 10. revizija, kao sledeće dijagnoze: hipertenzija, koronarne bolesti, kardiomiopatija, srčana insuficijencija i cerebrovaskularne bolesti. Korišćen je *top-down* metod, baziran na prevalenciji, kako bi se kvantifikovali godišnji kardiovaskularni troškovi. Troškovi smanjene produktivnosti su procenjeni korišćenjem dva pristupa: pristup ljudskom kapitalu (*human capital approach*) i metod frikcionih troškova (*friction cost method*). Za obračunavanje troškova u sadašnju vrednost korišćena je diskontna stopa od 5%. **Rezultati.** Ukupni direktni troškovi kardiovaskularnih bolesti u 2009. godini iznosili su 400 miliona evra. Rezultati pokazuju da je više od pola miliona radnih dana izgubljeno zbog nesposobnosti usled kardiovaskularnih bolesti, dajući ukupno 113,9 miliona evra indirektne troškove. Većina ukupnih troškova (514,3 miliona evra) bili su za: lekove (29,94%), hospitalizaciju (28,97%) i bolničko lečenje – hirurške intervencije i dijagnostiku (17,84%). Rezultati su bili robusni na promene od 20% u volumenu ili ceni pojedinih kategorija troškova, kao i na primenjenu diskontnu stopu od 2% i od 10%. **Zaključak.** Ukupni troškovi kardiovaskularnih bolesti u 2009. godini su predstavljali oko 1,8% bruto domaćeg proizvoda. Rezultati studije su značajni za kreiranje zdravstvene politike i premošćavanja jaza između uloženih sredstava i potreba, a u cilju poboljšanja ishoda kardiovaskularnih bolesti.

Ključne reči:
kardiovaskularne bolesti; zdravstvena zaštita, troškovi; srbija.

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Introduction

Cardiovascular disease (CVD) imposes a burden to society in terms' of mortality and morbidity, as well as an economic impact. Management of CVD consumes a large amount of healthcare resources. In America nearly 2,400 Americans die of CVD each day, an average of one death every 37 seconds [1]. CVD mortality in Eastern European countries is much higher than the European average as it reaches a value of 650 deaths per 100,000 in some countries [2] CVD dominated the burden of premature mortality in Serbia (48%) with almost 400,000 years of life lost [3].

Cost of illness (or burden of disease) analysis involves identification, measurement and valuation of resources related to the illness, in this case CVD. The economic burden of CVD consists of direct and indirect costs. Direct costs are associated with hospitalisations, physician visits, rehabilitation services and medications. Indirect costs represent losses to the economy due to premature mortality and morbidity, resulting in lost economic production and consumption and the associated effect on the functioning of the economy. The total costs of CVD in the European Union were over 168 billion Euros in 2003, of which direct healthcare costs represented 62% [4].

National health authorities have postulated in their report [5] that intensive research work is needed in the field of CVD, particularly the impact of hypertension and its major complications. Therefore, the purpose of this study was to estimate the economic burden of cardiovascular disease in Serbia in 2009 from the perspective of the society.

Methods

For the purpose of the study CVD was defined by the International Classification of Diseases, 10th revision as the following diagnosis: hypertension (I10–I15), coronary heart disease (I20–I25), cardiomyopathy (I42), heart failure (I50) and cerebrovascular disease (I60–I69). The method of prevalence was used to quantify the annual CVD costs for the total Serbian population in 2009 [6]. The analysis was performed from the societal perspective including direct healthcare costs, as well as indirect costs associated with productivity loss due to morbidity or premature death which were estimated.

We employed a top-down approach, using aggregate data on morbidity, doctor visits at primary care, hospitalisations, medications utilisation, rehabilitations and mortality. This approach was previously developed by Liu et al. [7] to estimate economic burden of coronary heart disease. Productivity losses were estimated using the human capital approach and the friction cost method [8]. All costs were expressed in Euros (€), using the 2009 average exchange rate to convert Serbian dinar (RSD) to the € (€1 = 94.12 RSD) [9].

Healthcare direct costs

Direct costs include the value of medical care resources used to treat a disease. Healthcare costs were calculated by assessing the value of resources used by patients for detection, treatment and rehabilitation of CVD.

The following items of healthcare service were included: primary care provided by general practice; emergency care; hospital care; diagnostic and surgical procedures; rehabilitation services and medication treatment.

The number of patients visits to general practice regarding CVD was obtained from the Republic Institute for Health Insurance (RIHI). The total number of hospital emergency visits and hospitalisations were provided by the RIHI; the average length of stay in hospital due to CVD was eight days. The number of surgical interventions: percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass grafting (CABG) and coronography was also obtained from RIHI, due to their complete coverage by the RIHI. The number of diagnostic procedures was estimated based on the prevalence of the given CVD and the current clinical practice and guidelines in Serbia [10–13]. The number of rehabilitations was obtained from the Republic Institute for Public Health; the average length of stay at the rehabilitation unit was 20.5 days. Utilisation of the medications in hospital and ambulatory settings aimed at prevention and treatment of CVD was provided from the RIHI. Data regarding the utilisation were considered for the following Anatomical Therapeutic Chemical (ATC) classification system medicines groups: B01 (antitrombotic agents), C01 (cardiac therapy), C02 (antihypertensives), C03 (diuretics), C07 (beta blocking agents), C08 (calcium channel blockers), C09 (agents acting on renin-angiotensin system) and C10 (serum lipid reducing agents).

The values of resources used were calculated by multiplying the resource quantities used in healthcare services for management of CVD with their unit costs. All costs are derived from the RIHI price list. The RIHI is a leading health care payer, responsible for the health care of almost the entire Serbian population (7.3 million). Hospitalisation daily costs are calculated as the average value of days spent in cardiovascular, neurological and neurosurgical unit, due to the fact that the RIHI charges hospitalisation cost *per diem*. Costs for a diagnostic procedures, both cerebrovascular disease and coronary heart disease, were calculated as the number of units consumed multiplied by the sum of different diagnostic procedures applied for given disease (e.g. neurological exam, computed tomography scan, magnetic resonance imaging of the head, basic analysis of blood, biochemical analysis of C-reactive protein, creatine kinase, electrocardiogram, etc.). The costs of rehabilitation were calculated as the average cost per day spent in rehabilitation centres, multiplied with the average length of stay (20.5) and the number of rehabilitation procedures. For ambulatory prescribed medicines we included the pharmacy mark-up and value added tax.

Healthcare indirect costs

Indirect costs included forgone earning related to mortality and morbidity due to CVD which were estimated using the human capital approach. The indirect cost from mortality was estimated considering the following: the number of premature deaths due to CVD [14], the number of working years left till retirement (65 years of age for men, 60 for women) [15], unem-

ployment rate and the 2009 average earnings [16]. We also calculated the productivity loss in retirement based on predicted years spent in retirement, life expectancy, and 2009 average pension earnings.

The value of indirect cost from morbidity was estimated as total number of days lost from work obtained from the RIHI multiplied by the average daily earnings [16]. According to the current legislation [17], the first 30 sick days are paid by the employer, additional sick days are charged to RIHI. Thus we estimated the number of employers' paid sick days as the total number of persons for which the RIHI paid sick allowances multiplied by 30 days. The authors did not find any way of estimate the number of days lost due to morbidity paid by employers not followed by the RIHI coverage.

Due to the fact that an estimate of lost production using the human capital approach tends to be overestimated, we also apply the friction cost method. In this case, production losses are calculated during "the friction period" (time between the start of absence from work and replacement). This is estimated to be about 90 days [18, 19]. The friction period adjusted productivity loss was calculated by multiplying the unadjusted productivity loss, obtained as described above, by the friction period (90 days) and then dividing this product by the average duration of work incapacity (calculated in this study, for CVD patient). A discount rate of 5% was used to convert all future lifetime earnings into the present value [20].

Sensitivity analysis

To examine the robustness of the results we performed one-way sensitivity analyses. We assessed the change in the estimated total cost of CVD resulting from a 20% change in the volume or the unit price of all direct and indirect costs; the 20% change was based on the cost of illness study conducted in EU [4]. The effect of discounting on indirect costs was assessed using the rates of 2% and 10%. All analyses were performed using the Microsoft Excel.

Results

In 2009 women were diagnosed more often with CVD than men (54% *vs* 46%). There were 338,279 patients diagnosed with hypertension; 505,358 patients with coronary heart diseases; 221,663 patients with cardiomyopathy; 61,713 patient diagnosed with heart failure and 187,564 patient with cerebrovascular disease. Most of the diagnosed diseases occurred in patients aged over 70 (Figure 1).

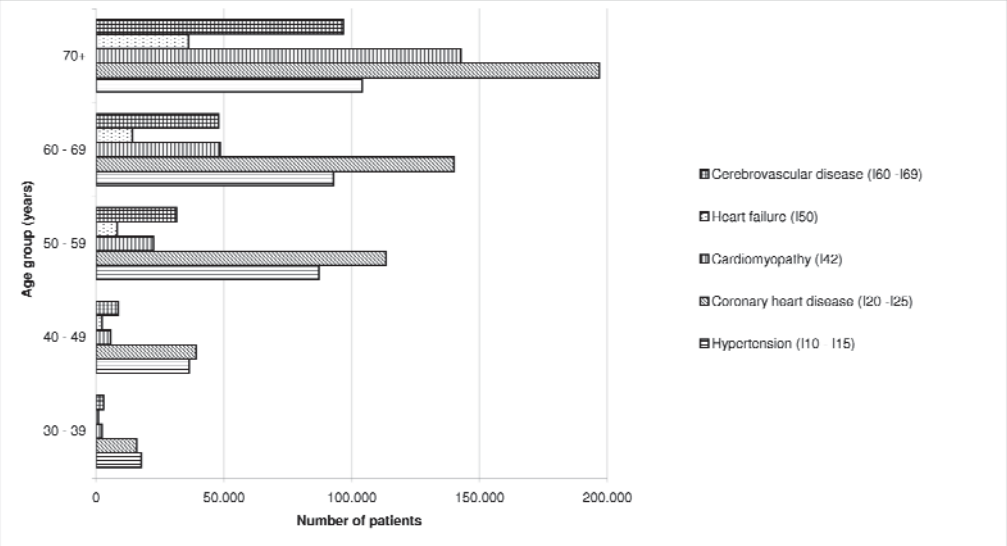

Fig. 1 – Number of patients diagnosed with cardiovascular diseases according to age. In brackets are the diagnoses defined by the International Classification of Disease, 10th revision.

Direct costs

The total direct costs of CVD in 2009 were € 400 million (Table 1). Hospitalisation and surgical and diagnostic procedures applied to hospitalised patients accounted for € 240.7 million, or 60.13% of the direct costs. The majority of costs attributable to surgical and diagnostic procedures were allocated on diagnostic of cerebrovascular sequel (25.48% of costs allocated on surgical and diagnostic procedures) and PTCA (30.77% of costs allocated on surgical and diagnostic procedures). Medication treatment accounted for 38.46% of total direct costs (€ 154 million). Of these costs, 87.2% was for prescription medicine covered by the RIHI used by outpatients. Physician visits at primary care and rehabilitation accounted just for 0.83% and 0.58%, respectively, of total direct costs.

Indirect costs

The results showed that more than a half million working days were lost due to incapacity resulting from CVD (Table 2). Cerebrovascular and coronary heart diseases caused the longest absence from work, on average, 112.8 days and 100.7 days, respectively. The average length of incapacity for all CVD patients was 95 days. The production losses due to CVD morbidity estimated using the human capital approach were € 11.6 million; with the friction cost method the estimate was lower at € 11 million. According to

Table 1

The direct costs of cardiovascular disease (CVD)

Direct costs	Number of units	Average cost *per* unit (€)	Average stay (days)	Total cost (€)
Doctor (GP) visit at clinic	1 596 638	2.07		3 312 193
Hospitalisations due to CV complications	521 514	35.71	8	149 000 960
Interventions due to CV complications				
PTCA (without stent)	13 500	1 455.79		19 653 171
drug eluting stent (DES)	6 750	1 058.23		7 143 042
bare metal stent (BMS)	6 750	211.65		1 428 638
Coronography	18 300	494.96		9 057 839
By-pass revascularisation; graft (CABG)	4 800	3 083.54		14 801 003
Diagnostic procedures for cerebrovascular disease*	146 024	160.04		23 369 984
Diagnostic procedures for coronary heart disease†	521 514	31.22		16 280 080
Rehabilitation due to CV complications	9 695	11.69	20.5	2 322 803
Medication treatment				
antitrombolytic medicine (utilised in hospital)	1 821 787.45			4 330 162
outpatient reimbursed medicine (covered by RIHI)	36 630 948.55			130 490 385
outpatient medicine (patient participation)	36 630 948.55			19 160 657
Total direct cost (€)				400 350 917

*Diagnostic procedures for cerebrovascular disease include: neurological exam, computed tomography (CT) scan, and magnetic resonance imaging (MRI) of the head; †diagnostic procedures for coronary heart disease include: basic analysis of blood, additional analysis of C-reactive protein (CRP), creatine kinase (CK), myoglobin, and troponin-I, echocardiogram, electrocardiogram, and ergometry; GP – general practitioner; CV – cardiovascular; PTCA – percutaneous transluminal coronary angioplasty; CABG – coronary artery bypass grafting; RIHI – Republic Institute for Health Insurance.

Table 2

The indirect costs of cardiovascular disease

Indirect costs	Number of units	Average cost *per* unit (€)	Total cost (€)
Mortality			
working years lost (men)	5 270.3	5 628.6*	19 738 507
working years lost (women)	1 857.7	5 628.6*	7 279 430
years lost in retirement (men)	8 693	2 523.6†	20 246 645
years lost in retirement (women)	26 080	2 523.6†	55 617 209
Morbidity (number of days lost from work)			
RIHI paid	353 130	22.3	7 472 293
employer paid	168 720	22.3	3 570 145
Total indirect cost (€)			113 924 229

*Average annual earnings in 2009. For all future years the values of annual earning were discounted at 5% depending on the age of death; †Average annual pension in 2009. For all future years the values of annual pension were discounted at 5% depending on the age at which death occurred.

the results, more than 7100 working years were lost from CVD; 74% of these years lost were from deaths in men. Of all working years lost in men 83.05% were in the 40–59 year age range; in women 74.92% of the working years lost were from deaths in the 40–59 year age range. The mortality costs due to CVD were estimated to be € 104.4 million. However, after adjustment for friction period estimate fell to € 102.9 million.

Total costs

Table 3 shows the total costs of CVD for Serbia in 2009. The total costs resulted in € 514.3 million; most of these costs were used for medication (29.94%), hospital days (28.97%) and hospital inpatient care – surgical and diagnostic interventions (17.84%). Indirect costs (mortality and morbidity) accounted for 22.15% of total costs.

Table 3

The total costs of cardiovascular disease

Type of costs	Value (€)	Percentage of total cost (%)
Direct costs		
doctor (GP) visit at clinic	3 312 193	0.64
hospitalisation	149 000 960	28.97
surgical and diagnostic procedures	91 733 757	17.84
rehabilitation	2 322 803	0.45
medication treatment	153 981 203	29.94
Total direct cost	400 350 917	77.85
Indirect costs		
mortality	102 881 791	20.00
morbidity	11 042 438	2.15
Total indirect cost	113 924 229	22.15
Total costs (€)	514 275 146	100

Sensitivity analysis

The baseline estimate of total cost related to CVD was not sensitive to changes in the input variables (Figure 2).

A change of 20% in volume or cost of hospitalisation and prescription medicine produced the largest variation in the baseline estimate of total cost of ± 5.79% and ± 5.07%, respectively. Regarding the variables included in indirect costs, the largest impact on total cost had 20% change in the number of years lost in retirement for women (± 2.16%) and change in discount rate. When a discount rate of 2% was applied, the baseline estimate changed for 2.31%; with the rate 10%, the total cost decline for - 2.80%. Changes in all other variables produced very small effects on the total cost estimate (between 0.06% and 1.03%).

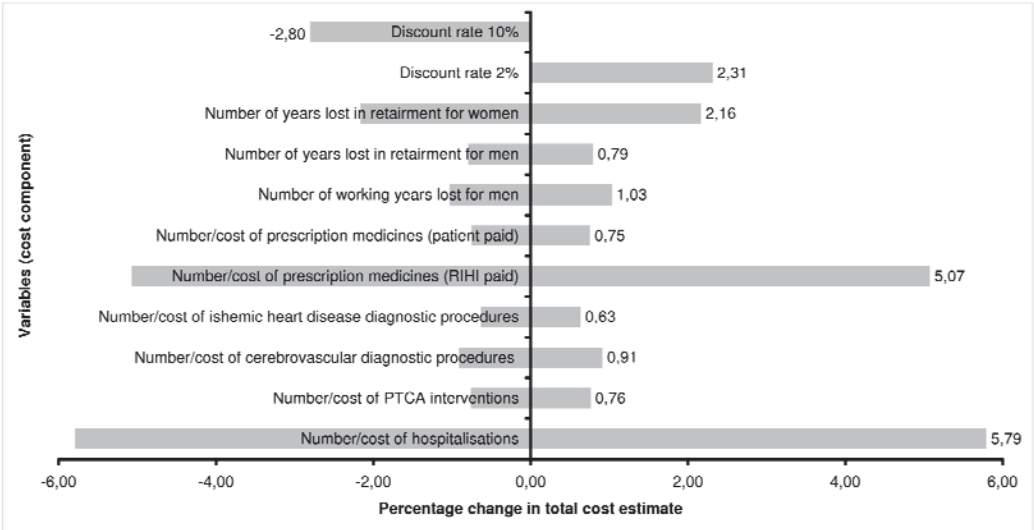

Fig. 2 – One-way sensitivity analyses of direct and indirect costs resulting from a ± 20% change in the volume or the unit price of all factors, and discount rates of 2% and 10%. The sensitivity analyses of the factors that resulted with an change in total cost estimate below ± 0.5% are not shown.

Discussion

CVDs are a costly group of diseases to the health care system. In 2009, the average CVD direct costs per patient were over € 300. The results of the direct costs of CVD show that more than 60% of the costs are attributable to hospitalisation and surgical/diagnostic procedures, while medication treatment represented over 38% of the direct costs. Primary care, which to some extent can be considered as preventive medicine, accounts only for 0.83% of direct costs. In the majority of studies on evaluation of economic burden of CVD, hospital costs were the most expensive direct category, with the values of 50–66% of total direct cost, followed by pharmaceutical expenditures [4, 7, 21, 22]. On the other hand, Maetzel et al. [23] showed that 51.2% of direct costs in hypertension were attributable to drugs, and only 20% to hospitalisation. Primary care accounted for only 8.8% in direct costs in EU, with significant variation between countries, from 0.7% in Greece to 15.9% in Germany [4].

The estimated total indirect costs of CVD in Serbia were almost € 114 million, with more than 90% attributable to mortality. The cost of CVD in EU revealed that almost 70% of indirect costs are attributable to mortality; in some EU countries, like Latvia, mortality represented 90.63% [4].

The total cost of CVD in Serbia was estimated to be over € 514 million. Only a quarter (22.15%) of total cost was estimated to be indirect cost (Table 3). In contrast, studies conducted in UK, Canada, Finland and Mexico [7, 21, 22, 24] noted that much higher percent (in some cases over 50%) of total cost is attributable to indirect costs. Such difference could be explained by much lower average earning than in the above mentioned, Western market economics. Bloom et al. [25], in the review article of published cost-of-illness studies on US populations, estimated that more than 48% of total cost was attributable to indirect costs. A study by Leal et al. [4], showed that daily and annual earnings in newer EU countries (like Estonia, Latvia or Lithuania) in 2003, were more than eight times lower than in older EU countries. Similar results were observed in other transition countries, after conducting the different cost of illness studies [26, 27]. The magnitude of the total costs devoted to CVD prevention and treatment can be best represented as a % of gross domestic product (GDP). According to our results, total cost of CVD comprises approximately 1.78% of the total Serbian GDP [15]. In 2009 the total CVD cost was 3.37% of the total American GDP [1]. On the other hand, results of study conducted in China in 2003, showed that 0.62% of the China GDP was attributable to direct costs only [28].

In Serbia, women were diagnosed more often with CVD, primarily coronary heart disease. Similar results were shown in other studies [1, 23] where the prevalence of CVD was higher in women. As it would be expected the majority of diagnosed patients were older than 70 years. The prevalence of CVD increases with age, from 38.2% in the age group 40–59 to 82.6% among those aged 80 years or older [1].

A sensitivity analysis indicates that volume or cost of hospitalisations and medicines are components which are

most likely to affect estimated total cost but the overall impact is small, less than 6% on total cost estimate (Figure 2).

This study has limitations because estimates of costs are likely to be underestimated. The authors did not include preventive actions like anti-smoking campaigns in the analysis, because of unavailability of the quantity of promotion activities and the amount of money devoted to them in Serbia. A study on coronary heart disease costs in UK [7] estimated that less than 0.002% of total cost was attributable to prevention, so the authors believe that this omission would not affect our results substantially.

The authors analysis also did not consider the cost of patient travel expenses. However these costs make up only a small percentage of CVD costs [29]. The out-of-pocket expenditures for different Over the Counter (OTC) medicines or dietary supplements for CVD were not considered in the analysis due to no published data regarding the consumption. As the authors postulated in the Methods section the sick leave less than 30 days was not included in the study due to the absence of the data. Also, since we used the prevalence, top-down approach, productivity losses were estimated as average earning ad pension. The cost estimate would probably be different if patient population was divided into subgroups according to the education level and socioeconomic status. However, due to the absence of these data, a more accurate estimate was impossible.

Authors did not focus on the clinical guidelines and protocols. It would be interesting for further research to evaluate compliance of the prescribers with the clinical guidelines, especially with the recommendations regarding antihypertensive medications, since our study showed that almost 30% of a total cost is attributable to medications cost.

In spite of its limitations, this is the first cost of illness study that estimated direct and indirect costs associated with CVD in Serbia. Cost of illness studies cannot determine whether healthcare system is spending too much in a particular area, in this case CVD, but it has the potential to identify main cost drivers of the disease. This helps in allocating scant health care resources efficiently, and consequently leads to improved clinical and economic outcomes, reducing the morbidity and mortality of CVD, resulting with the substantial financial savings. Also, the magnitude and pattern of expenditure can guide research priorities and decision makers in the development of better action plan in order to decrease the burden of CVD.

Conclusion

CVD is a high costly group of diseases with the heavy burden to society. The total costs in 2009 represented approximately 1.8% of the Serbian GDP. The authors believe that the results of this study would be of special interest for national health policy makers to bridge the gap between invested resources and needs, in order to improve CVD outcomes. High efforts should be made and taken to prevent CVD in order to reduce medical costs and productivity losses to society.

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REFERENCES

1. *Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K,* et al. Heart disease and stroke statistics-2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2009; 119(3): e21−e181.
2. European health for all database. Available from: http://data.euro.who.int/hfadb/ [accessed 2011 May 26].
3. *Vlajinac H, Marinkovic J, Kocev N, Sipetic S, Bjegovic V, Jankovic S.* Years of life lost due to premature death in Serbia (excluding Kosovo and Metohia). Public Health 2008; 122(3): 277−84.
4. *Leal J, Luengo-Fernandez R, Gray A, Petersen S, Rayner M.* Economic burden of cardiovascular diseases in the enlarged European Union. Eur Heart J 2006; 27(3): 1610−9.
5. Ministry of Health. Final report of population health research in 2006. Available from: http://www.zdravlje.gov.rs/showpage.php?id=142 [accessed 2011 May 26].
6. *Rice D.* Cost-of-illness studies: fact or fiction? Lancet 1994; 344(8936): 1519−20.
7. *Liu JY, Manadakis N, Gray A, Rayner M.* The economic burden of coronary heart disease in the UK. Heart 2002; 88(6): 597−603.
8. *Drummond MF, Sculpher MJ, Torrance GW, O'Brien BJ, Stoddart GL.* Methods for the economic evaluation of health care programes. 3rd ed. New York: Oxford University Press; 2005.
9. National Bank of Serbia. Exchange rates. Available from: http://www.nbs.rs/export/internet/english/80/index.html [accessed 2011 May 26].
10. *Ministry of Health.* National Guideline of Clinical Practice: Prevention of ischemic heart disease Belgrade: Faculty of Medicine University of Belgrade; 2005. (Serbian)
11. *Ministry of Health.* National Guideline of Clinical Practice: Acute ischemic cerebral infraction. Belgrade: Faculty of Medicine University of Belgrade; 2004. (Serbian)
12. *Ministry of Health.* Guidelines for prevention, diagnosis and treatment of patients with chest pain. Belgrade: Faculty of Medicine University of Belgrade; 2002. (Serbian)
13. *Ministry of Health.* National Guideline of Clinical Practice: Arterial hypertension. Belgrade: Faculty of Medicine University of Belgrade; 2005. (Serbian)
14. *Institute of Public Health of Serbia.* Health Statistical Yearbook of Republic Serbia 2009. Available from: http://www.batut.org.rs/download/publikacije/pub2009.pdf [accessed 2011 February 06].
15. Law on Pension and Social Security. ("Official Gazette of the Republic of Serbia" 101/2010). (Serbian)
16. Statistical Office of the Republic of Serbia. Statistical Database. Available from: http://webrzs.stat.gov.rs/WebSite/public/ReportView.aspx [accessed 2011 February 6].

17. Law on healthcare insurance. ("Official Gazette of the Republic of Serbia" 109/2010). (Serbian)
18. *Koopmanschap MA, Rutten FF, van Ineveld BM, van Roijen L.* The friction cost method for measuring indirect costs of disease. J Health Econ 1995; 14(2): 171−89.
19. *Koopmanschap MA, van Ineveld BM.* Towards a new approach for estimating indirect costs of disease. Soc Sci Med 1992; 34(9): 1005−10.
20. *Shaffer PA, Haddix AC.* Time Preference. In: *Haddix AC, Teutsch SM, Shaffer PA, Dunet DO,* editors. Prevention Effectiveness: A Guide to Decision Analysis and Economic Evaluation. New York: Oxford University Press; 1996. p. 76−84.
21. *Chan B, Coyte P, Heick C.* Economic impact of cardiovascular disease in Canada. Can J Cardiol 1996; 12(10): 1000−6.
22. *Kiiskinen U, Vartiainen E, Pekurinen M, Puska P.* Does prevention of cardiovascular diseases lead to decreased cost of illness? Twenty years of experience from Finland. Prev Med 1997; 26(2): 220−6.
23. *Maetzel A, Li LC, Pencharz J, Tomlinson G, Bombardier C.* The economic burden associated with osteoarthritis, rheumatoid arthritis, and hypertension: a comparative study. Ann Rheum Dis 2004; 63(4): 395−401.
24. *Arredondo A, Zuniga A.* Epidemiological changes and economic burden of hypertension in Latin America. Am J Hypertens 2006; 19(6): 553−9.
25. *Bloom BS, Bruno DJ, Maman DY, Jayadevappa R.* Usefulness of US cost-of-illness studies in healthcare decision making. Pharmacoeconomics 2001; 19(2): 207−13.
26. *Grover S, Avasthi A, Bhansali S, Chokrabarti S, Kulhara P.* Cost of ambulatory care of diabetes mellitus: a study from north India. Postgrad Med J 2005; 81(956): 391−5.
27. *Poulos C, Riewpaiboon A, Stewart JF, Clemens J, Guh S, Agtini M,* et al. Cost of illness due to typhoid fever in five Asian countries. Trop Med Int Health 2011; 16(3): 314−23.
28. *Yang L, Wu M, Cui B, Xu J.* Economic burden of cardiovascular diseases in China. Expert Rev Pharmacoecon Outcomes Res 2008; 8(4): 349−56.
29. *Dewey HM, Thrift AG, Mihalopoulos C, Carter R, Macdonell RA, McNeil JJ,* et al. Cost of stroke in Australia from a societal perspective: results from the North East Melbourne Stroke Incidence study (NEMESIS). Stroke 2001; 32(10): 2409−16.

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Cost-effectiveness analysis of tocilizumab in combination with methotrexate for rheumatoid arthritis: A Markov model based on data from Serbia, country in socioeconomic transition

Analiza odnosa troškova i efekata tocilizumaba u kombinaciji sa metotreksatom u lečenju reumatoidnog artritisa: Markovljev model baziran na podacima iz Srbije, države u socio-ekonomskoj tranziciji

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Abstract

Background/Aim. Recent studies have shown that biological treatments for rheumatoid arthritis can change the course of rheumatoid arthritis and improve functional ability of patients with rheumatoid arthritis. In spite of this fact, use of biological therapy is still limited by high prices of these medicines, especially in countries in socioeconomic transition. The aim of our study was to compare cost-effectiveness of a combination of tocilizumab and methotrexate with methotrexate alone for rheumatoid arthritis in Serbia, a country in socioeconomic transition. **Methods.** For the purpose of our study we designed a Markov model using data on therapy efficacy from the available literature, and data on the costs of health states calculated from records of actual patients treated in the Clinical Center Kragujevac, Serbia. The duration of one cycle in our model was set at one month, and the time horizon was 480 months (40 years). The study was done from the social perspective, and all the costs and outcomes were discounted for 3% per year. **Results.** Treating rheumatoid arthritis with disease-modifying antirheumatic drugs (DMARDs) alone was more cost-effective in comparison with a combination of biologic treatment with tocilizumab and DMARDs. The total costs for treating a patient with DMARDs for one year were on average 261,945.42 RSD, or 2,497.70 Euro and the total costs for treatment with tocilizimab plus DMARDs were on average 1,959,217.44 RSD, or 18,659.20 Euro. However, these results are susceptible to changes in costs and treatment effects of tocilizumab in patients with more severe forms of rheumatoid arthritis. **Conclusion.** Our results show that the use of tocilizumab for rheumatoid arthrits in economic environment of Serbia is not cost-effective. Use of tocilizumab for treating rheumatoid arthritis can become affordable, if costs of its use become lower. In order to start using expensive biologic medicines in patients in transitional countries, special strategy and pricing policy of international pharmaceutical companies are necessary, which would include calculation of prices of biologic medicines on the basis of local pharmacoeconomic studies.

Key words:
arthritis rheumatoid; economics, pharmaceutical; biological therapy; methotrexate; serbia.

Apstrakt

Uvod/Cilj. Nedavne studije ukazale su da biološka terapija za reumatoidni artritis može menjati tok bolesti i popraviti funkcionalnu sposobnost obolelih. Uprkos tome, upotreba bioloških lekova ograničena je visokom cenom ovih lekova, posebno u zemljama koje su u socioekonomskoj tranziciji. Cilj ovog istraživanja bio je da se uporede troškovi i efekat kombinacije tocilizumaba i metotreksata sa metotreksatom u terapiji reumatoidnog artritisa u Srbiji, zemlji u socioekonomskoj tranziciji. **Metode.** Za potrebe ovog istraživanja konstruisan je Markovljev model na osnovu podataka o efikasnosti iz dostupne literature, dok su podaci o troškovima za sva zdravstvena stanja procenjeni iz dostupne dokumentacije obolelih od reumatoidnog artritisa koji se leče u Kliničkom centru Kragujevac, Srbija. Jedan ciklus u modelu trajao je jedan mesec, a ukupan vremenski horizont bio je 480 meseci, odnosno 40 godina. Studija je izvedena sa aspekta društva u celini, a svim troškovima i ishodima je pridodata diskontna stopa od 3%. **Rezultati.** Lečenje reumatoidnog artritisa standardnom, nebiološkom terapijom je u pogledu odnosa troškova i efekata povoljnije u poređenju sa biološkom terapijom tocilizumabom u kombinaciji sa standardnom nebiološkom terapijom. Ukupni troškovi lečenja re-

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umatoidnog artritisa standardnom nebiološkom terapijom tokom jedne godine lečenja po bolesniku iznose 261 945,42 dinara Republike Srbije, odnosno 2 497,70 eura, a ukupni troškovi lečenja tocilizumabom u kombinaciji sa standardnom nebiološkom terapijom u toku jedne godine po pacijentu iznose 1 959 217,44 dinara Republike Srbije, odnosno 18 659,20 eura. Ipak, ovi rezultati su podložni promenama i uticaju troškova i efekata terapije tocilizumabom kod bolesnika sa težom formom bolesti. **Zaključak.** Rezultati našeg istraživanja pokazuju da primena tocilizumaba u lečenju reumatoidnog artritisa nije farmakoekonomski isplativa. Primena tocilizumaba za lečenje reumatoidnog artritisa može postati isplativija u farmakoekonomskom smislu, ukoliko cena tocilizumaba postane niža. Upotreba skupe biološke terapije kod obolelih od reumatoidnog artritisa u zemljama u socioekonomskoj tranziciji može biti izvesna jedino uz postojanje posebne strategije i cenovne politike internacionalnih farmaceutskih kompanija, što podrazumeva određivanje cene ovih lekova na bazi lokalnih farmakoekonomskih studija.

Ključne reči:
artritis, reumatoidni; farmakoekonomika; biološka terapija; metotreksat; srbija.

Introduction

Rheumatoid arthritis is a chronic disease characterized by systemic inflammation and continous irreversible destruction of joints mediated with immunological mechanisms [1]. Rheumatoid arthritis affects 0.5–1% of general population, with severe destructions of joints encountered in 15% of patients. The prevalence of rheumatoid arthritis is 3–4 times higher in women than in men, with the tendency to rise with aging [2, 3]. Diagnosis of rheumatoid arthritis is based on the criteria established by the American College of Rheumatology (ACR), and 4 of 7 criteria must be present: morning stiffness, arthritis in 3 or more joint areas, arthritis of hand joints (more than 1 joint), symmetrical arthritis, rheumatoid nodules, elevated serum rheumatoid factor and typical radiographic changes (with exception for the two last criteria, the listed changes must persist for at least 6 weeks) [4]. These criteria have been lately recognized as less sensitive and updated by the European League Against Rheumatism (EULAR) during 2010, with special concern for early arthritis [5]. The Health Assessment Questionnaire (HAQ) is a dominant instrument for capturing a state of disability in patient with rheumatoid arthritis. The HAQ estimates functional status of patients in several domains: disability, pain and discomfort, adverse drug reactions and economic issues of treating rheumatoid arthritis. Nowdays, HAQ is the most widely used techinque for evaluating disability in patients with rheumatoid arthritis [6].

The therapeutic approach in rheumatoid arthritis involves two strategies: to prevent the spread of chronic inflammatory process and to ensure protection of affected joints from further deterioration [7]. Treatment of rheumatoid artritis with standard disease modifying anti-rheumatic drugs (DMARDs), and newer biologic therapy, alone or in combination, has proven efficacy. Among DMARDs, methotrexate, sulphasalasine and leflunomide with their immunosupressant actions have shown the greatest impact on the course of rheumatoid arthritis [8]. Methotrexate is considered to be the gold standard for treatment of rheumatoid arthritis because of its good efficacy and moderate adverse reactions. However, the response to methotrexate is sometimes inadequate or unsatisfying, so biologic medicines remain the only solution [7]. The targets of biologic medicines are different cytokines or their receptors, and these medicines (etanercept, adalimumab, infliximab, tocilizumab, rituximab and others) have shown beneficial effect on the course of rheumatoid arthritis [7, 9, 10]. Biologic therapy use differs among Europian countries and depends mostly on available budgets for buying these medicines. High prices of biologic medicines are the main reason for restrictive utilization of these medicines [11]. The majority of European countries uses similar criteria for reimbursing prescription of a biologic medicine like those recommended by the National Institute for Clinical Excellence (NICE) from U.K.: treatment with biologic medicine (mostly with a TNF blocker) is given to a patient whose response to methotrexate is poor and incomplete; if there is no response to the first biologic medicine after 3 to 6 months of treatment, the patient should be switched to another biologic medicine [12–14].

In spite of large evidence on therapeutic effects of biologic medicines on rheumatoid arthritis, the data is limited to economic aspects of this therapy, especially with newer biologic medicines. The question of cost-effectiveness ratio is important issue nowadays, especially in countries in socioeconomic transition, since introduction of new medicines often means a substantial increase in total health care costs. Economic burden of rheumatoid arthritis involves direct and indirect costs, and it depends mostly on prices of a prescribed medication [11].

The aim of this study was to compare cost-effectiveness of two therapeutic strategies in patients with rheumatoid arthritis: treatment with DMARDs alone or in combination with tocilizumab using a Markov model based on data on efficacy from published clinical trials and costs sampled from the economic environment in Serbia.

Methods

The Markov model was designed in order to compare the cost-effectiveness of two therapeutic strategies for patients with rheumatoid arthritis. The startegies were therapy with DMARDs alone and therapy with a combination of DMARDs and tocilizumab. For the purpose of modelling we presented rheumatoid arthritis as 5 primary health states based on the Health Assesment Questionnaire (HAQ), according to Kobelt et al. [15]. These states reflect chronic course and severity of rheumatoid arthritis: HAQ score less than 0.6, HAQ score from 0.6 to 1.1, HAQ score from 1.1 to 1.6,

HAQ score from 1.6 to 2.1 and HAQ scores higher than 2.1. For every node we added death as potential state. After taking into account activity of the disease, each primary HAQ state was subdivided into two new states: one with high and another with low activity. Each state in the model except death was not definitive, and a hypothetic patient could move from one to another state, depending on natural course of the disease and experiences from clinical trials. The initial patient distribution, transitional probabilities, utilities, and effectiveness of the two treatment options were obtained from the available literature [15–17], while the costs of health states were calculated from records of actual patients treated in Clinical Center Kragujevac, Serbia.

The duration of one cycle in our model was one month, and the time horizon was 480 months (40 years). All the costs and outcomes were analyzed from social perspective and discounted for 3% annually. For the purpose of modelling, we conducted a pilot survey to estimate costs of rheumatoid arthritis. We analyzed all the aspects of economic issues of treating rheumatoid arthritis. Using interview techniques we collected data from our patients about direct (costs of medicines, hospitalization, diagnostic procedures, medical exams etc.) and indirect costs (costs of transportation, lost wages etc.) of treating rheumatoid arthritis. All the costs were expressed in 2010 Serbian dinars (RSD) and the data on health services utilization were collected from files of rheumatoid arthritis patients, for each HAQ state and disease activity score separately. The patients were randomly chosen from the population of patients with rheumatoid arthritis treated in the Clinical Center Kragujevac, Serbia, during one year (from June 2009 to June 2010). The prices of health services were obtained from the Republic Institute for Health Insurance (RIHI) Tariff Book and prices of medicines were those from the list of medicines financed by the RIHI, issued in 2010 [18]. The process of modelling requires a definition of willingness to pay, i.e. how much a society is willing to pay for one quality-adjusted life year (QALY) gained with certain treatment of the disease. For societies in socioeconomic transition there is a recommendation from the World Bank that the value of willingness to pay should be equal to two to three multiples of gross national income *per* capita. In case of Serbia, gross national income *per* capita (GDP/capita) was 563,400 dinars (RSD) in 2009 [19]. We also used the value of average monthly net income in Serbia during 2009 to calculate the costs of lost wages.

The model was constructed using TreeAge pro® software, version 2006 [20]. We performed Monte Carlo simulations using microsimulation trial, where cohorts of virtual patients, which consist of 1,000 virtual patients, passed through all hypothetical scenarios. The model of Monte Carlo simulation randomly chooses patients from the cohort, and every patient from the cohort runs through different scenarios and results are the summaries as incremental cost-effectiveness ratio [21–23]. For each therapeutic option we calculated the mean costs and the mean effects, and expressed them also as incremental cost-effectiveness ratio. We followed the following outcomes: gains in utility for each therapy option, expressed as QALYs gained, and total and mean costs incurred by each therapeutic option. Incremental cost effectiveness ratio for tocilizumab *vs* DMARDs therapy as baseline was also calculated. Two-way sensitivity analysis (± 50% of baseline values of a variable) was performed in order to check for robustness of the model results, and its outcome is shown as a Tornado diagram.

Results

Treating rheumatoid arthritis with DMARDs alone was more cost-effective than a combination of biologic treatment with tocilizumab and DMARDs. The total costs for treating a patient with DMARDs for one year (2009–2010) were on average 261,945.42 RSD, or 2,497.70 Euro (on August 12, 2010) and total costs for treatment with tocilizimab plus DMARDs were on average 1,959,217.44 RSD, or 18659,20 Euro (on August 12, 2010) (Figure 1).

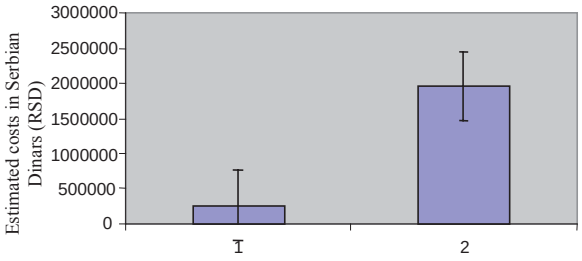

Fig. 1 – Total costs for one year-treatment (2009–2010) *per* patient for DMDARs and a combination of tocilizumab and metotrexate (prices on August 12, 2010).

Using the cost-effectiveness calculation method we compared total costs per QALY gained for both examined therapeutic options. The results of this method indicate that standard non-biological therapy requires much less investment than therapy with a combination of tocilizumab and methotrexate for higher gain in QALY. Treatment with standard non-biological therapy for gain of one QALY requires investment of 1,446,640.78 RSD, which is more cost-effective than treatment with tocilizumab and methotrexate together which costs 6,171,321.57 RSD *per* QALY gained. The results of cost-effectiveness analysis are shown in Table 1.

The distribution of incremental cost-effectiveness ratios (ICERs) calculated by Monte Carlo simulations (using a cohort of 1,000 virtual patients) for total costs *per* QALY is shown in Figure 2. For therapeutic option combination of tocilizumab and metotrexate the calculated ICERs (with only methotrexate as baseline comparator) for the majority of virtual patients fall on the left side of willingness-to-pay line, which indicates that this kind of biological therapy for rheumatoid arthritis in Serbian socioeconomic enviroment is not cost-effective.

In order to check robustness of our conclusion, we made two-way sensitive analysis using a Tornado diagram. In this analysis, all the parameters were varied simultaneously in the range ± 50%. The most influential variables

Table 1

Cost effectiveness analysis of the two therapeutic strategies: disease modifying anti-rheumatic drugs (DMARDs) only and tocilizumab in combination with methotrexate

Therapeutic option	Costs (RSD)	The difference in costs (RSD)	Effectiveness expressed in quality adjusted life years (QALY)	The difference in effectiveness (QALY)	Cost-effectiveness ratio (RSD/QALY)	Incremental cost effectiveness ratio (ICER)
DMDARs	7.788.768,97		5.38		1.446.640,78	
Tocilizumab + methotrexate	20.731.954,15	12.943.185,18	3.36	-2.02	6.171.321,38	(Dominated)

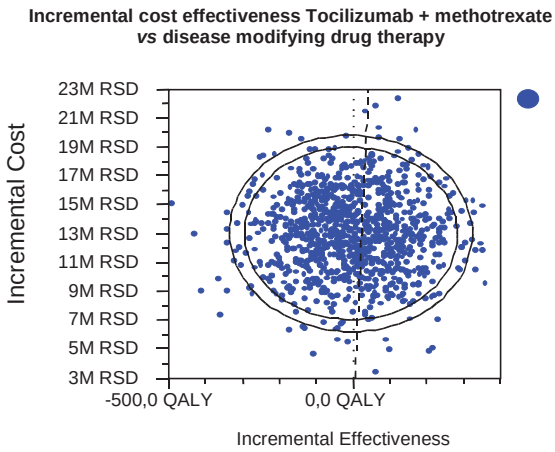

Fig. 2 – Distributions of the incremental cost-effectiveness ratio (ICER) calculated by Monte Carlo simulation for the total costs *per* quality-adjusted life years (QALY) for tocilizumab and metotrexate comparing with the standard nonbiological therapy

were those which describe state with HAQ score higher than 2.1: the costs for non-biological therapy for HAQ states higher than 2.1, discount rate for the costs, the costs for biological therapy for HAQ state higher than 2.1 with low activity of the disease, utility score for HAQ state higher than 2.1 with high activity of the disease and discount rate for effects of the treatment. With changes in these variables, the value of the net monetary benefit becomes negative, within the range from -7.3 to -2.8 milions of Serbian dinars, which means that our conclusion is susceptible to changes in costs and treatment effects of tocilizumab in patients with more severe forms of rheumatoid arthritis.

Discussion

Efficacy of tocilizumab has already been tested in a recent randomized controlled clinical trial, and because it achieved a significant benefit on the course of rheumatoid arthritis, it was approved for treatment of rheumatoid arthrits in Europian Union [24, 25]. Nevertheless, pharmacoeconomic studies on tocilizumab in rheumatoid arthritis have not been conducted to this date, and certainly not based on socioeconomic enviroment of Balkan countries in transition from controlled economy to free market.

The use of biological therapy for rheumatoid arthritis is not common within the Serbian health system, and is limited mostly by high prices of these medications and restrictive treatment guidelines. Similiar experience has been gained by physicians and patients in most countries of Balkan region [11].

The results of our model suggest that therapy with a combination of tocilizumab and methotrexate in comparison with methotrexate alone is not cost-effective. A gain in QALYs is lower and costs are higher with tocilizumab and methotrexate together than with methotrexate alone. Apart from relatively moderate clinical effect of tocilizumab, an important reason for such an outcome was a large disproportion between prices of medicines, which are almost the same in Serbia and in developed European countries, and prices of health care services, which are 10–100 times lower in Serbia than in developed countries. Therefore, beneficial effects of tocilizumab on decrease in health care utilization do not translate to significant savings in costs. The prices of health care services in Serbia are controlled by the RIHI, which publishes them periodically in its internal publications, which are not accessible to general public. To show how unrealistic these prices are, we mention the prices of one hospital day for basic care, which range from 10 to 20 euro, depending on the branch of medicine. Actually, we have two systems operating in the same time: free market rules for medicines, and controlled economy rules for health care services. Such duality inevitably creates paradoxical results of health economics studies situated in Balkan countries in socioeconomic transition.

The sensitivity analysis shows that this conclusion could be changed if the effectiveness of tocilizumab is increased in more severe forms of rheumatoid arthritis (which is unlikely to happen, since the degree of efficacy was well-established) or if the price of tocilizumab goes down and the prices of health care services go up. The second option (decrease in the price of medicine) could happen if the producer of tocilizumab finds interest to increase the volume of its sales in transitional and other poor countries and maintain profit selling more of the less expensive medicine. For the time being, this is also the only option how patients with severe forms of rheumatoid arthritis in transitional Balkan countries could reach this effective but very expensive medicine.

In order to start using expensive biologic medicines in patients in transitional countries, special strategy and pricing policies of international pharmaceutical companies are necessary. The prices of biologic medicines should be calculated on the basis of local pharmacoeconomic studies (like this

one), up to the point where the prices make the studies outcomes cost-effective. This would provide for the acceptance of such medicines by the local health insurance funds and sufficient financing to make registration of the medicine in such a country profitable for the pharmaceutical companies [26].

Conclusion

Due to the progressive nature and chronic course of rheumatoid arthritis, it is important to estimate cost-effectiveness of new medicines for rheumatoid arthritis by pharmacoeconomic modelling. Cost-effectiveness ratio of tocilizumab could be acceptable if the price of tocilizumab reaches a lower value. Further research is necessary to indentify a subset of patients with rheumatoid arthritis in which tocilizumab could be cost-effective therapy.

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R E F E R E N C E S

1. *Firestein GS*. Evolving concepts of rheumatoid arthritis. Nature 2003; 423(6937): 356–61.
2. *Firestein GS*. Rheumatoid arthritis. In: *Ruddy S, Harris ED, Sledge CB, Budd RC, Sergent JS,* editors. Kelley's Textbook of rheumatology. 6th ed. Philadelphia: W.B. Saunders Company; 2001. p 921−67.
3. *Bush M, Emery P*. The aetiology and pathogenesis of rheumatoid arhtritis. Hospital Pharmacist 2002; 9: 5−11.
4. *Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS,* et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3): 315−24.
5. American College of Rheumatology. The 2010 ACR-EULAR classification criteria for rheumatoid arthritis [Internet]. Available from: http://www.rheumatology.org/practice/clinical/classification/ra/ra_2010.asp [updated 2011 January 24].
6. *Bruce B, Fries JF*. The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation. J Rheumatol 2003; 30(1): 167−78.
7. *Schett G, Stach C, Zwerina J, Voll R, Manger B*. How antirheumatic drugs protect joints from damage in rheumatoid arthritis. Arthritis Rheum 2008; 58(10): 2936−48.
8. *van Vollenhoven RF*. Treatment of rheumatoid arthritis: state of art 2009. Nat Rev Rheumatol 2009; 5: 531−41.
9. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, et al. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998; 41(9): 1552−63.
10. *Fan PT, Leong KH*. The use of biological agents in the treatment of rheumatoid arthritis. Ann Acad Med Singapore 2007; 36(2): 128−34.
11. *Kobelt G, Kasteng F*. Access to innvative treatments in rheumatoid arthritis in Europe. A report prepared for the Europian Federation of Pharmaceutical Industry Associations (EFPIA). Stockholm: Lunds University; 2009.
12. National Institute for Health and Clinical Excellence. Nice clinical guideline 79. The reumatoid arthritis. The menagment of rheumatoid arthritis in adults 2009. Available from: www.nice.org.uk/CG79 [cited 2010. October 21].
13. National Institute for Health and Clinical Excellence. Nice clinical guideline 130. Adalimumab, etanercept and infliximab for the treatment of rheumatoid arthritis 2010. Available from: www.nice.org.uk/TA130 [cited 2010. October 21].
14. National Institute for Health and Clinical Excellence. NICE technology appraisal guidance 195. Adalimumab, eternecept, infliximab, rituximab and abatacept for the treatment for rheumatoid arthritis after the failure of a TNF inhibitor 2010. Available from: www.nice.org.uk/guidance/TA195 [cited 2010. October 21].
15. *Kobelt G, Lindgren P, Singh A, Klareskog L*. Cost effectiveness of etanercept (Enbrel) in combination with methotrexate in the treatment of active rheumatoid arthritis based on the TEMPO trial. Ann Rheum Dis 2005; 64(8): 1174−9.
16. *St. Clair EW, Pisetsky DS, Haynes BF*. Rheumatoid arthritis. Philadelphia: Lippincott Williams & Wilkins; 2004.
17. *Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E*, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. Lancet 2008; 371(9617): 987−97.
18. Tariff book of health care services in health facilities of Republic of Serbia. Belgrade: Republic Institute for Health Insurance; 2008.
19. The World Bank. Data and Statistics. Available from: hptt://web.worldbank.org./wbsite/external/datastatistics/0. [updated 2010 September 12].
20. Tree Age Pro. Healthcare software. Tree Age Software Inc [release 2005,1998-2005]. Available from: www.softscout.com/software/healthcare-and-Human Services/Diagnosis
21. *Janković S*. Modelling in Pharmacoeconomy In: *Prostran M, Stanulović M, Marisavljević D, Đurić D,* editors Pharmaceutical Medicine. Beograd: Hemofarm AD Vršac; 2009. p. 526−33. (Serbian)
22. *Drummond MF, Barbieri M, Wong JB*. Analytic choices in economic models of treatments for rheumatoid arthritis: What makes a difference? Med Decis Making 2005; 25(5): 520−33.
23. Markov Modeling. In *Rascati K*, editor. Essentials of Pharmacoecmics. Philadelphia: Lipincott Williams&Wilkins; 2009. p. 155−75.
24. *Nishimoto N, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, Azuma J*. Long-term safety and efficacy of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in monotherapy, in patients with rheumatoid arthritis (the STREAM study): evidence of safety and efficacy in a 5-year extension study. Ann Rheum Dis 2009; 68(10): 1580−4.
25. National Institute for Health and Clinical Excellence. NICE technology appraisal guidance 198. Tocilizumab for the treatment of rheumatoid arthritis. Available from: http://guidance.nice.org.uk/TA198/Guidance/pdf/English [cited 2011 August 23].
26. The Prices of Medication in Serbia, from July/2010. ("RS Official Gazette", No 45/2011). (Serbian)

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ORIGINAL ARTICLE

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The role of biochemical markers as early indicators of cardiac damage and prognostic parameters of perinatal asphyxia

Uloga biohemijskih markera kao ranih indikatora oštećenja srca i prognostičkih parametara perinatalne asfiksije

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Abstract

Background/Aim. In recent years, the focus of interest of the scientific community is the application of heart markers as early indicators and prognostic parameters of perinatal asphyxia (PA). The aim of this study was to evaluate the significance of clinical application of heart markers in term newborns with perinatal asphyxia. **Methods.** During a 3-year period we analyzed 91 full-term newborns (55 with and 36 without perinatal asphyxia). In all the subjects within the first 24–48 h after birth, we simultaneously determined serum concentrations of cardiac troponin I, brain natriuretic peptide, MB fraction of creatine kinase (CK-MB) and C-reactive protein. **Results.** In the group of full-term neonates with PA significantly higher levels of cardiac troponinI (p = 0.000), CK-MB fraction (p = 0.000), brain natriuretic peptide (p = 0.003) and C-reactive protein (p = 0.017) were found, compared to the group of healthy full-term newborns. In merged group (n = 91) cardiac troponin I level correlated with the fifth minute Apgar score (r = -0.637, p = 0.000) and the serum lactate concentration in the first 12h after birth (r = 0.529, p = 0.000). Early increase in cardiac troponin I > 0.135 μg/L predicted the risk of death with the sensitivity of 84.6% and specificity of 85.9%, while the increase in CK-MB fraction, brain natriuretic peptide and C-reactive protein did not have a predictive value with respect to a mortality outcome. **Conclusion.** Among the tested cardiac markers, cardiac troponin I is the most sensitive and the only reliable early predictor of mortality in full-term neonates with perinatal asphyxia.

Key words:
perinatology; asphyxia; biological markers; heart failure; troponin I; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Poslednjih godina u žiži interesovanja naučne javnosti je primena srčanih markera kao ranih indikatora i prognostičkih parametara perinatalne asfiksije. Cilj ovog rada bio je da se proceni značaj kliničke primene srčanih markera kod terminske novorođenčadi sa perinatalnom asfiksijom. **Metode.** Tokom trogodišnje studije analizirano je 91 terminsko novorođenče (55 sa i 36 bez perinatalne asfiksije). Kod svih ispitanika određivana je simultano serumska koncentracija srčanog troponina-I, moždanog natriuretskog peptida, MB frakcije kreatin kinaze i C-reaktivnog proteina u prvih 24–48 h po rođenju. **Rezultati.** U grupi terminske novorođenčadi sa perinatalnom asfiksijom registrovan je značajno viši nivo srčanog troponina-I (p = 0,000), CK-MB frakcije (p = 0,000), moždanog natriuretskog peptida (p = 0,003) i C-reaktivnog proteina (p = 0,017), u odnosu na grupu zdrave, terminske novorođenčadi. Nivo srčanog troponina-I bio je u korelaciji sa Apgar skorom u petom minutu (r = -0,637; p = 0,000) i koncentracijom laktata u prvih 12 h po rođenju (r = 0,529; p = 0,000). Rani porast srčanog troponina-I > 0,135 μg/L ukazivao je na rizik od smrtnog ishoda, sa senzitivnošću 84,6% i specifičnošću 85,9%, dok porast CK-MB frakcije, moždanog natriuretskog peptida i C-reaktivnog proteina nije bio pouzdan prediktor mortaliteta. **Zaključak.** Srčani troponin-I je najsenzitivniji i jedini pouzdan prediktor mortaliteta kod terminske novorođenčadi sa perinatalnom asfiksijom.

Ključne reči:
perinatologija; gušenje; biološki pokazatelji; srce, insuficijencija; troponin I; testovi, prognostička vrednost.

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Introduction

Three groups of cardiac markers are routinely used in adult clinical cardiology: markers of cardiac function, markers of necrosis and inflammation markers [1]. Markers of cardiac function (cardiac natriuretic peptides) are used in diagnosis, monitoring, prognosis and treatment of heart failure [1, 2]. Markers of myocyte necrosis, cardiac troponin I (cTnI) and cardiac troponin T (cTnT), are included in the new international guidelines for diagnosis and treatment of acute myocardial infarction [1, 3]. Markers of inflammation, particularly C-reactive protein (CRP), play an important role in risk stratification and application of appropriate therapy in acute coronary syndrome [1, 4].

Acute coronary syndrome (ACS) refers to a spectrum of clinical presentations ranging from unstable angina, to non-ST-segment elevation myocardial infarction (NSTEMI) and, finally, to ST-segment elevation myocardial infarction (STEMI). Patients with unstable angina can be separated from those with NSTEMI by measuring the levels of troponin as cardiac-specific markers which can reveal minimal (microscopic) myocardial necrosis. With that in mind, it is of paramount importance to determine troponin reference values and detection limits, which is the subject of extensive evaluation and standardization at the international level [5].

The application of biochemical markers in perinatal asphyxia (PA) has not been sufficiently studied in the literature. McAuliffe et al. [6] in the study of 110 infants, mean gestational age of 39.9 weeks (33.4–42.3 weeks), were analyzed for cTnI levels and correlated with intrapartal risk factors and blood pH levels. These authors found a significant difference in the level of cTnI in the observed groups. In 84/110 (76%) of the newborns they found the mean value (median) of cTnI to be 0.03 ng/mL (0.03–0.881 ng/mL) and in 26/110 (23.6%) it was 0.5 ng/mL (0.00–4.3 ng/mL). Also, they found that 90 percentile for healthy neonatal population represents the value of cTnI < 0.05 ng/mL, reported in 12/110 (10.9%) of respondents, on the basis of their results. In 5/110 (4.5%) of respondents in this study, the cTnI value was registered > 0.1 ng/ml, which according to the reference values for adults, might indicate cardiac morbidity and significant consequences. Comparing the group of patients with normal and high concentrations of cTnI, McAuliffe et al. [6] noted significantly lower blood pH (7.24 ± 0.09) in the patients with intrapartal risk factors and high levels of serum cTnI, compared with the group of healthy term newborns (pH = 7.32 ± 0.07). The results of these and other authors suggested that troponin I could be used as early indicator of PA [6–10].

Most of the neonatal studies are focused on changes in serum concentrations of cardiac troponins (cTnI/cTnT) and natriuretic peptides (BNP/NT-pro-BNP) with respect to gestational age, patent ductus arteriosus, degree of respiratory distress syndrome and/or perinatal asphyxia, applied ventilatory, inotropic and other therapies. It was found that the increase in these biochemical markers was often in proportion with the diameter of patent ductus arteriosus, degree of respiratory distress syndrome and perinatal asphyxia, hypoxic ischemic encephalopathy and other serious conditions which are the cause of significant mortality and long-term morbidity [7, 11–18].

In adult patients with chronic heart failure, increased levels of cardiac troponins or BNP / NT-pro-BNP have a prognostic significance. High values of these biomarkers were demonstrated to be associated with higher mortality and higher rates of repeated hospitalizations [1, 3, 19–22]. Elevated levels of these biomarkers in acute coronary syndrome were also associated with increased mortality and increased incidence of recurrent ischemic events [23, 24].

In neonatal studies, increased BNP / NT-pro-BNP levels, as markers of heart failure, positively correlated with elevated levels of serum troponins as markers of myocyte necrosis, indicating severe myocardial damage with possible fatal outcome [13].

C-reactive protein is a highly sensitive but not a specific marker of acute inflammation [4, 25]. In newborn infants infection is a common cause of neonatal morbidity and mortality. Early and rapid diagnosis of systemic infection is very important for the timely treatment, and the role of CRP, as an early marker of inflammation, is very important. On the other hand, a well-known prognostic significance of increased CRP levels in adults with coronary ischemia open the discussion on the possibilities of implementing CRP as a prognostic marker of post-asphyctic myocardial lesions in newborns [25].

The aim of this study was to assess whether there is a significant difference in serum cardiac troponin I, creatine kinase MB fraction, brain natriuretic peptide and CRP between the groups of term neonates, with and without PA. The aim of this study was also to precisely determine the predictive value of each of the above mentioned biomarkers with respect to fatal outcome in the examined group of term newborns with PA.

Methods

This study was conducted at the Center of Neonatology, Pediatric Clinic and Maternity Gynecology and Obstetrics Clinic, Clinical Centre Kragujevac, during the period August 2007 – January 2010. The study was retrospective-prospective and non-interventional. Not a single diagnostic procedure was performed solely for the purpose of the study but was conducted within the framework of referent neonatal protocols and was approved both by the parents written consent, and the Ethics Committee, Clinical Center in Kragujevac, No. 01-613.

A previously conducted pilot study, determined that to get a statistically significant difference in the level of troponin I compared to the group of neonates without PA (power of the study 80%, statistical significance 0.05), the minimal number of examinees in the group of neonates with PA was 36.

During a 3-year study we analyzed 108 subjects, 17 neonates were excluded from the study. Exclusion criteria were: proven congenital heart defect (1 hypoplastic left heart syndrome, ventricular septal defect and pulmonary artery

stenosis and 2 atrial septal defects), chromosomal aberrations (1 Edwards and 2 Downs syndrome), and conatal sepsis (9 patients with positive blood cultures).

The study included a total of 91 full-term neonates (55 with and 36 without perinatal asphyxia). The clinical diagnosis of PA was based on the criteria Caliskan et al. [12] and Zupan Simunek [26].

Inclusion criteria for the study were: the history of fetal asphyxia and gynecology/obstetric complications; cardiorespiratory and neurological depression defined by Apgar score < 4 in the 1st minute and < 7 in the 5th minute after delivery; metabolic acidosis (defined as a lactate level > 3.7 mmol/L in the first 1–12 hours after birth); respiratory distress; convulsions, coma or hypotonia in the first 48 h after birth; hypotension and/or oliguria; multiorgan failure.

Following variables were analyzed in both the groups of examinees: the 5th minute Apgar score; blood lactate levels in the first 1–12 h after birth (capillary blood sample, analyzed by a gas analyzer Gem Premier 3000; reference values 0.3–3 mmol/L); serum level of the second generation troponin I (cTnI-Ultra) determined simultaneously with other biomarkers (CK-MB, BNP and CRP) in the first 24–48 h after birth [enzyme-linked immunosorbent method on a Biomerieux mini Vidas ELFA ("enzyme-linked fluorescent assay")]. For this type of analyzer in the adult population, normal values (99. percentile) of serum level of cTnI-Ultra were < 0.01 μg/L with coefficient of variation of 10% (from 0.01 to 0.11 μg /L) [27]. For the neonatal population, the reference value for the second-generation cTnI is still not known, whereas the first generation cTnI reference range is from 0.01 to 2.8 μg/L, depending on authors [28–30]; creatine kinase MB fraction (CK-MB) level was determined by a biochemical analyzer Beckman Coulter. For such analysis, the adult population reference range is 2–25 U/L, while for the neonatal population 95th percentile for healthy full-term newborns is 72 U/L [10]; The level of "brain" natriuretic peptide (BNP) was determined from the same sample of blood on the immunochemical analyzer Axsym. In the adult population BNP reference value is < 108 pg/mL while in the neonatal population it varies from 231.6 ± 197.5 pg/mL in the first week of life, to 48.449 ± 49.1 pg/mL in the later period [31]; serum concentration of C-reactive protein (CRP) was determined by the biochemical analyzer Beckman Coulter. The reference value in the Clinical Center Kragujevac laboratory, irrespective of age, is < 5 ng/mL.

To analyse of basic respondent's clinical characteristics we used descriptive statistics – mean and standard deviation. To display the mean values of biochemical markers and other variables, whose distribution was not normal we used descriptive statistics – median and quartiles. To compare the mean values of variables two populations were used: Mann-Whitney-test and ANOVA. The correlation of two numerical characteristics was examined using Spearman's and Pearson's correlation coefficient. The suitability of numeric variables was tested using ROC (receiver operating characteristic) curves.

Results

In the group of 55 asphyxiated newborn infants there were 31 (56.4%) males and 24 (43.6%) females. The average gestational age (GA) was 39.5 ± 1.3 weeks and the average birth-weight (BW) 3429 ± 571 g. All of them presented with fetal distress syndrome and/or abnormal obstetric history. Furthermore, all had clinical signs of cardiorespiratory and neurological disability [i.e., Apgar score recorded at the first minute < 4, Apgar score at the 5th minute < 7, seizures (< 48 h after birth), hypotonia or comma, hypotension and/or oliguria and multiorgan dysfunction with postnatal blood lactate level > 3.7 mmol/L (1–6 h after birth)]. Nineteen out of 55 newborns (34.5%) were delivered by caesarean section. Thirty-one out of 55 (56.4%) newborns required respiratory and 23 (41.8%) pressure support; 13 (23.6%) had critical cardiorespiratory problems or multiorgan dysfunction and died. The median 5th minute Apgar score in this group of newborn infants was 5 (range 3–7), and mean value of lactate levels 8.63 ± 4.43 mmol/L. Median CRP concentration was 4.2 mg/L (range 1.9–12.1 mg/L). The median value of CK in the same group of neonates was 1,550 U/L (range 608–4736 U/L) and the mean value of CK-MB fraction 240.7 ± 212.1 U/L. The median level of cTnI in the group of asphyxiated newborn infants (both survived and non-survived) was 0.08 μg/L (range 0.02–0.17 μg/L).

In the control group of 36 non-asphyxiated healthy newborn infants there were 17 males and 19 females. The average BW was 3,455 ± 352 g and GA 39.8 ± 1.1 weeks. All the participants had Apgar score > 8 at 5th minute (median 9) and their lactate levels were 1.04 ± 0.36 mmol/L.

Table 1 shows the average serum concentrations of the analyzed biochemical markers in the groups of full-term newborns with and without perinatal asphyxia, measured during the first two days of life. There was a statistically significant difference in concentrations of all the investigated biochemical markers (CRP, cTnI, CK-MB and BNP) between the examined groups of neonates.

Table 1

Average value of the analyzed biochemical markers in the observed groups

Analyzed biochemical markers	Asphyxiated newborns (n = 55)	Healthy newborns (n = 36)	*p*
Lactate (mmol/L), $\bar{x}$ ± SD	8.63 ± 4.43	1.04 ± 0.36	0.000
C-reactive protein (mg/L)	4.20 (IQR 1.9–12.1)	2.60 (IQR 0.8–4.8)	0.017
Cardiac troponin I (μg/L)	0.08 (IQR 0.02–0.17)	0.01 (IQR 0.01–0.01)	0.000
Creatine kinase-MB (U/L), $\bar{x}$ ± SD	240.69 ± 212.13	78.83 ± 39.14	0.000
B-type natriuretic peptide (pg/mL), $\bar{x}$ ± SD	993.05 ± 1259.51	278.98 ± 190.47	0.003

***p* – statistical significance; IQR – interquartile range.**

In both groups of patients (n = 91), all the investigated biochemical parameters (CRP, cTnI, CK-MB and BNP) correlated with the parameters of perinatal asphyxia (5th minute Apgar score and lactate concentration) (Table 2). However, in the group of full-term neonates with perinatal asphyxia, 5th minute Apgar score and lactate concentration significantly correlated only with cTnI and CK-MB levels (Tables 2 and 3).

CTnI level negatively correlated with the 5th minute Apgar score (unified group r = -0.637; group with PA r = -0.318), and positively correlated with the serum lactate level (r = 0.529 in unified group and r = 0.399 in PA group) and the concentration of CK-MB (r = 0.507 in the unified group and r = 0.410 in the PA group). The correlation between cTnI and CRP was less pronounced (unified group r = of 0.345, a PA group r = 0.290), whereas there was no correlation between the concentrations of cTnI and BNP (unified group r = 0.279; group with PA r = 0.115) (Tables 2 and 3).

Table 2

Correlation of perinatal asphyxia indicators with cardiac damage parameters showed by correlation coefficients (r) in the merged group (newborns with and without perinatal asphyxia) (n = 91)

Analyzed markers	Lactate	CRP	cTnI	CK-MB	BNP
Apgar scores at 5th min	r = -0.839 p = 0.000	r = -0.228 p = 0.032	r = -0.637 p = 0.000	r = -0.449 p = 0.000	r = -0.341 p = 0.022
Lactate (mmol/L)	–	r = 0.348 p = 0.001	r = 0.529 p = 0.000	r = 0.533 p = 0.000	r = 0.613 p = 0.000
CRP (mg/L)	–	–	r = 0.345 p = 0.001	r = 0.337 p = 0.002	r = 0.340 p = 0.021
cTnI (μg/L)	–	–	–	r = 0.507 p = 0.000	r = 0.279 p = 0.061
CK-MB (U/L)	–	–	–	–	r = 0.405 p = 0.005

***p* – statistical significance; CRP – C-reactive protein; cTnI – cardiac troponin I; CK-MB – creatine kinase-MB; BNP – B-type natriuretic peptide.**

In the merged group of patients (n = 91) CRP (p = 0.181) and BNP (p = 0.095) there were no reliable predictors of death. CK-MB had a borderline predictive value for a mortality outcome (p = 0.017). Among the tested biochemical markers only the cardiac troponin I with the area under the ROC curve of 0.896 and serum lactate levels with an area under the ROC curve of 0.894 had a highly significant predictive value for fatal outcome (p = 0.000) (Table 4). For a threshold of 0.135 mg/L, cTnI was a predictor of death with sensitivity of 84.6% and specificity 85.9%.

Table 3

Correlation of indicators of perinatal asphyxia with cardiac damage parameters showed by correlation coefficients (r) in the group with perinatal asphyxia (n = 55)

Analyzed markers	Lactate	CRP	cTnI	CK-MB	BNP
Apgar score 5th min	r = -423 p = 0.000	r = -0.086 p = 0.541	r = -0.318 p = 0.019	r = -0.286 p = 0.051	r = -0.408 p = 0.148
Lactate (mmol/L)	–	r = 0.242 p = 0.090	r = 0.399 p = 0.004	r = 0.318 p = 0.035	r = 0.494 p = 0.073
CRP (mg/L	–	–	r = 0.290 p = 0.033	r = 0.279 p = 0.057	r = 0.203 p = 0.469
cTnI (μg/L)	–	–	–	r = 0.410 p = 0.004	r = 0.115 p = 0.684
CK-MB (U/L)	–	–	–	–	r = 0.277 p = 0.317

***p* – statistical significance; CRP – C-reactive protein; cTnI – cardiac troponin I; CK-MB – creatine kinase-MB; BNP – B-type natriuretic peptide.**

Table 4

Analysis of serum biochemical markers after asphyxia as a predictor of death by ROC (receiver operating characteristic) curve in the merged groups of patients (n = 91)

Analyzed biochemical markers	AUC	Statistical values			
		p	Cut-off	Sensitivity	Specificity
Lactate (mmol/L)	0.894	0.000	8.65	83.3%	84.0%
CRP (mg/L)	0.616	0.181	5.0	61.5%	71.4%
Troponin I (μg/L)	0.896	0.000	0.135	84.6%	85.9%
CK-MB (U/L)	0.717	0.017	126	83.3%	63.9%
BNP (pg/mL)	0.791	0.095	372.45	100%	65.1%

***p* – statistical significance; AUC – area under the curve; CRP – C-reactive protein; cTnI – cardiac troponin I; CK-MB – creatine kinase-MB; BNP – B-type natriuretic peptide.**

Discussion

The diagnosis of myocardial damage in newborn infants was previously based on clinical examination, suggestive electrocardiographic or echocardiographic examinations and increasing value CK-MB isoenzyme. Numerous studies have shown that CK-MB isoenzyme, and particularly total creatine kinase, cannot be regarded as specific cardiac enzymes in the neonatal period, but the interpretation of their increasing concentrations in infants must be viewed with extreme caution [32].

Major goals of neonatal studies were both to define normal values of cardiac biochemical markers in the neonatal population, and to evaluate factors that may have impact on their serum concentrations [6–10, 28–30].

In our study the reference value of troponin I Ultra in healthy newborn infants was 0.0183 ± 0.026; mediana 0.01 (0.01–0.01) μg/L. A statistically significant higher mean concentration of cTnI and others investigated biochemical markers of perinatal asphyxia (lactate, CRP, CK-MB and BNP) was found in the group of asphyxiated full-term newborn infants compared to the group of healthy full-term neonates. Similar to our results, Costa et al. [33] and Rajakumar et al. [34] in two separate studies found a correlation of increased cTnT and signs of myocardial damage in newborns with PA. Szymankiewicz et al. [35] studied 39 asphyxiated newborn versus 44 nonasphyxiated newborns and tried to relate the cTnT to echocardiographic findings of myocardial damage. The cTnT was measured within 12 and 24 hours of life. Asphyxiated infants had higher levels of cTnT (0.141 versus 0.087 ng/mL) nonasphyxiated infants ($p < 0.01$).

In asphyxiated newborns heart failure is the consequence of "hypoxic-ischemic lesions or hypotensive necrosis" [33], and it can accurately be assessed through measurement of cardiac troponin I serum concentrations. At birth, cardiac troponin I is not found in skeleton muscles and other tissues, but only in the myocardium, and its level does not change under the influence of regenerative or degenerative processes in muscles [30, 32, 33]. Iacovidou et al. [36] analyzed changes in the level of cTnI in fetuses with intrauterine arrest, due to chronic malnutrition and hypoxia of the fetus, and found a correlation between cTnI levels in neonates and pregnant women, assuming that the increase in neonatal cTnI level is the result of transplacental cTnI transit from mother to fetus. On the other hand, Trevisanuto et al. [37], similar to our results, showed a significant increment in cTnI level in asphyxiated newborn infants. Comparing levels of cTnI in asphyxiated full-term neonates (gestational age 34–40 weeks), with cTnI levels in serum of their mothers, these authors found no significant association, which is similar to the results of Alexandre et al. [38], who also founded that transplacenar cTnI passage is not possible. Based on such findings Trevisanuto et al. [37], concluded that increased cTnI level is not related to the mother, but is strictly the consequence of increased fetal and neonatal production due to organ lesions in perinatal asphyxia.

In the group of full-term neonates with PA we found a significant correlation between increased serum cTnI levels and standard clinical markers of perinatal asphyxia such as the 5th minute Apgar score and serum lactate levels [6, 15]. The 5th minute Apgar score and serum lactate levels also positively correlated with serum CK-MB levels but less significantly than with cTnI, similar to other authors. This is in agreement with recently published reports showing that CK-MB is both less specific and less sensitive in detecting cardiac involvement and in early prediction of poor outcome/death in neonates with PA [10, 12, 39].

We found that cTnI is a highly sensitive and specific marker of myocardial damage as part of terminal multisystem failure [8, 10, 12, 15, 16].

In recent years, an increasing number of neonatal study is trying to determine the value of cardiac troponin I and T, as early indicators of critically ill newborns with PA, which would in future allow monitoring of therapeutic response and improvement of cardioprotective strategies.

Türker et al. [40] in their original study compared the levels of cTnI in 109 critically ill (mechanically ventilated neonates) with cTnI levels in the control group (48 healthy and 48 newborn infants requiring only the first stage of intensive care unit). According to the results of these authors in a group of critically ill, mechanically ventilated infants, there was a significant increase in cTnI (median 1.4 ng/mL, the min. 0 to max. 13.0 ng/mL; $p < 0.001$), compared to the control group (median 0, min from 0 to max. 1.84 ng/mL). Also in the group of critically ill children with fatal outcome there has been a significant increase in cTnI ($p < 0.001$), (6.6 ng/mL; 1.3–13.0 ng/mL) compared to the patients who survived (1.3 ng/mL; 0–8.0 ng/mL). Reciver-operator curve showed that early increase in cTnI could be a sensitive predictor of death in critically ill newborns with the confidence interval of 96%.

Similar to other authors, we found an association of increased cTnI values with several variables related to illness severity. A statistically significant higher mean concentration of cTnI was associated with the need of respiratory support: 0.11 μg/L (0.04–0.18); $p = 0.039$ and to the use of inotropic drugs: 0.15 μg/L (0.06–0.56); $p = 0.006$, compared to the group without cardio-respiratory support: 0.01 μg/L (0.01–0.04). The results of our study suggest that early increase in cTnI could be used as an important prognostic marker, since serum cTnI > 0.135 mg/L predicted a mortality outcome with sensitivity of 84.6% and specificity 85.9% [40, 41].

CRP, as a non-specific indicator of tissue damage [42], and BNP, as insufficiently sensitive indicator of perinatal asphyxia, neither correlated with 5th minute Apgar score and serum lactate levels, nor were reliable predictors of mortality outcome in neonates with PA, in our study. One-time blood samples in a wide interval of 24–48 h could have a limiting effect on results analysis according to the different period of elimination of observed biochemical markers [43]. On the other hand, such findings could be explained by the fact that BNP is secreted primarily from the myocardium of heart chambers in response to pressure/volume overload [1, 2, 13], while the increase in cTnI is the result of hypoxia and/or myocardial ischemia [1, 3, 5, 29, 33, 43]. Heart failure is a complex clinical syndrome and a single biochemical marker, such as BNP, may not reflect all of its features. Measurement of both serum BNP levels, as markers of cardiac load, and cTnI levels, as

markers of myocardial damage, could open new perspectives in diagnosis, prognosis and monitoring of critically ill asphyxiated newborns with heart failure [44].

Conclusion

Cardiac troponin I, a highly specific and sensitive marker of myocardial damage, can be used as a prognostic marker of perinatal asphyxia in full-term newborn infants. Increase in cardiac troponin I > 0.135 mg/L in the first 24–48 h after birth may predict fatal outcome with sensitivity of 84.6% and specificity 85.9%.

Creatinine kinase MB fraction is both sensitive and specific marker of myocardial damage, but its predictive value is less significant than cTnI.

C-reactive protein is not sensitive indicator of perinatal asphyxia and, accordingly, its increase in the serum cannot be used for early prediction of outcome.

The increase in serum BNP levels in the population of full-term newborns, in our study did not appear to be a reliable predictor of perinatal asphyxia. Further studies on more patients are necessary to assess its predictive capacity in terms of mortality outcome in full-term neonates with perinatal asphyxia.

R E F E R E N C E S

1. M*ilutinovic S*, *Karadzic* R, *Pavlovic M*, *Tomasevic M*, *Stankovic A*. Biochemical cardiac markers' application in cardiology. Apoll Med Aesculap 2006; 4(3−4): 16−22. (Serbian)
2. Givertz MM, Braunwald E. Neurohormones in heart failure: predicting outcomes, optimizing care. Eur Hear J 2004; 25(4): 281−2.
3. *Panteghini M*. The new definition of myocardial infarction and the impact of troponin determination on clinical practice. Int J Cardiol 2006; 106(3): 298−306.
4. *Fichtlscherer S, Breuer S, Schachinger V, Dimmeler S, Zeiher A*. C-reactive protein levels determine systemic nitric oxid bioavailability in patients with coronary artery disease. Eur Heart J. 2004; 25(16): 1412−8.
5. *Majkic-Singh N*. The use of biochemical markers for diagnosis of the acute coronary syndromes. Jugoslav Med Biohem. 2003; 22: 289−301. (Serbian)
6. *McAuliffe F, Mears K, Fleming S, Grimes H, Morrison JJ*. Fetal cardiac troponin I in relation to intrapartum events and umbilical artery pH. Am J Perinatol 2004; 21(3): 147–52.
7. *El-Khuffash AF, Molloy JE*. Serum troponin in neonatal intensive care. Neonatology 2008; 94(1): 1−7.
8. *Trevisanuto D, Picco G, Golin R, Doglioni N*, *Altinier S*, *Zaninotto M*, et al. Cardiac troponin I in asphyxiated neonates. Biol Neonate 2006; 89(3): 190-3.
9. *Simovic AM, Knezevic J, Igrutinovic Z, Stojanovic N, Kocic S*. Cardiac troponin as biochemical marker of perinatal asphyxia and hypoxic myocardial injury. Vojnosanit Pregl 2009; 66(11): 881−6. (Serbian)
10. *Panteghini M, Agnoletti G, Pagani F, Spandrio M*. Cardiac Troponin T in Serum as Marker for Myocardial Injury in Newborns. Clin Chem 1997; 43(8 Pt 1): 1455−7.
11. *Da Graca RL, Hassinger DC, Flynn PA*, *Sison CP*, *Nesin M*, *Auld PA*. Longitudinal change of brain-type natriuretic peptide in preterm neonates. Pediatrics 2006; 117(6): 2183−9.
12. *Çaliskan E, Doger E, Cakiroglu Y*. Cord blood cardiac troponin I and creatine kinase MB in poor outcomes. J Turkish-German Gynecol Assoc 2006; 7(2): 98−102.
13. *EL-Khuffash AF, Davis PG, Walsh K, Molloy EJ*. Cardiac troponin T and N-terminal-pro-B type natriuretic peptide reflect myocardial function in preterm infants. J Perinatol 2008; 28(7): 482−6.
14. *Trevisanuto D, Zaninotto M, Lachin M, Altinier S, Plebani M, Ferrarese P*, et al. Effect of patent ductus arteriosus and indomethacin treatment on serum cardiac troponin T levels in preterm infants with respiratory distress syndrome. Eur J Pediatr 2000; 159(4): 273−6.
15. *Vento M, Sastre J, Asensi MA, Viña J*. American Thoracic Society: Understanding Cardiac Troponin T in the Newborn Period. Am J Respir Crit Care Med 2006; 173(7): 816−7.
16. *Correale M, Nunno L, Ieva R*, *Rinaldi M*, *Maffei G*, *Magaldi R*, et al. Troponin in Newborns and Pediatric Patients. Cardiovas Hematol Agents Med Chem 2009; 7(4): 270−8.
17. *Venge P*, *James S*, *Jansson L*, *Lindahl B*. Clinical performance of two highly sensitive cardiac troponin I assays. Clin Chem 2009; 55(1): 109−16.
18. *Clark SJ, Newland P, Yoxall CW, Subhedar NV*. Concentrations of cardiac troponin T in neonates with and without respiratory distress. Arch Dis Child 2004; (8): 348–52.
19. *Kearnev M, Marber M*. Trends in incidence and prognosis of heart failure; You always pass failure on the way to success. Eur Heart J 2004; 25(4): 283−4.
20. *Vergès B, Zeller M, Desgrès J, Dentan G, Laurent Y, Janin-Manificat L*, et al. High plasma N-terminal pro-brain natriuretic peptide level found in diabetic patients after myocardial infarction is associated with an increased risk of in-hospital mortality and cardiogenic shock. Eur Heart J 2005; 26(17): 1734−41.
21. *Hole T, Hall C, Skaerpe T*. N-terminal proatrial natriuretic peptide predicts two-year remodelling in patients with acute transmural myoacardial infaretion. Eur Heart J 2004; 25(5): 416−23.
22. *Latini R, Masson S, Ananad I, Salio M, Hester A, Judd D*, et al. The comparative prognostic value of plasma neurohormones at baseline in patients with heart failure enrolled in Val-HeFT. Eur Heart J 2004; 25(4): 292−9.
23. *Bazzino O, Fuselli JJ, Botto F, Perez de Arenaza D, Bahit C, Dadone J*, et al. Relative value of N-terminal probrain natruiretic peptide, TIMI rise seore, ACC/AHA prognostic classification and other rise markers in patients with non-ST-elevation acute coronary syndromes. Eur Heart J 2004; 25(10): 859−66.
24. *Matunovic R, Stojanovic A, Mijailovic Z, Rabrenovic M*. Therapeutic and prognostic significance of cardiac biomarkers in patients with the acute coronary syndrome. Srp Arh Cel Lek. 2006; 134(3−4): 162−5. (Serbian)
25. *Abbate A*, *Biondi-Zoccai GG*, *Brugaletta S*, *Liuzzo G*, *Biasucci LM*. C-reactive protein and other inflammatory biomarkers as predictors of outcome following acute coronary syndromes. Semin Vasc Med 2003; 3(4): 375−84.
26. *Zupan Simunek V*. Definition of intrapartum asphyxia and effects on outcome. J Gynecol Obstet Biol Reprod. 2008; 37(1): 7−15.
27. *Apple FS*. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem 2009; 55(7): 1303−6.
28. *Baum H, Hinze A, Bartels P, Neumeier D*. Reference values for cardiac troponins T and I in healthy neonates. Clin Biochem 2 004; 37(12): 1079−82.
29. *Bader D, Kuqelman A*, *Lanir A*, *Tamir A*, *Mula E*, *Riskin A*. Cardiac troponin I serum concentrations in newborns: a study and review of the literature. Clin Chim Acta 2006; 371(1−2): 61−5.

30. *Araújo K, da Silva J, Sañudo A, Kopelman B*. Plasma concentrations of cardiac troponin I in Newborn Infants. Clin Chem 2004; 50(9): 1717−8.
31. *Koch A, Singer H*. Normal values of B type natriuretic peptide in infants, children and adolescent. Heart 2003; 89(8): 875−8.
32. *Babuin L, Jaffe AS*. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005; 173(10): 1191−202.
33. *Costa S, Zecca E, De Rosa G, De Luca D*, *Barbato G*, *Pardeo M*, et al. Serum troponin T a useful marker of myocardial damage in newborn infants with perinatal asphyxia? Acta Paediatr 2007; 96(2): 181–4.
34. *Rajakumar PS, Bhat BV, Sridhar MG, Balachander J*, *Konar BC*, *Narayanan P*, et al. Cardiac enzyme levels in myocardial dysfunction in newborns with perinatal asphyxia. Indian J Pediatr 2008; 75(12): 1223–5.
35. *Szymankiewicz M, Matuszczak D,* Vidyasagar and *Gadzinowski J*. Retrospective diagnosis of hypoxic myocardial injury in neonates. J Perinat Med 2006; 34(3): 220–5.
36. *Iacovidou N, Boutsikou M, Gourgiotis D, Briana DD*, *Baka S*, *Vraila VM*, et al. Perinatal changes of cardiac troponin I in normal and intrauterine growth restricted pregnancies. Mediators Inflamm. 2007; 2007: 53921.
37. *Trevisanuto D, Doglini N, Altinier S, Zaninotto M*, *Plebani M*, *Zanardo V*. Cardiac troponin I at birth is of fetal - neonatal origin. Arch Dis Child Fetal Neonatal Ed 2009; 94(6): 464−6.
38. *Alexandre SM, D'Almeida V*, *Guinsburg* R, *Nakamura MU*, *Tufik S*, *Moron A*. Cord blood cardiac troponin I, fetal dopler velocimetry and acid base status at birth. Int J Gynecol Obstet 2008; 100(2): 136−40.
39. *Yan A, Yan RT, Chow CM, Fitchett D, Stanton E, Langer A*, et al. Troponin is more useful then creatin kinase in predicting one-year mortality among acute coronary syndrome patients. Eur Heart J 2004; 25(22): 2006−12.
40. *Tűrker G, Sarper N, Babaoglu K*, *Gökalp AS*, *Duman C*, *Arisoy AE*. Early prognostic significance of umbilical cord troponin I in critically ill newborns. Prospective study with a control group. J Perinat Med 2005; 33(1): 54−9.
41. *Setiadi BM, Lei H, Chang J*. Troponin not just a simple cardiac marker: prognostic significance of cardiac troponin. Chin Med J 2009; 122(3): 35−8.
42. *Jialal I, Devaraj S*, *Venugopal SK*. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension. 2004; 44(1): 6−11.
43. *Steen H, Futterer S, Merten C, Jünger C, Katus HA, Giannitsis E*. Relative role of NT-pro BNP and cardiac troponin Tat 96 hours for estimation of infarct size and left ventricular function after acute myocardial infarction. J Cardiovasc Magn Reson 2007; (9): 749–58.
44. *Bhayana V, Henderson AR*. Biochemical markers of myocardial damage. Clin Biochem 1995; 28(1): 1–29.

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Clinical features of endobronchial tuberculosis

Kliničke karakteristike endobronhijalne tuberkuloze

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Abstract

Background/Aim. Endobronchial tuberculosis (EBTB) is a specific type of pulmonary tuberculosis which often affect the tracheobronchial tree, and can be microbiologically and/or pathohistologically confirmed. The aim of the study was to determine the clinical features and diagnostic aspects of EBTB. **Methods.** This retrospective study was conducted at the Clinic for Lung Diseases, Clinical Center of Serbia, Belgrade, from January 1997 to December 2007. All patients with EBTB confirmed by bronchoscopy with biopsy during a study period were analysed. Data included the patient's medical history, a physical exam, chest X-ray, mycobacterial analysis of sputum samples, endoscopic types and patohistological confirmation. **Results.** In the study, 57.6% of the patients were males. The most frequent symptoms were cough (71.2%), malaise (54.2%), fever (49.2%), weight loss (40.7%), and hemoptysis (13.6%). Most of the patients were diagnosed within 30 days of symptoms onset. Sputum examination showed acid-fast bacilli in 31.4% of the patients, while sputum culture for tuberculosis bacilli were positive in 55.9% of the patients. The most common radiographic localization was in the upper lung lobes (63.5%). Cavities were present in 60.4% of the patients. The most common endoscopic subtype determined by bronchoscopy were nonspecific bronchitis (39.9%) and edematous-hyperemic subtype (36.4%). **Conclusion.** EBTB was more frequent among men, and among people in their fifties in our country. Detailed bronchoscopic examination, correlated with clinical and laboratory findings, will improve diagnostic rate and provide timely therapy.

Key words:
tuberculosis, pulmonary; diagnosis; signs and symptoms; radiography; bronhoscopy; histological techniques.

Apstrakt

Uvod/Cilj. Endobronhijalna tuberkuloza (EBTB) je tuberkuloza traheobronhijalnog stabla, mikrobiološki i/ili patohistološki potvrđena. Cilj rada bio je da se odrede kliničke karakteristike i dijagnostički aspekt endobronhijalne tuberkuloze. **Metode.** Ova retrospektivna studija sprovedena je u Klinici za plućne bolesti Kliničkog centra Srbije u Beogradu u periodu od januara 1997. do kraja decembra 2007. god. Analizirani su svi bolesnici sa EBTB koja je potvrđena bronhoskopski i biopsijom u navedenom periodu. Prikupljeni su anamnestički podaci bolesnika, podaci o fizikalnom pregledu i radiografiji pluća, laboratorijske analize sputuma, uz bronhoskopsku i patohistološku potvrdu. **Rezultati.** U studiji je bilo 57,6% osoba muškog pola. Najčešći simptomi bolesti bili su kašalj (71,2%), znojenje (54,2%), povišena telesna temperatura (49,2%), gubitak telesne mase (40,7%) i hemoptizije (13,6%). Kod većine bolesnika dijagnoza je postavljena unutar 30 dana od početka simptoma. U sputumu je kod 31,4% bolesnika potvrđen *Mycobacterium tuberculosis*, a kultura je bila pozitivna kod 55,9% bolesnika. Na radiografskom nalazu najčešće su bili zahvaćeni gornji režnjevi pluća (kod 63,5% bolesnika). Kaverne je imalo 60,4% bolesnika. Bronhoskopski nalaz je kod najvećeg broja pokazivao nespecifični bronhitis (39,9%) i edemohiperemični podtip EBTB (36,4%). **Zaključak.** U našoj zemlji EBTB je značajno češća kod muškaraca i osoba u pedesetim godinama života. Bronhoskopski nalaz koreliše sa kliničkim i laboratorijskim nalazom i omogućava lakše postavljanje dijagnoze i pravovremenu terapiju.

Ključne reči:
tuberkuloza pluća; dijagnoza; znaci i simptomi; radiografija; bronhoskopija; histološke tehnike.

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Introduction

Endobronchial tuberculosis (EBTB) is a specific type of pulmonary tuberculosis (TB) which often injuries the tracheobronchial tree, and can be microbiologically and/or pathohistologically confirmed [1]. The diagnosis of EBTB is frequently delayed until the onset of serious bronchial stenosis with resultant atelectasis and bronchiectasis [2]. EBTB often presents a diagnostic challenge because the clinical presentation varies, and some patients can have normal chest radiography, even though the acid-fast bacilli (AFB) positive sputum sample is present [3]. EBTB may have a prolonged and insidious course and mimic lung cancer, or an acute course, and imitate bronchial obstruction, aspiration of a foreign body or pneumonia, and sometimes the clinical course can be asymptomatic. Because of the complexity and different prognosis, an endoscopic EBTB classification was adopted based on morphological characteristics of the seven subtypes (actively caseating, edematous hyperemic, fibrostenotic, tumorous, granular, ulcerative, and nonspecific bronchitic) proposed by Chung and Lee [4]. The course of EBTB differs according to the endoscopic type. Actively caseating, edematous hyperemic, and fibrostenotic subtypes most frequently lead to the formation of bronchostenosis that is the most serious complication of EBTB [5]. All subtypes can be transformed one into another. The outcome of the treatment for all subtypes is predictable, except for the tumorous form. Fibrostenosis may develop later, after a nine-month doctrinaire treatment (a combination regimen composed of four kinds of anti-tuberculosis drugs – the commonly used drugs include isoniazid, rifampicin, pyrazinamide and ethambutol), which was observed in one third of cases. Endoscopic type can predict bacillar prominence and anticipate the extent and duration of the disease (the edematous hyperemic form, the granulous form, and non-specific bronchitis occur in the early stages, while other forms indicate a widespread disease). The proportion of EBTB in pulmonary TB is about 10–40% [3]. Although the TB incidence has decreased during the last few decades in Serbia [6], this disease remains an important public health problem especially among patients older than 65 years [7]. The incidence rates being for the last few decades 32–36 *per* 100,000 inhabitants, up to the last few years when they decreased to 24–26 *per* 100,000 inhabitants. However, there are a few data about EBTB in our country, and in the region of Balkan and Southern Europe as well, even in the countries with high TB prevalence [8].

The aim of this study was to determine common clinical features and diagnostic aspects of EBTB in our patients.

Methods

This retrospective study was conducted at the Clinic for Lung Diseases, Clinical Center of Serbia, Belgrade, from January 1997 to December 2007. A total of 118 consecutive patients with EBTB were hospitalized and treated in our hospital, during the study period. All the cases with EBTB confirmed by bronchoscopy with biopsy during study period were analysed. For each patient we had a questionnaire filled out. Data included the patient's medical history, physical exam, chest X-ray, bronchial examination with bronchial biopsies and mycobacterial analysis of sputum samples. Statistical Package for Social Sciences (SPSS) program-version 15.0 was used for data entry and analysis. Statistical differences were evaluated using the chi-square test and *t*-test.

The Institutional Ethics Committee approved the study.

Results

There were 68 (57.6%) men and 50 (42.4%) women, the ratio being 1.4 : 1. The mean age was 44.3 ± 4.32 years (ranged from 16 to 73 years). Twenty four (20.3%) of the patients were older than 60 years. At the time of diagnosis, there was a significant tendency for the women that had EBTB to be younger than 40 years, compared to the men ($p = 0.05$) (Figure 1).

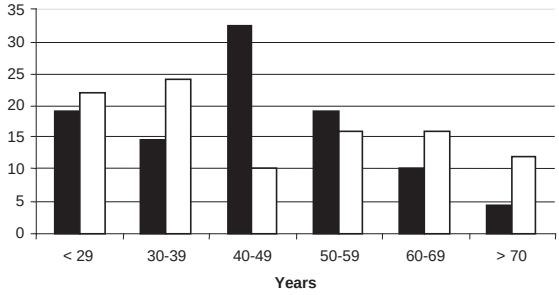

Fig. 1 – Percentage of patients with endobronchial tuberculosis according to age and sex.
Closed bars – men; open bars – women.

Nearly half (47.8%) of the patients were diagnosed within 30 days of symptom onset. The clinical presentation of EBTB varied. Five (4.2%) patients were asymptomatic, all the other patients have at least one symptom of TB. Symptoms and their duration before diagnosis are presented in Table 1, the dominant being cough 84 (71.2%) and general weakness 64 (54.2%), followed by fever 58 (49.2%), weight loss 48 (40.7%), and hemoptysis 16 (13.6%), respectively. Hemoptysis were significantly more frequent in males than in females ($p = 0.04$), but without differences according to age. Beside that, other complaints included chest pain (31.4%), sweating (17.8%), and dyspnea (12.4%), and 3.4% patients were hoarse. A predisposing disease or condition was recorded in 43 (36.4%) patients. Forteen (11.9%) patients had diabetes mellitus. A small number of patients, 5 (4.2%) had been previously treated for TB, on average 28.5 years earlier, ranging between 10 and 40 years.

In patients with EBTB, a unilateral process was more frequent (59.5%) than bilateral (41.5%). Upper lung fields were the most frequently involved in 63.5% of the patients (Table 2). Cavernous lesions were notified in 60.4% patients.

TB was confirmed by direct microscopy and/or culture. Sputum examination for acid-fast bacilli (AFB) was positive in 31.4%, and culture in 55.9% of the patients.

The most common bronchoscopic findings were nonspecific bronchitis and edematous-hyperemic subtype (Table 3).

Table 1

Symptoms of endobronchial tuberculosis

Symptoms	Patients n (%)	Duration of symptoms (days) x̄ ± SD	median	min-max
Cough	84 (71.2)	98.6 ± (96.9)	60.0	1–450
Hemoptysis	16 (13.6)	55.7 ± (110.7)	10.0	1–365
Fever	58 (49.2)	47.3 ± (37.8)	30.0	2–150
General weakness	64 (54.2)	87.4 ± (84.9)	60.0	10–450
History of weight loss	48 (40.7)	90.1 ± (91.8)	60.0	20–450

▪**The total number of patients is higher than 118 because some of the patients had more than one symptom.**

Table 2

Roentgenographic site in 118 patients with endobronchial tuberculosis

Localisation	Patients, n (%)
Right upper lobe	27 (22.9)
Left upper lobe	22 (18.6)
Both side – upper lobes	26 (22.0)
Middle lobe	6 (5.1)
Right lower lobe	8 (6.8)
Left lower lobe	6 (5.1)
Both side – diffusely	23 (19.5)
Total	118 (100.0)

Table 3

The bronchoscopic findings in 118 patients with endobronchial tuberculosis

Bronchoscopic findings	Patients, n (%)
Actively caseating	10 (8.5)
Edematous-hyperemic	43 (36.4)
Fibrostenotic	10 (8.5)
Tumorous	5 (4.2)
Granular	3 (2.5)
Nonspecific bronchitis	47 (39.9)
Total	118 (100.0)

Of the 118 patients, 111 (94.0%) cases presented with chronic tuberculous bronchitis on the pathological findings of biopsy obtained by bronchoscopic procedures while only 6% cases presented tuberculous granuloma.

Discussion

This research encompassed one of the largest numbers of a series of EBTB cases in the region of Balkan. We found that there were more male than female ETBT patients in the fifth decade of life (sex ratio 1.4 : 1), which is confirmed by papers from different regions (Hong Kong, Korea, China, Japan, Brazil) [9–13]. In contrast, the preponderance of females among patients with EBTB is stated in the works of other authors [14].

Respiratory symptoms in EBTB, according to our results, were nonspecific. Cough and fatigue dominate, followed by fever, weight loss and hemoptysis. Cough was the most common symptom according to the results of other studies [1, 15]. Thoracic pain was present in almost a third of our patients (31.4%), whereas this symptom was less frequently observed (15%) by other authors [3, 9]. In our study group, 13.6% of the patients had hemoptysis, while according to other papers, 25% to 40.2% of the patients had hemoptysis [9]. General symptoms, such as weakness, fever, and weight loss, were present in a significant number of patients, according to the results of this study and other studies, as well [3, 9]. As for the duration of symptoms prior to diagnosis of TB, according to our results, the interval varies from 1 week to 1 year, but the severity of symptoms (most commonly cough and fatigue) most likely contributed to the fact that almost half of patients came for a checkup during the first 30 days of the onset of symptoms. Short average period from disease onset to diagnosis of EBTB, when compared to overall pulmonary TB in the same settings, could be explained by clear clinical symptoms that lead to suspicion to EBTB and faster implementation of diagnostic procedure. Other authors describe precisely this diversity of symptoms and their intensity: with symptoms that mimic asthma [9, 13, 14], aspiration of a foreign body, and lung cancer [16–18]. An asymptomatic form of the disease is also possible and it was observed in five patients in our study.

In terms of the bacilarity of sputum in EBTB, several different results have been published. Thus, some studies [1, 4, 13] confirm the assumption of high infectivity of patients with EBTB by the presence of simple sputum positive for AFB in 51.8–91% patients. On the other hand, some studies [1, 9, 14] report on a significantly lower (9.1–17%) positive sputum for AFB. The results of the present study confirm AFB in one third of the patients, while the Löwenstein-Jensen culture of sputum samples were positive in over half the cases, which is consistent with the results reported by other authors [4]. Certainly, different histological types EBTB correspond to these contradictory findings. It was noted that the endoscopic ulcerative form and actively caseating type of EBTB have higher sputum positivity, which is not the case with edematous-hyperemic and fibrostenotic type [5], so that in these cases the histological confirmation of disease fiberbronchoscopy is required, while negative sputum for AFB does not exclude the diagnosis of EBTB.

Radiographic analysis of the lungs of the patients in our study showed that cavernous changes in the lungs were present in about two-thirds of the patients with EBTB and this finding did not differ significantly from the results in the literature [13]. None of the patients had chest radiography without pathological changes, in our study. In contrast to our work, many studies have ascertained the presence of normal chest radiographs in 10–20% of cases [9, 10, 14, 19]. Normal chest radiography is a diagnostic trap for EBTB that is why the diagnosis of the disease is often delayed [20]. The authors agree

that the most common radiographic localization of pulmonary TB is in the upper lobes which was confirmed by our results, as well (63.5%). The middle and lower lobes, as atypical radiographic presentations, in our work were affected in 17% of the patients, while the same results, were found in up to 42–90% of patients, according to the literature [21, 22].

The most common endoscopic forms of EBTB determined by bronchoscopy in our study were nonspecific bronchitis in 39.8% of the patients, followed by the edematous hyperemic form in 36.4%, while the granular form was recorded in only 2.5% of the cases, and no patient had ulcerative EBTB form. These results differ from those in the study by authors who made a proposal of classification, Chung and Lee [4] and Lee et al. [23], which showed the most frequent occurrence to be the actively caseating form (43%), while nonspecific bronchitis (7.9%) and ulcerative type (2.7%) were the rarest represented. The differences could most likely be explained by the duration of the disease until bronchoscopy, that is, endoscopic exploration. In our study, in approximately half of the patients the disease was diagnosed by bronchoscopy during the first month, which resulted in the highest frequency of nonspecific bronchitis and edematous-hyperemic type, which confirmed the observation that these forms occur in the early stages of EBTB [8]. Bronchoscopy was performed in order to obtain good aspirate sample for bacteriologic analysis, and later to use the same sample to test the susceptibility to antituberculosis (AT) drugs. Concerning difficulties of *Mycobacterim tuberculosis* isolation, it was the case in the past from time to time up to 3–4 years ago, due to the periods when sputum induction could not be performed at all because of a lack of essential equipment (the nineties of 20th century and first few years of new millenium). This diagnostic procedure was conducted in the patients with radiological findings which were not enough persuasive of TB process, and in a certain proportion in the patients without bacteriologic confirmation of TB. They were treated with AT drugs for 3–4 weeks, and when at the first check-up, chest x-ray did not show any initial improvement (initial regression of lesions) and who did not provide good or not at all sputum sample for bacteriologic diagnosis, even after sputum induction, so there was a problem how to confirm the suspition of TB process. Bronchoscopy was also used to exclude any other underlying or concommitant disease. Due to a serious problem of increasing incidence of lung cancer in our country, in the last decade more than 27% [24], it is very important to use bronchoscopy as diagnostic tool.

A histologic confirmation of EBTB is of great importance in the prevention of further spread of TB and to eliminate the suspicion of malignancy [25]. It is also useful in differentiation from the other granulomatosis, mostly sarcoidosis. We found TB granuloma in 6% of the patients. When EBTB cannot be diagnosed by histologic confirmation findings after repetitious examinations, diagnosis should be established by a combination of clinical, bacteriological and bronchoscopic findings and aggressive treatments must be performed to eradicate *Mycobacterium tuberculosis* to avoid tracheobronchial stenosis [26].

Conclusion

In our patients, EBTB was more frequent among men, and among people in their fifties. Detailed bronchoscopic examination is unavoidable as being a simple and reliable diagnostic procedure, correlated with clinical and laboratory findings. This procedure alone will improve diagnosis accuracy rate and provide timely therapy.

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REFERENCES

1. *Hoheisel G, Chan BK, Chan CH, Chan KS, Teschler H, Costabel U.* Endobronchial tuberculosis: Diagnostic features and therapeutic outcome. Respir Med 1994; 88(8): 593 –7.
2. *Kashyap S, Mohaparta PR, Saini V.* Endobronchial Tuberculosis. Indian J Chest Dis Allied Sci 2003; 45(4): 2472–6.
3. *Tetikkurt C.* Current perspectives on endobronchial tuberculosis. Pneumon 2008; 21(3): 239–5.
4. *Chung HS, Lee JH.* Bronchoscopic assesment of the evolution of endobronchial tuberculosis. Chest 2000; 117(2): 385– 92.
5. *Kim YH, Kim HT, Lee KS, Uh ST, Cung YT, Park CS.* Serial fiberoptic bronchoscopic observations of endobronchial tuberculosis before and early after antituberculosis chemotherapy. Chest 1993; 103(3): 673– 7.
6. *Jovanovic D, Skodric-Trifunovic V, Markovic-Denic L, Stevic R, Vlajinac H.* Clinical and epidemiological evaluation of tuberculosis in Serbia, 1990–2004. Int J Tuberc Lung Dis 2007; 11(6): 647–51.
7. *Pešut DP, Gledovic ZB, Grgurevic AD, Nagorni Obradovic LM, Adzic TN.* Tuberculosis incidence in elderly in Serbia: key trends in socioeconomic transition. Croat Med J 2008; 49(6): 807– 12.
8. *Zutić H, Dizdarević Z, Ustamujić A, Hadzimurtezić Z.* More than ten years of DOTS in Bosnia and Herzegovina. Bosn J Basic Med Sci 2008; 8(1): 52–7.
9. *Ip MS, So SY, Lam WK, Mok CK.* Endobronchial tuberculosis revisited. Chest 1986; 89(5): 727– 30.
10. *Hoheisel G, Chan BK, Chan CH, Chan KS, Teschler H, Costabel U.* Endobronchial tuberculosis: diagnostic features and therapeutic outcome. Resp Med 1994; 88(8): 593–7.
11. *Wang SY, Zhang XS.* Endobronchial tuberculosis. Report of 102 cases. Chest 1994; 105(6): 1910−1.
12. *Kurasawa T, Kuze E, Kawai M, Amitani R, Murayama T, Tanaka E,* et al. Diagnosis and management of endobronchial tuberculosis. Intern Med 1992; 31(5): 593–8.
13. *Morrone N, Abe NS.* Bronchoscopic findings in patients with pulmonary tuberculosis. J Bronchol 2007; 14(1): 15–8.
14. *Lee JH, Park SS, Lee DH, Shin DH, Yang SC, Yoo BM.* Endobronchial tubeculosis. Clinical and bronchoscopic features in 121 cases. Chest 1992; 102(4): 990–4.

15. *Sucena M, Amorim A, Machado A Hespanhol V, Magalhães A.* Endobronchial tuberculosis-clinical and bronchoscopic features. Rev Port Pneumol 2004; 10(5): 383−91.
16. *Cordovilla R, Jimenez-Massa A.* Endobronchial tuberculosis. a case report and review of the literature. J Bronchol Intervent Pulmonol 2009; 16(2): 121–3.
17. *Lohani S, Chadwick PR, Armstrong G, O' Driscoll R, Taggart S.* Endobronchial Mimics of Lung Cancer. J Bronchol 2005; 121: 56–8.
18. *Kreisel D, Arora N, Weisenberg SA, Saqi A, Krupnick A, Demetriades A, et al.* Tuberculosis presenting as an endobronchial mass. J Thorac Cardiovasc Surg 2007; 133(2): 582–4.
19. *Lee JH, Chung HS.* Bronchoscopic, radiologic and pulmonary function evaluation of endobronchial tuberculosis. Respirology 2000; 5(4): 411–7.
20. *Golshan M.* Tuberculous bronchitis with normal chest x ray among a large bronchoscopic population. Ann Saudi Med 2002; 22(1–2): 98−101.
21. *Berger HW, Granada MG.* Lower lung fields tuberculosis. Chest 1974; 65(5): 522–6.
22. *Kim HC, Kim HS, Lee SJ, Jeong YY, Jeon KN, Lee JD, et al.* Endobronchial tuberculosis presenting as right middle lobe syndroma: clinical characteristics and bronchoscopic findings in 22 cases. Yonsei Med J 2008; 49(4): 615–9.
23. *Lee JH, Park SS, Lee DH, Shin DH, Yang SC, Yoo BM.* Endobronchial tuberculosis. Clinical and bronchoscopic features in 121 cases. Chest 1992; 102(4): 990−4.
24. *Marković-Denić L, Vlajinac H, Zivković S, Miljus D.* Cancer mortality among men in Central Serbia: 1985-2006 survey study. Croat Med J 2008; 49(6): 792–8.
25. *Stojsic J, Jovanovic D, Sudjic E.* Pathohistological diagnosis of endobronchial tuberculosis. Int J Tuberc Lung Dis 2005; 9(Suppl1): 11.
26. *Xue Q, Wang N, Xue X, Wang J.* Endobronchial tuberculosis: an overview. Eur J Clin Microbiol Infect Dis 2011; 30(9): 1039– 44.

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Blunt chest trauma – An audit of injuries diagnosed by the MDCT examination

Tupa trauma grudnog koša – pregled povreda dijagnostikovanih MDCT pregledom

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Abstract

Background/Aim. Multidetector computed tomography (MDCT) characterised by speed and precision is increasingly accessible in emergency wards. The aim of our study was to determine the most common injuries to the chest region, as well as type associated extrathoracic injuries, and the treatment outcome. **Methods.** This prospective study included 61 patients with blunt trauma who were submitted to computed tomography (CT) of the thorax. The number of injuries was evaluated by organs and organ systems of the chest. The cause of the injury, the length and the outcome of the treatment, and the presence of injuries in other regions were assessed. **Results.** Chest injuries were associated with injuries to other regions in 80.3% cases, predominantly injuries to extremities or pelvic bones in 54.1% cases, followed by head injuries in 39.3% patients. Associated thoracic injuries were present in 90.9% of patients with lethal outcome. Lung parenchymal lesions, pleural effusions and rib fractures were the most common injuries affecting 77.1%, 65.6% and 63.9% of the cases, respectively. **Conclusion.** Blunt chest trauma is a significant problem affecting predominantly males in their forties and it is usually caused by a motor vehicle accident. In case of pneumomediastinum or mediastinal haematoma, the use of 3D reconstructions is advised for diagnosing possible tracheobronchial ruptures and thoracic aorta injuries. Increased resolution of CT scanners yielded a large number of findings that are occult on radiography, especially in the event of lung parenchymal and pleural injuries. However, none imaging modality can replace surgical judgement.

Key words:
wounds, nonpenetrating; thorax; diagnosis; tomography, x-ray computed; multiple trauma; treatment outcome.

Apstrakt

Uvod/ Cilj. Multidetektorska kompjuterizovana tomografija (MDCT) koja se odlikuje brzinom i preciznošću sve više se koristi u odeljenjima urgentne medicine. Cilj istraživanja bio je da se utvrde najčešće povrede grudnog koša, udružene vantorakalne povrede, kao i da se ustanovi ishod lečenja. **Metode.** Ova prospektivna studija obuhvatila je 61 bolesnika sa tupom traumom. Svakom bolesniku urađen je CT grudnog koša, kao deo inicijalne dijagnostike. Procenjen je broj povreda po organima i organskim sistemima grudnog koša. Evidentiran je uzrok povrede, dužina i ishod lečenja, kao i prisustvo povreda u drugim regijama. **Rezultati.** Povreda grudnog koša bila je udružena sa drugim regijama kod 80,3% bolesnika i to najčeće sa povredama ekstremiteta ili kostiju karlice kod 54.1% bolesnika, potom sa povredama glave kod 39,3% bolesnika. Kod 90,9% bolesnika sa smrtnim ishodom postojale su pridružene povrede grudnog koša. Lezije plućnog parenhima, pleuralni izlivi i frakture rebara bile su najčešće povrede kod 77,1%, 65,6% i 63,9% bolesnika, respektivno. **Zaključak.** Tupa trauma grudnog koša je značajan problem prevashodno kod muškarca u četrdesetim godinama i obično je uzrokovana saobraćajnim nezgodama. U slučaju pneumomedijastinuma ili medijastinalnog hematoma, savetuje se korišćenje 3D rekonstrukcija u postavljanju dijagnoze potencijalne traheobronhijalne povrede i povrede grudne aorte. Povećana rezolucija CT skenera dala je veliki broj nalaza koji su teško vidljivi na radiografiji, naročito u slučaju povrede plućnog parenhima i pleure. Trebalo bi imati na umu međutim, da nijedna tehnika snimanja ne može da bude jedini faktor pri odlučivanju o hirurškom lečenju.

Ključne reči:
povrede, zatvorene; toraks; dijagnoza; tomografija, kompjuterizovana, redgenska; povrede, multiple; lečenje, ishod.

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Introduction

The clinical presentation of thoracic trauma ranges from a minimum of pain to a state of shock, and about a third of thoracic injuries require hospital treatment [1]. Two thirds of patients with multiple blunt injuries have a chest injury, whereas severe chest injuries are associated with other injuries in 70–90% of patients [2]. What matters is whether a patient is in immediate danger and if radiological examinations can be made safely [3]. After initial clinical evaluation and stabilisation of traumatised patients, radiological methods play an important role in evaluation of injuries [4]. The initial diagnostic approach to chest trauma is typically based on chest X-ray at admission. With respect to its limits, chest X-ray can be a valuable diagnostic tool providing a wide range of information. However, it is well-known that information provided by standard chest X-ray could be insufficient in diagnosing both vascular and non-vascular thoracic injuries [3, 5–8]. Computed tomography (CT) was primarily used for thoracic aortic injuries, but numerous studies found that CT is more sensitive to other thoracic injuries [2, 4, 9–11]. Due to the lack of time and in order to avoid unnecessary radiation, chest CT is often performed in injured patients when CT examination of the abdomen or head has already been indicated, because of the frequent association between thoracic and extrathoracic injuries [4, 12]. Multidetector computed tomography (MDCT), a new valuable tool in modern medicine that is characterised by speed and precision, is increasingly accessible in emergency wards. However, regardless the growing application of MDCT examinations in emergency departments, it should be borne in mind that MDCT examination should not delay necessary surgery [13].

The aim of this study was to determine the most common type of injuries to the chest region, associated extrathoracic injuries, and the treatment outcome.

Methods

This prospective study included 61 patients (mean age 43.9 years) with blunt trauma who were treated in our clinical center and who were submitted to CT scan of the thorax as part of their initial assessment. It should be emphasised that the patients got their CT examination report within clinically reasonable time, independently on our study.

These CT examinations were recorded and saved, to let us evaluate the number of injuries by organs or organ systems of the chest. The description thereof was carried out by entering each patient's data separately into the protocol of the study. These data included the following injuries: rib fracture, vertebral fracture, sternal fracture, scapular fracture, clavicular fracture, subcutaneous emphysema, chest wall haematoma, pneumothorax, pleural effusion, lung injury, pneumomediastinum, mediastinal haematoma, tracheobronchial rupture, oesophageal injury, aortic injury, pneumopericardium, pericardial effusion, and diaphragmatic rupture.

After transferring or discharging a patient, we assessed the cause and the time of injury, the length and the treatment outcome, and the presence of injuries in other regions.

The description of numeric variables was performed using classical methods of descriptive statistics (arithmetic mean) and measures of variability (standard deviation, minimum and maximum values). Relative values were used in tables. Nonparametric analysis of variance (F) for the comparison of three or more groups of data was also used. The value of $p < 0.05$ was considered significant.

All CT studies were performed using Siemens 16 and 64-section MDCT (120 kV, 220 mAs/slice, 5 mm section thickness, pitch of 1.4). Approximately 1.2 m of iodinated contrast agent (Ultravist 370 or Omnipaque 350) *per* kilogram of body mass was injected intravenously using a mechanical power injector at 2 mL/s. The volumetric MDCT data were reconstructed into axial and MPR 1-mm-thick sections.

Results

The average age of the patients included in this study was 43.9 years (min 14.0, max 82.0, SD 17.7 years). The number of male patients was 46 (75.4%), whereas the number of female patients was 15 (24.6%).

The length of treatment, meaning the length of hospitalisation at the Institute for Surgery, Clinical Center of Vojvodina, ranged from 1 to 64 days, with the average of 13.8 days and standard deviation of 14.9. The most common cause of injury was traffic accident (39; 63.9%). The differences in the number of patients in relation to the type of injury were statistically highly significant ($F = 8.014$, $df = 3$, $p < 0.01$), because traffic accidents greatly exceed other causes of injury (Table 1).

Table 1

Causes of blunt chest injuries

Mechanism of injury	Patients	
	n	%
Traffic accident	39	63.9
Fall from height	12	19.7
Severe blow with a heavy blunt object	6	9.8
No reliable data	4	6.6
Total	61	100.0

Upon observing the association of thoracic injuries to other body regions, it can be seen that chest injury was associated with injuries of other regions in 49 (80.3%) patients (Figure 1). Thoracic injuries were associated with injuries to

extremities or pelvic bones in 33 (54.1%) cases, head injuries in 24 (39.3%) patients, abdominal and/or pelvis injuries in 16 (26.2%) patients and spine injuries in 15 (24.6%) patients.

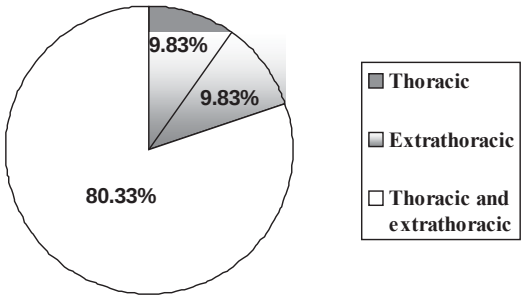

Fig. 1 – Association of thoracic injuries with injuries to other regions of the body.

The analysis of the treatment outcome of the patients showed that the patients were usually (20; 32.8%) discharged to home treatment (Table 2). Lethal outcome was recorded in the smallest number of the cases (11 patients, 18%). Comparing the fatal outcome with the type of injury, we found that associated thoracic injuries were present in 10 (90.9%) out of 11 exited patients, whereas an isolated spine injury existed in only one patient. Independent isolated thoracic injuries did not lead to death.

According to the protocol of the study, Table 3 presents the number of patients with injuries in various regions of the chest. Lung parenchymal lesions, pleural effusions and rib fractures were the most common injuries affecting 77.1%, 65.6% and 63.9% of the cases, respectively.

Subcutaneous emphysema was detected in 21 (34.4%) patients. By comparing the association between subcutaneous emphysema and rib fractures we observed that following patients had a rib fracture: 7 (87.5%) out of 8 patients with subcutaneous emphysema on the right; 7 (77.8%) out of 9 patients with subcutaneous emphysema on the left; and 3 (75%) of the 4 patients who had bilateral subcutaneous emphysema. We also found that 11 (47.8%) out of a total of 23 patients with right-sided rib fractures had subcutaneous emphysema, whereas 19 (59.4%) out of the 32 patients with left-sided rib fractures had subcutaneous emphysema.

Mediastinal haemathoma was diagnosed in 14 (22.9%) of the patients, while none of them had an aortic rupture as a source of bleeding (Figure 2).

Table 2

The outcome of treatment

Outcome	Patients	
	n	%
Discharged	20	32.8
Transferred to the home institution	12	19.7
Transferred to the Institute for Pulmonary Diseases	18	29.5
Lethal outcome	11	18.0
Total	61	100.0

Table 3

Frequency of injuries to the chest structures

Type of injury	Patients	
	n	%
Lung injury	47	77.1
Pleural effusion	40	65.6
Rib fracture	39	63.9
Pneumothorax	31	50.8
Subcutaneous emphysema	21	34.4
Vertebral fracture	17	27.9
Chest wall haematoma	14	23
Mediastinal haematoma	14	22.9
Scapular fracture	11	18
Sternal fracture	10	16.4
Pneumomediastinum	10	16.4
Clavicular fracture	7	11.5
Tracheobronchial rupture	1	1.6
Pneumopericardium	1	1.6
Pericardial effusion	1	1.6
Oesophageal injury*	1	1.6
Aortic injury	0	0
Diaphragmatic rupture	0	0

***No clinical feedback confirmed the diagnosis of oesophageal injury.**

Discussion

Our study indicates that chest trauma is an important issue affecting younger population, aged 43.9 on average. Liman et al. [14] reported similar mean age (45 years), whereas Shorr et al. [15] and Wagner et al. [16] presented even lower val-

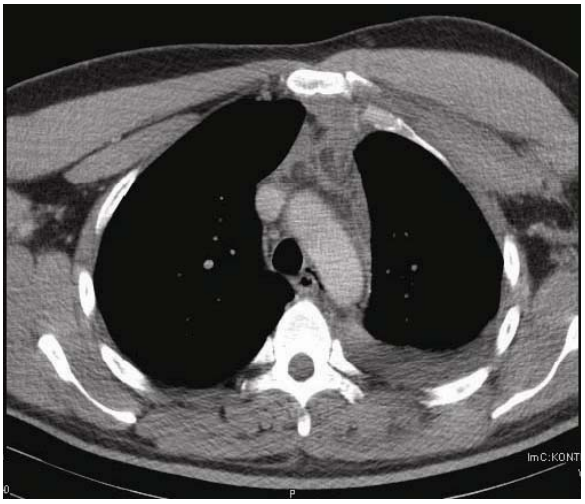

Fig. 2 – Contrast enhanced axial computed tomography (CT) slice shows a mediastinal haematoma, pneumomediastinum, pleural effusion, rib fracture with dislocation and chest wall haematoma on the left. Multiplanar reformations excluded the possibility of thoracic aortic injury as a source of mediastinal bleeding.

ues with the mean age of 32.2 and 36.7 years, respectively. The fact that males were injured three times more than females is in accordance with previous publications that reported the participation of male patients in more than 70% of cases [2, 14, 15].

The length of treatment was 1–64 days (13.8 days on average). From the Institute for Surgery, Clinical Center of Vojvodina, the patients were transferred to the home institution, the Institute for Pulmonary Diseases, rehabilitation centres, or were discharged to home treatment.

Traffic accidents were the most common cause of trauma and they accounted for 63.9% of the cases. For this reason, they are considered the primary and most common cause of blunt chest trauma [2, 12, 15]. The second most common cause of injury in our study was fall from height, which occurred in 19.7% of the patients, and this is also consistent with the aforesaid publications [2, 12, 15].

Observing the association of thoracic injuries to other body regions in our group of patients, it can be seen that the chest injury was associated with injuries to other body regions in 49 (80.3%) patients. Traub et al. [2] and Trupka et al. [17] also found a very frequent association of the chest injuries with extrathoracic injuries, namely in 70–90% and 91.3% of the cases. Sampson et al. [6] and Shorr et al. [15] reported that thoracic injuries were most commonly associated with head injuries. In our report, thoracic injuries were associated with head injuries in 39.3% of the cases, which were the second most frequent associated injury after limb injuries, which, though often not diagnosed by CT, were the most frequent associated injury (54.1%).

The patients' condition as equal as imaging findings played important part in the management, which was implemented according to the modern recommendations [18]. For example: the cases with pulmonary contusions were treated supportively, with early detection and treatment of complications; the finding of a pleural effusion or pneumothorax was most often followed by tube thoracostomy drainage of the respective pleural space; most of the rib fractures were treated conservatively, except in the cases of flail-chest when surgical intervention was indicated.

Our finding of 18% of fatal outcomes is similar to that reached by Wicky et al. [12], that reported the mortality rate of 15.5%. Regarding the outcome of the treatment of polytraumatised patients with or without a chest injury, it can be concluded that an associated chest injury existed in 10 (90.9%) out of 11 decesed patients, while the isolated spinal injury was diagnosed in only one case. This observation is supported by the fact that a polytraumatised patient with an associated chest injury is severely injured by classification. Such a patient requires a longer hospital stay and suffers lethal outcome in a greater number of cases than patients with serious injuries without an associated thoracic injury [19]. Additionally, independent isolated thoracic injuries did not lead to death.

Primack and Collins [4] found the occurrence of rib fractures in over 50% of the patients, as was the case in our study (64%). Previous studies [4, 5, 12, 20] presented data in sternal fracture occurring in 7–10% of patients, whereas in our study this percentage was higher (16.4%) and referred to the number of fractures identified by MDCT examination. Fractures of the scapula were also slightly higher than in previous studies [4, 5, 12, 20], although Traub et al. [2] had found a similar percentage of clavicular fractures, namely in 9.2% of patients.

It can be observed that at least 75% of patients with subcutaneous emphysema, seen as stripes and lines of air in the chest wall, have at least one fractured rib. This percentage is significantly higher compared to the data presented by Liman et al. [14], according to which 18.4% of the patients with fractures of the ribs had an associated subcutaneous emphysema. The difference in the results is probably due to the fact that a number of our patients already had a tube thoracostomy because of pneumothorax, as well as because the results reported by Liman et al. [14] did not apply to the MDCT examination but to the standard axial CT examination.

Pulmonary contusions appear as geographic, non-segmental areas of ground glass or nodular opacities on CT that do not respect lobar boundaries [10]. On the one hand, while pulmonary contusions have been estimated to affect 30–70% of the injured patients [4, 20, 21], our study reported the slightly higher rate of 77.1%. On the other hand, Sampson et al. [6] and Traub et al. [2] stated that lung contusions comprised 40% of cases, which is significantly less than in our study. Fluid collections in dependent part of a pleural space were attributed to pleural effusions [10]. The frequency of pleural effusion in our study was higher compared to the studies of other authors [2,6], whereas our previous report [22] detected the presence of pleural effusion in 73% of the 36 patients who suffered a blunt trauma. Pneumothorax was detected as an accumulation of air in the pleural space [23]. The fact that more than a half of the patients had been diagnosed with pneumothorax and that even a small one could enlarge under positive mechanical ventilation emphasises the importance of early diagnosis, for which CT had high sensitivity [23]. The increasing use of CT scanners has led to defining the term 'occult injuries' of pulmonary parenchyma and pleural space, which are radiographically hard or impossible to detect. Consequently, adequate treatment of these injuries has been and still remains controversial [24]. Certainly, more research is necessary to understand the clinical significance of these findings.

The rate of mediastinal haematoma in our study (14 patients, 22.9%) is slightly higher than in previous studies, which reported the frequency of 7% and 17.7%, respectively [2, 6]. The presence of this haematoma, which is reflected through increase in density of mediastinal fat, should always give rise to a suspicion of thoracic aorta injury [24]. However, even though thoracic aorta injury was assumed in two patients due to clinical findings and chest x-ray (Figure 2), detailed MDCT examination using 3D reconstructions excluded this possibility. Therefore, mediastinal haemorrhage in our patients could be attributed to lesions of the small veins and fractures of the bone structures, whose prevalence is estimated at 87.5% in the literature [12].

Pneumomediastinum was observed on CT as streaks of air surrounding and paralleling the bronchovascular bundles [24]. It was registered in 16.4% of the cases, which is higher than what was stated by other authors [4, 25]. Neither tracheal rupture nor oesophageal perforation was diagnosed in the 9 out of 10 (90%) patients with traumatic pneumomediastinum. This could be explained by the Macklin effect, according to which an alveolar rupture is followed by centripetal air dissection through the pulmonary interstitium into the mediastinum [26]. Tracheobronchial rupture is rare and occurs in 0.4–1.5% of cases [5, 12], like in our study, where it was registered in only one (1.6%) patient. Even on the axial CT slices, we assumed that the tracheobronchial rupture existed due to pronounced subcutaneous emphysema, pneumomediastinum and pneumothorax. Nonetheless, the site of injury could only be located using MDCT 3D reconstruction. The site of the rupture was unusually high in the trachea, unlike frequently described locations, namely 2.5 cm from the carina for bronchial injuries and 2 cm above the carina for tracheal injuries [23].

The diagnosis of oesophageal injury was made in one (1.6%) patient, due to the typical appearance of the mediastinum around the oesophagus that included indirect signs such as the presence of cervical and mediastinal emphysema, pleural effusion, pneumothorax, change of mediastinal contour due to a leak of fluid and/or mediastinal haemorrhage [23]. Unfortunately, the lack of clinical feedback precluded a complete evaluation of the patient. In making a diagnosis of oesophageal injury, usually perforation, CT could be a diagnostic modality in patients who are too ill to cooperate in oesophagography or a supplement in contrast-enhanced luminal studies, so as to further delineate the extent of the disease and assess the complications [27].

Diaphragmatic injury was not observed in our study, probably confirming the fact that this kind of injury is more associated with abdominal rather than thoracic injuries [12]. Although some 19 CT signs of diaphragmatic injury were detected, no single CT sign could be considered a marker leading to the correct diagnosis of a blunt diaphragmatic rupture. Instead, accurate diagnosing requires analysis of all the signs present [28].

Conclusion

Blunt chest trauma is a significant problem affecting predominantly males in their forties and it is usually caused by motor vehicle accidents. In more than 80% of cases it is associated with extrathoracic injuries, predominantly injuries to extremities or pelvic bones, followed by head injuries.

The widespread use of MDCT facilitates the diagnosis of clinically relevant injuries. The existence of subcutaneous emphysema should always spark suspicion that rib fracture exists. In any case of pneumomediastinum, especially in the abundant ones, we advise the use of 3D reconstructions in search of potential tracheobronchial and oesophageal injuries. The integrity of the thoracic aorta must be evaluated in all the planes so as to exclude its contribution to mediastinal haemorrhage.

Increased resolution of CT scanners yielded a large number of findings that are occult on radiography, especially in the event of lung parenchymal and pleural injuries. However, no imaging modality can replace surgical judgement.

REFERENCES

1. *Scaglione M, Pinto A, Pedrosa I, Sparano A, Romano L.* Multidetector row computed tomography and blunt chest trauma. Eur J Radiol 2008; 65(3): 377–88.
2. *Traub M, Stevenson M, McEvoy S, Briggs G, Lo SK, Leibman S,* et al. The use of chest computed tomography versus chest X-ray in patients with major blunt trauma. Injury 2007; 38(1): 43–7.
3. *Westaby S, Brayley N.* ABC of major trauma. Thoracic trauma-I. BMJ 1990; 300(6740): 1639–43.
4. *Primack SL, Collins J.* Blunt nonaortic chest trauma: radiographic and CT findings. Emerg Radiol 2002; 9(1): 5–12.
5. *Gavelli G, Canini R, Bertaccini P, Battista G, Bnà C, Fattori R.* Traumatic injuries: imaging of thoracic injuries. Eur Radiol 2002; 12(6): 1273–94.

6. *Sampson MA, Colquhoun KB, Hennessy NL.* Computed tomography whole body imaging in multi-trauma: 7 years experience. Clin Radiol 2006; 61(4): 365−9.
7. *Omert L, Yeaney WW, Protetch J.* Efficacy of thoracic computerized tomography in blunt chest trauma. Am Surg 2001; 67(7): 660−4.
8. *Ho ML, Gutierrez FR.* Chest radiography in thoracic polytrauma. AJR Am J Roentgenol 2009; 192(3): 599−612.
9. *Elmali M, Baydin A, Nural MS, Arslan B, Ceyhan M, Gürmen N.* Lung parenchymal injury and its frequency in blunt thoracic trauma: the diagnostic value of chest radiography and thoracic CT. Diagn Interv Radiol 2007; 13(4): 179−82.
10. *Miller LA.* Chest wall, lung, and pleural space trauma. Radiol Clin North Am 2006; 44(2): 213−24.
11. *Mirvis SE.* Diagnostic imaging of acute thoracic injury. Semin Ultrasound CT MR 2004; 25(2): 156−79.
12. *Wicky S, Wintermark M, Schnyder P, Capasso P, Denys A.* Imaging of blunt chest trauma. Eur Radiol 2000; 10(10): 1524−38.
13. *Vergnion M, Lambert JL.* A protocol of trauma care in the emergency service including MDCT imaging. Acta Anaesthesiol Belg 2006; 57(3): 249−52.
14. *Liman ST, Kuzucu A, Tastepe AI, Ulasan GN, Topcu S.* Chest injury due to blunt trauma. Eur J Cardiothorac Surg 2003; 23(3): 374−8.
15. *Shorr RM, Crittenden M, Indeck M, Hartunian SL, Rodriguez A.* Blunt thoracic trauma. Analysis of 515 patients. Ann Surg 1987; 206(2): 200−5.
16. *Wagner RB, Crawford WO Jr, Schimpf PP.* Classification of parenchymal injuries of the lung. Radiology 1988; 167(1): 77−82.
17. *Trupka A, Waydhas C, Hallfeldt KK, Nast-Kolb D, Pfeifer KJ, Schweiberer L.* Value of thoracic computed tomography in the first assessment of severely injured patients with blunt chest trauma: results of a prospective study. J Trauma 1997; 43(3): 405−11.
18. *Weyant MJ, Fullerton DA.* Blunt thoracic trauma. Semin Thorac Cardiovasc Surg 2008; 20(1): 26−30.
19. *Shirley PJ.* Trauma and critical care III: chest trauma. Trauma 2005; 7(3): 133−42.
20. *Sangster GP, González-Beicos A, Carbo AI, Heldmann MG, Ibrahim H, Carrascosa P,* et al. Blunt traumatic injuries of the lung parenchyma, pleura, thoracic wall, and intrathoracic airways: multidetector computer tomography imaging findings. Emerg Radiol 2007; 14(5): 297−310.
21. *Pal JM, Mulder DS, Brown RA, Fleiszer DM.* Assessing multiple trauma: is the cervical spine enough? J Trauma 1988; 28(8): 1282−4.
22. *Petrović K, Stojanović S, Till V, Vučaj-Ćirilović V, Nikolić O, Ničforović D.* Blunt injuries of the lungs, pleura and chest bone structures: Computed Tomography contribution. Med Pregl 2008; 61(1−2): 83−6. (Serbian)
23. *Oikonomou A, Prassopoulos P.* CT imaging of blunt chest trauma. Insights Imaging 2011; 2(3): 281-95.
24. *Milia DJ, Brasel K.* Current Use of CT in the Evaluation and Management of Injured Patients. Surg Clin North Am 2011; 91(1): 233−48.
25. *Wintermark M, Schnyder P.* The Macklin effect: a frequent etiology for pneumomediastinum in severe blunt chest trauma. Chest 2001; 120(2): 543−7.
26. *Assenza M, Passafiume F, Valesini L, Centonze L, Romeo V, Modini C.* Pneumomediastinum and pneumoperitoneum after blunt chest trauma: the Macklin effect. J Trauma Treatment 2012; 1(1): 1−5.
27. *Young CA, Menias CO, Bhalla S, Prasad SR.* CT features of esophageal emergencies. Radiographics 2008; 28(6): 1541−53.
28. *Desir A, Ghaye B.* CT of blunt diaphragmatic rupture. Radiographics 2012; 32(2): 477−98.

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Assessment of health status and quality of life of homeless persons in Belgrade, Serbia

Procena zdravstvenog stanja i kvaliteta života beskućnika u Beogradu

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Abstract

Background/Aim. Homelessness is a problem with social, medical, economic, political and other implications. Despite a large number of studies, reports about health-related quality of life (HRQoL) of homeless persons remain sparse. There is a summary of consistent evidence that homeless people have higher prevalence of chronic disease (mental and somatic) than general population. The aim of this study was to assess HRQoL and depression in homeless persons in Belgrade, to describe their sociodemographic factors and health status (the presence of chronic mental and somatic diseases and addiction disorders) and analyse impact of sociodemographic factors and health status to HRQoL and depression of homeless persons. **Methods.** The study was conducted in the Shelter for Adult and Elderly Persons in Belgrade, from January 1 to January 31, 2012. A set of questionnaires used in survey included Serbian translation of SF-36 questionnaire, Serbian translation of Beck Depression Inventory-II (BDI-II) and sociodemographic questionnaire. Statistical analysis was performed by descriptive and analytic methods. **Results.** Our study sample consisted of 104 adult participants. The majority of them were male (74%) and the mean age in the sample was 48.2 ± 13.0 years. We have found that 35.6% participants had lifetime diagnosis of psychiatric disorder, most frequently depression (lifetime prevalence of 15.4% in the study group). The history of suicide attempts was registered in 28 (26.9%) participants. Lifetime illicit drugs use was reported by 12.5%, daily smoking by 82.7% and daily alcohol consumption by 8.7% of the participants. Most common somatic chronic diseases were cardiovascular while chronic lung diseases were the second most frequent. Single chronic disaese was present in 33 (31.7%) of the participants and comorbidity of 2 chronic diseases was present in 20 of them. A statistically significant difference between participants` HRQoL SF-36 domain scores and norms of general population was found only for role physical domain (lower in homeless, $p < 0.001$). ANOVA showed no statistically significant difference in SF-36 HRQoL domain and composite scores between different age groups, nor did marital status, education level, length of homelessness, alcohol use or smoking significantly affect the HRQoL. The mean BDI-II score in the studied population was 19.1 ± 11.6. Severe depression was registered in 20.2% of the participants, moderate in 23.1%, mild in 19.2% and minimal in 37.5%. A highly significant negative correlation was verified between BDI-II and all domains and composite scores of SF-36 ($p < 0.001$). **Conclusion.** Measures for prevention of homelessness should include: foundation of national registry of homeless persons, development of systemic multisectorial cooperation and special psychosocial intervention strategies. In homeless population, health care measures should be focused on prevention and treatment of mental health disorders and chronic somatic diseases.

Key words:
homeless perons; health status; quality of life; serbia.

Apstrakt

Uvod/Cilj. Beskućništvo predstavlja problem sa širokim društvenim, zdravstvenim i ostalim implikacijama. Postoje brojni dokazi da beskućnici imaju višu prevalenciju hroničnih (mentalnih i somatskih) oboljenja u odnosu na opštu populaciju. Cilj rada je bio utvrđivanje kvaliteta života (KŽ) i depresivnosti kod beskućnika, socijalnodemografskog i zdravstvenog statusa u ovoj populaciji, te analiza faktora koji utiču na KŽ i depresivnost beskućnika. **Metode.** Istraživanje je sprovedeno u Centru za smeštaj odraslih i starih lica u Beogradu tokom januara 2012. godine. Korišćen je komplet upitnika: SF-36 za ispitivanje KŽ, Bekova skala depresije II (BDI-II) i sociodemografski upitnik. Analiza je

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obavljena metodama deskriptivne i analitičke statistike. **Rezultati.** Studija je obuhvatila 104 ispitanika. Većinu su činili muškarci (74%), a prosečna starost je iznosila 48,2 ± 13,0 godina. Kod 35,6% ispitanika utvrđena je dijagnoza psihijatrijske bolesti (najčešće depresije). Samoubistvo je pokušalo 28 (26,9%) ispitanika. U uzorku je bilo 82,7% pušača, a najčešće hronične somatske bolesti su bile kardiovaskularne bolesti. Komorbiditet više somatskih bolesti je bio prisutan kod trećine ispitanika. Fizička uloga je jedini domen KŽ koji je bio niži nego u opštoj populaciji ($p < 0,001$). Depresija teškog stepena utvđena je kod 20,2% ispitanika. Negativna korelacija postojala je između skorova BDI-II i svih skorova KŽ ($p < 0,001$). **Zaključak.** Mere za prevenciju beskućništva bi trebalo da uključe formiranje nacionalnog registra beskućnika, razvoj sistemske međusektorske saradnje i primenu specijalnih psihosocijalnih interventnih strategija. Kod beskućnika zdravstveni sistem treba da bude fokusiran na prevenciju i lečenje mentalnih poremećaja i hroničnih somatskih oboljenja.

Ključne reči:
beskućnici; zdravstveno stanje; kvalitet života; srbija.

Introduction

Homelessness is a multidimensional problem of contemporary society with social, medical, economic, political and other implications. Thus, there is no single definition of homelessness. In 2009, at the United Nations Economic Commission for Europe Conference of European Statisticians (CES), the Group of Experts on Population and Housing Censuses approached homelessness by defining two types of this phenomenon: (a) Primary homelessness (or rooflessness) that includes persons living in the streets without a shelter that would fall within the scope of living quarters, and (b) Secondary homelessness attributed to persons with no place of usual residence who move frequently between various types of accommodations (including dwellings, shelters and institutions for the homeless or other living quarters) [1]. The United States Department of Housing and Urban Development issued a document that explains homelessness as condition of people without regular dwelling (lack of regular, safe, and adequate housing or fixed, regular, and adequate night-time residence) [2].

The approach by European experts underlines evidences that homelessness is more likely to be a temporary than a prolonged state. Today, researchers in different fields analyse homelessness as complex problem emerging through collision of individual and structural factors [3]. Epidemiological data from different parts of developed world give further perspective on homelessness: estimated 700,000 homeless living in USA [4], 84,900 households classified as homeless in England [5], in 2010 some 248,000 people were homeless in Germany [6] while only 5,000 were registered in the Republic of Korea in 2011 [7].

Despite a large number of studies published mainly by social scientists and medical researchers, reports about health-related quality of life (HRQoL) of homeless persons remain sparse. On the other hand, multitude of investigations address mental health issues in this population, either by simply describing prevalence of psychiatric disorders or by trying to establish causal relation between lack of regular home and mental disturbances or *vice versa* [4, 8–10].

There is a summary of consistent evidence that homeless people have a higher prevalence of chronic disease than the population as a whole, including both mental and somatic disease [11]. In 1999, Barrow et al. [12], the Columbia University Center for Homelessness Prevention Studies, found a 4 times higher age-adjusted death rates of homeless persons when compared to those of general US population and 2 to 3 times in comparison to New York City population. Currently, high morbidity and mortality in this marginal social group is attributed to numerous health risks explainable by homelessness: increased risk of suffering from violence or abuse, reduced access to health care, low hygiene, difficulties in obtaining and storing food, as well as privacy needed for sleep [13]. Mental illnesses (including addiction) are often recognized as important etiological factor for homelessness, along with other causative factors such as physical disability, substance abuse and social exclusion [14, 15].

The problem of homelessness in Serbia is mostly neglected by public and scientific community. This is in contrast to current Serbian social context which provides ground for spread of homelessness (poverty, high unemployment rate, transition, high number of refugees, proliferation of non-hygienic dwellings, insufficient homelessness prevention strategy). There are no official data nor relevant estimates on the number of homeless persons in Serbia. Our research is motivated by the lack of investigations, especially health-related, in this marginal population. We hypothesized that homeless persons have significant health issues, both mental and physical, that could be addressed through health-related quality of life investigations.

The aims of this study were to assess HRQoL and depression in homeless persons in Belgrade, to describe their sociodemographic factors and health status (the presence of chronic mental and somatic diseases and addiction disorders) and analyse impact of sociodemographic factors and health status to HRQoL and depression of homeless persons.

Methods

The study was conducted in the Shelter for Adult and Elderly Persons in Belgrade within January 2012. The Shelter for Adult and Elderly Persons is governed by the City of Belgrade and is defined as an institution of urgent social care with task to provide shelter and protection to persons with urgent social need. Capacities include 105 regular beds with the possibility of acquiring additional beds during winter time (up to 20%). It is the only institution of its kind on the City of Belgrade teritory, for 1.6 milion inhabitants in the metropolitan area.

A set of questionnaires used in the survey included the Serbian translation of SF-36 questionnaire, Serbian translation of Beck Depression Inventory – II (BDI-II) and sociodemographic questionnaire. SF-36 is a well-known and widely used generic HRQoL instrument that measures eight domains of HRQoL: physical functioning (PF), role functioning physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role functioning emotional (RE), and mental health (MH). Calculation of SF-36 composite scores is performed by standardized formula that results in physical health composite score (PCS), mental health composite score, and SF-36 total composite score (TCS). The SF-36 domains are scored by a 0 to 100 scale with zero representing the lowest possible score, highest defined with 100. In general population, used as a norm-based reference group, 50 represents the mean score. Higher values mean better HRQoL domains in the tested subjects. Scoring of SF-36 scales and further were performed according to Ware's survey manual recommendations. Depression was measured with the Serbian translation of Beck Depression Inventory – II (BDI-II) [16]. Depression was designated as minimal if the BDI-II score range was within 0–13, as mild if 14–19, as moderate if 20–28 and severe depression if scores ranged above 28 [16]. The sociodemographic and health status questionnaire was designed specifically for this study and it included 27 questions. The questions in this part of the survey instrument covered social, demographic and health status that the authors considered important on the basis of previous studies on homelessness-related medical issues [17–19]. Health status was investigated through the questions on current and lifetime alcohol, tobacco and illicit drugs use and current or lifetime presence of chronic disease (including mental disorders). Somatic chronic diseases that were included in the questions of the survey instrument were: epilepsy, asthma, chronic obstructive pulmonary disease, ischemic heart disease, chronic heart failure, heart arrhythmia, arterial hypertension, chronic liver disease, chronic renal insufficiency, rheumatic diseases, cancer, diabetes mellitus, cerebrovascular diseases, AIDS and tuberculosis (diagnoses based on ICD-10).

All the three questionnaires were designed to be self-administered and only completely filled data were used in further analysis. The set of questionnaires was offered to all homeless persons staying in the Shelter for Adult and Elderly Persons in Belgrade during a study period and the response rate was 84.7%. The individuals were considered as eligible for the study if they were able to communicate with the investigators and self-administer the test. Additional data on participants' health were retrieved from individual dossiers attributed to each person in the Shelter and containing thorough medical and social history.

Descriptive statistics, such as mean ± standard deviation (SD) on the collected data were calculated. Assessing the difference of SF-36 scores and BDI-II score between different homeless subgroups (based on sex, marital status, educational level, length of homelessness and morbidity) was performed by the *t*-test or ANOVA. Pearson correlation coefficients were used to examine the relation between the SF-36 scores and the scores of BDI-II. We used *t*-test to compare SF-36 domain, composite and total scores of the studied group to general population standard. The chi-square test was used to determine if severity of depression (groups designated as minimal, mild, moderate and severe) was related to gender, age group, educational level, marital status, length of homelessness, suicide attempt and the presence of chronic disease.

The statistical significance level was set at $p < 0.05$.

Results

Our study sample consisted of 104 adult participants. The majority of them were male (74%). The average age in the whole sample was 48.2 ± 13.0 years (range from 19 to 74), with 31.7% participants in the age group between 51 and 60 years. We registered that only 4 (3.8%) participants were living in marital community, while others were single (never married, divorced or widowed). The majority of participants had finished high school (54.8%), 11.5% had higher education, while others had lesser than high school. Homelessness longer than 5 years was reported by 36 (34.6%) participants and this was the largest subgroup, while second largest portion of the participants (33.6%) were homeless for less than one year. The majority of the participants had no income (salary or social welfare) what soever. Detailed demographic data of the participants are represented in Table 1.

Table 1

Social and demographic characteristics

Variable	Homeless persons, n (%)
Age (years)	
18–30	9 (8.7)
31–40	17 (16.3)
41–50	23 (22.1)
51–60	33 (31.7)
> 60	22 (21.2)
Marital status	
married	4 (3.8)
divorced	39 (37.5)
widowed	12 (11.5)
never married	49 (47.2)
Education	
no completed school	8 (7.7)
elementary school	27 (26)
high school	57 (54.8)
higher education	12 (11.5)
Length of homelessness	
less than 1 year	35 (33.6)
1–2 years	14 (13.5)
2–3 years	10 (9.6)
3–4 years	3 (2.9)
4–5 years	5 (4.8)
more than 5 years	36 (34.6)
Income status	
salary	19 (18.3)
social welfare	12 (11.5)
no income	73 (70.2)

Regarding mental illness in the studied population, we found that 35.6% of the participants had been diagnosed with psychiatric disorder, most frequently with depression (life-

time prevalence of 15.4% of the whole study group) and schizophrenia (10.6%). The history of suicide attempts was registered in 28 (26.9%) participants, and currently present insomnia in 6.7%. During 6 months prior to the study, 33.7% participants were prescribed with psychiatric drugs (mostly benzodiazepines and antidepressants). Lifetime illicit drugs use was reported by 13 (12.5%) of the studied homeless persons (8 with heroin addiction). Use of illicit drugs within 12 months prior to the study was reported by 4 (3.8%) subjects. Daily smoking was present in 82.7% of the participants while 8.7% had daily alcohol consumption. More detailed data on mental and addiction disorders in our sample are represented in Table 2.

Table 2

Mental and addiction disorders

Variable	Homeless persons, n (%)
Lifetime prevalence of mental disorders	
depression	16 (15.4)
schizophrenia	11 (10.6)
neurotic disorders	10 (9.6)
Lifetime prevalence of illicit drug use	
marijuana	4 (3.8)
cocaine	4 (3.8)
heroin	8 (7.7)
morphine	2 (1.9)
synthetic drugs	6 (5.7)
Alcohol use	
daily	9 (8.7)
weekly	15 (14.4)
monthly	8 (7.7)
less frequent than monthly	11 (10.6)
do not drink	61 (58.7)
Smoking	N (%)
>20 cigarettes/day	29 (27.9)
10-20 cigarettes/day	34 (32.7)
<10 cigarettes/day	23 (22.1)
do not smoke	18 (17.3)
Use of psychiatric medicaments during past 6 months	
benzodiazepines	21 (20.2)
antidepressants	7 (6.7)
antipsychotics	7 (6.7)
disulfiram	1 (0.9)
methadone	1 (0.9)
Lifetime suicide attempts prevalence	
suicide attempted	28 (26.9)
BDI-II score depression severity	
minimal depression	39 (37.5)
mild depression	20 (19.2)
moderate depression	24 (23.1)
severe depression	21 (20.2)

BDI-II – Beer Depression Inventary

The most common somatic chronic diseases in the studied population of homeless were cardiovascular: 21 of the participants had the diagnosis of arterial hypertension and 16 chronic heart failure. Chronic lung disease were second most frequent chronic health issues of homeless in our group since 13 participants had the diagnosis of chronic obstructive pulmonary disease (COPD) and 11 were diagnosed with asthma. The absence of chronic somatic disease was noted in 37 (35.6%) participants, while single chronic disease was present in 33 (31.7%) participants. Comorbidity of 2 chronic diseases was present in 20 (19.2%) homeless persons, and 5 or more somatic chronic diseases were simultaneously present in 3.9% participants. Complete data on chronic somatic disease status in sample are depicted in Table 3.

Table 3

Prevalence of chronic somatic disease and comorbidity

Variable	Homeless persons, n (%)
Lifetime presence of chronic somatic diseases	
yes	66 (63.5)
Lifetime prevalence of cardiovascular diseases	
arterial hypertension	21 (20.2)
heart failure	16 (15.4)
cardiac arrhythmia	7 (6.7)
myocardial infarction	6 (5.8)
Lifetime prevalence of chronic lung diseases	
chronic obstructive pulmonary disease	13 (12.5)
asthma	11 (10.6)
Lifetime prevalence of neurologic diseases	
epilepsy	8 (7.7)
cerebrovascular disease	5 (4.8)
Lifetime prevalence of other diseases	
chronic liver diseases	9 (8.7)
diabetes mellitus type 2	8 (7.7)
degenerative rheumatism	7 (6.7)
chronic renal failure	6 (5.8)
Comorbidity of chronic somatic diseases	
2 diseases	20 (19.2)
3 diseases	8 (7.7)
4 diseases	2 (1.9)
> 4 disease	4 (3.9)

The results of health-related quality of life in 104 homeless participants are shown in Figure 1. A statistical

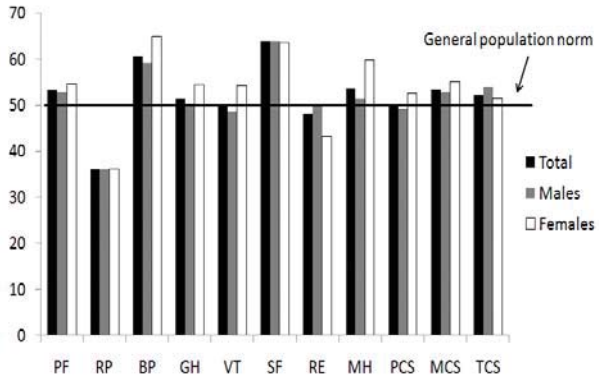

Fig. 1 – Health related quality of life

PF – physical functioning; RP – role physical; BP – bodily pain; GH – general health; VT – vitality; SF – social functioning; RE – role functioning emotional; MH – mental health; MCS – mental composite score; PCS – physical composite score; TCS – total composite SF-36 score.

significance between participants` HRQoL SF-36 domain scores and norms of general population was found for role physical domain (RP – lower in homeless, $p < 0.001$) and bodily pain and social functioning (BP, SF – higher values in participants than in general population, $p < 0.001$). When

comparison of SF-36 scores was done between sexes, no statistical significance was found; we remark that all domains of HRQoL were slightly higher in female homeless persons with the exception of role emotional domain scoring higher (without statistically significant difference) in males.

The ANOVA showed no statistically significant difference in SF-36 HRQoL domain and composite scores between different age groups, nor did marital status, education level, length of homelessness, alcohol use or smoking significantly affect the HRQoL. The presence of insomnia had statistically highly significant negative impact on mental health (MH) SF-36 domain (*t*-test, $p < 0.007$), and broader negative effect was noted in the participants with the history of suicide attempt who had significantly lower scores for PF, RP, VT, PCS and TCS. The diagnosis of depression or schizophrenia did not significantly affect HRQoL. Use of illicit drugs during 6 months prior to the study was related to significantly lower PF, RP, BP, RE, PCS scores. In participants with chronic lung disease (chronic obstructive pulmonary disease or asthma) VT and SF domains were found to have significantly lower values than in other participants, while diabetes mellitus was associated with significantly lower PF, RP and PCS scores. We found a strong negative correlation between comorbidity of somatic chronic diseases and all domains and composite scores of SF-36 and BDI-II score (Pearson corelation, $p < 0.001$).

The mean BDI-II score in the studied population was 19.1 ± 11.6, while median value was 19. Severe depression (BDI-II score above 28) was registered in 21 (20.2%) of the participants, moderate in 24 (23.1%), mild in 20 (19.2%) and minimal in 39 (37.5%). A statistically highly significant negative correlation was verified between BDI-II on one side and all domains and composite scores of SF-36 on the other (Pearson correlation, $p < 0.0001$). The χ^2 test showed that depression severity was not influenced by the age, gender, marital status, educational level, length of homelessness, alcohol use, smoking or a previously diagnosed chronic psychiatric condition (including depression). On the other hand, the χ^2 revealed a significantly higher presence of suicide attempts among the participants with the current moderate and severe depression tested by BDI-II ($p < 0.05$). Also, lifetime illicit drug abuse was founded in a significantly higher presence within moderate and severe depression subgroups of the participants, while the use of drugs during the recent 6 months was not significantly different between the depression subgroups. In the participants with severe and moderate depression a significantly higher number had chronic somatic disease, and comorbidity of 3 or more chronic diseases was associated with severe depression (χ^2, $p < 0.003$).

Discussion

The majority of the participants in the study were male, which is in accordance to previous studies on homeless populations [7, 14, 20]. Observation that more than 50% of the participants were older than 50 years of age is higher than expected. On the other hand, the trend of homeless population becoming significantly older over the last two decades has already been noted [21]. Similar studies revealed that apart from changes in age, there is a higher prevalence of chronic homelessness, schizophrenia, chronic somatic disease and restriction of social support among contemporary homeless persons [22]. In Serbian context, a higher risk of elderly persons for homelessness could be attributed to inadequate housing, chronic health issues, poverty, loneliness and family violence [23].

Over a third of the surveyed participants have the diagnosis of mental illness. This is in accordance to previous studies from different countries that show higher prevalence of mental disorders among homeless (ranging from 30% to 50%) when compared with general population [8, 9, 11, 24]. In our group depression is the most prevalent chronic mental illness (43.2% of all mental illnesses in the group), followed by schizophrenia; data from literature confirm that psychotic and affective disorders are the most fruequent mental illnesses in homeless population [8, 9]. In recent publication on sociodemographic aspects of homelessness in Serbia, similar data were retrieved for the prevalence of mental disorders [23]. Since mental disorders are usually seen both as risk factor and consequence of homelessness, the findings of this study correspond to the high presence of this type of morbidity in homeless population. Explanation for depression as leading mental illness in Serbian homeless could be also attributed to their health status (HRQoL and the prevalence of chronic disease) as well as to other well-known factors: low self-esteem, losing of life chances, family dissolution, poverty, unemployement.

Twenty eight (26.9%) of the participants have the history of suicide attempt. Our instrument, however, did not include questions which could identify homeless persons with current suicidal ideation regardless of past suicide attempts. According to studies with the focus on suicide phenomena in homeless population, a high prevalence of suicidal attempts could have been expected, with the possibility of substantialy present suicidal ideation [25, 26]. For example, study by Desai et al. [25] showed lifetime prevalence of suicidal ideation (66%) and attempts (51%) in a large sample of homeless in eastern USA. The results of Canadian study at the beginning of the last decade are more similar to our findings with 28% of homeless men and 57% of women reporting suicide attempt during lifetime [26]. Among the participants, there was no significant difference in this matter between genders, with slightly higher prevalence in women (less than 1% difference in prevalence). Previous studies on relation between homelessness and suicide indicated that the history of childhood homelessness, current homelessness longer than 6 months and the presence of mental illness were most important risk factors for suicide in homeless [26]. A contribution of our study could be found in the result that homeless persons with the history of suicide attempt had significantly lower scores of several HRQoL scores (PF, RP, VT, PCS and TCS) and a significantly higher prevalence of moderate and severe depression. The presence of lifetime history of suicide indicated prolonged and serious symptomatology of depression in large portion of subjects. We assume that a higher level of current depression and lower HRQoL in participants with suicide attempt history reveals a pattern of depression con-

tinuation that should be more thoroughly addressed in this subset of homeless.

In our study group, lifetime illicit drugs use was reported by 12.5% of the participants (most with heroin addiction), while more than two thirds of the participants reported the absence of regular alcohol use. In comparison to published data from Western hemisphere [4, 8], we report a lower prevalence of drugs and alcohol use. Large meta-analysis that included 29 studies on mental disorders among homeless reported addiction disorders as leading mental problem in this population (the prevalence of 37.9% for alcoholism and 24.4% for illicit drugs) [4]. There is also a report on 6–7 fold higher risk for alcohol and substance abuse among homeless than in general population, but the same study reveals a current trend towards predominance of illicit drugs use (especially synthetic drugs) over alcoholism [27]. In Serbia, there is a lack of studies describing epidemiology of drug use. Since more than 70% of the studied group is older than 40 years of age, a low prevalence of illicit drug use (especially synthetic) could be attributed to the structure of sample.

Smoking was present in 82.7% participants which is in accordance to the multitude of previous studies [28, 29]. We did not find any significant difference regarding gender among homeless smokers in contrast to recent Czech study [30]. A higher prevalence of smoking in homeless when compared to general population of Serbia [31] could be attributed to stressful living conditions of homeless persons.

Data contracted from the questions that tried to capture epidemiology of chronic somatic illnesses in the studied population are mostly coherent with previous investigations abroad [32, 33]. In our study group, almost two thirds of homeless had at least one chronic somatic disorder which is comparable to findings by Schanzer et al. [34] and significantly higher than the prevalence reported by Plumb [33]. Cardiovascular diseases (mainly arterial hypertension and chronic heart failure) were recognized as the most prevalent chronic somatic disorders among homeless by other researchers and these results are similar to ours [35]. High prevalence of cardiovascular disorders among homeless has already been linked to higher presence of smoking, diabetes and stress when compared to general population [29, 36, 37]. We identified chronic respiratory diseases as the second most prevalent group of somatic chronic diseases in our participants. Similar observations were made with reports of 15% prevalence of obstructive lung disease in homeless persons which is significantly higher than in general population [38]. Also, a high prevalence of comorbidity that we found has been previously elucidated, especially a combined presence of physical, mental and substance abuse disorders [7, 39].

The results of SF-36 HRQoL instrument applied in the sample showed a significantly lower score only for the role physical domain. This stands in contrast to findings that mental scores are usually lower in homeless than in general population [40, 41]. However, there are reports that underline lower physical QoL domains in homeless which is even more pronounced in mentally ill homeless persons [41, 42]. In this study, lower HRQoL domain scores were found in subgroups of homeless with insomnia, lifetime suicide attempt and illicit drugs use during 6 months prior to study. As expected, the presence of specific chronic somatic diseases (COPD, diabetes) was linked to lower physical health scores. Also, comorbidity of chronic diseases was significantly negatively correlated with all domains of HRQoL, occurrence previously elaborated by Wright and Tompkins [43]. These authors suggest a spectrum of possible health promoting interventions that includes primary prevention measures (vaccination and hygiene promotion) and management of addiction disorders. In the study group, however, chronic infections do not pose a significant part of morbidity. Interventions based on our research should be directed primarily towards early identification and better treatment options for chronic cardiovascular and pulmonary disorders.

All the domains of HRQoL were significantly negatively correlated to depression of the participants measured by BDI-II. A strong correlation of the results generated by SF-36 questionnaire and BDI-II have been documented in populations other than homeless, showing that depression level and HRQoL have a strong mutual interrelation in different settings [44].

The mean BDI-II score found in the group was similar to that of other homeless populations [45]. High rates of depression on the basis of Beck Depression Inventory scales were previously described in homeless, with severe depression prevalence ranging as high as 41% [46]. Finding that the participants` current BDI-II scores are significantly lower in those with previous suicide attempts, suggests that suicidal ideation is one of the strongest indicators of severe depression. A similar effect on depression severity was found for lifetime illicit drug users, but not for the participants with alcohol abuse or smokers. Comorbidity of 3 or more chronic disease was associated with severe depression which is expected in the context of abundant evidence provided by other researchers [47–49]. Since we established no significant difference in BDI-II scores between the participants with history of treated depression and other participants, we can speculate that depression is underdiagnosed in this marginal population. Insufficent access of sheltered homeless persons to psychiatric evaluation and care could be the main cause of lately diagnosed or unrecognized depression in homeless.

Conclusion

A high prevalence of severe and moderate depression in our study group is in accordance to international studies and provides an insight for strategic planning of eventual psychosocial interventiolns in homeless persons. A significant presence of chronic mental and somatic illnesses in homeless persons and its impact on the quality of life implies that this part of population should not be of interest only for social wellfare system but also for health institutions. To our best knowledge, this is the first study on HRQoL and depression in Serbian homeless persons, so we believe that further studies are needed to elucidate in more details numerous issues described in our investigation. In Serbia, there is no clear health and social policy toward homelessness. Homelessness in Serbia is not properly sta-

tistically evaluated, it is not in focus of media or public attention and there is no official and updated definition of this phenomenon. Prevention measures could include: foundation of national registry of homeless persons, development of systemic multisectorial cooperation (social welfare, health system, employment service) and special psychosocial intervention strategy such as psychological strengthening, professional training programs, alleviation of social stigma and family counseling. In homeless population, health care measures should be focused on prevention and treatment of mental health disorders and chronic somatic diseases.

REFERENCES

1. *United Nations Economic and Social Council.* Enumeration of Homeless People. Economic Commission for Europe Conference of European Statisticians, Group of Experts on Population and Housing Censuses; Twelfth Meeting; 2009 Oct 28–30, Geneva: UNECE; 2009. p. 3−4.
2. *United States Department of Housing and Urban Development.* Federal Definition of Homeless. [cited 2012 April 19]; Available from: http://portal.hud.gov/hudportal/HUD?src=/topics/homelessness/definition
3. *ETHOS – European Typology on Homelessness and Housing Exclusion.* [cited 2012 Apr 19]; Available from: http://www.feantsa.org/code/EN/pg.asp?Page=484
4. *Fazel S, Khosla V, Doll H, Geddes J.* Prevalence of mental disorders among the homeless in Western countries: Systematic review and meta-regression analysis. PLoS Med 2008; 5(12): e225.
5. Department for Communities and Local Government (2007) Homelessness statistics September 2007 and rough sleeping: 10 years on from the target. London: Crown Copyright; 2007.
6. FAENTSA Flash November 2011. [cited 2012 May 23]. Available from: http://www.bag-wohnungslosenhilfe.de/index2.html
7. *Yoon C, Ju Y, Kim C.* Disparities in Health Care Utilization Among Urban Homeless in South Korea: A Cross-Sectional Study. J Prev Med Public Health 2011; 44(6): 267–74.
8. *Reinking DP, Wolf JR, Kroon H.* High prevalence of mental disorders and addiction problems among the homeless in Utrecht. Ned Tijdschr Geneeskd 2001; 145(24): 1161−6. (Dutch)
9. *North CS, Eyrich KM, Pollio DE, Spitznagel EL.* Are rates of psychiatric disorders of the homeless population changing? Am J Public Health 2004; 94(1): 103−8.
10. *Henry JM, Boyer L, Belzeaux R, Baumstarck-Barrau K, Samuelian JC.* Mental disorders among homeless people admitted to a French psychiatric emergency service. Psychiatr Serv 2010; 61(3): 264−71.
11. *Martens WH.* A review of physical and mental health in homeless persons. Public Health Rev 2001; 29(1): 13−33.
12. *Barrow SM, Herman DB, Córdova P, Struening EL.* Mortality among homeless shelter residents in New York City. Am J Public Health 1999; 89(4): 529–34.
13. *Amster R.* Lost in Space: The Criminalization, Globalization, and Urban Ecology of Homelessness. New York: LFB Scholarly; 2008.
14. *Folsom D, Hawthorne W, Lindamer L, Gilmer T, Bailey A, Golshan S.* Prevalence and risk factors for homelessness. Am J Psychiatry 2005; 162(8): 370−6.
15. *Snow DA, Anderson L.* Down and Out in America. The Origins of Homelessness. Peter H. Rossi. University of Chicago Press, Chicago, 1989. xii, 247 pp. $15.95. Science 1990; 247(4949): 1485−6.
16. *Beck AT, Steer RA, Brown, GK.* Beck Depression Inventory. 2nd ed. San Antonio, TX: The Psychological Corporation; 1996.
17. *Hwang SW.* Homelessness and health. CMAJ 2001; 164(2): 229−33.
18. *Trevena LJ, Nutbeam D, Simpson JM.* Asking the right questions of disadvantaged and homeless communities: the role of housing, patterns of illness and reporting behaviours in the measurement of health status. Aust N Z J Public Health 2001; 25(4): 298−304.
19. *Wiersma P, Epperson S, Terp S, Lacourse S, Finton B, Drenzek C*, et al. Asking the right questions of disadvantaged and homeless communities: the role of housing, patterns of illness and reporting behaviours in the measurement of health status. South Med J 2010; 103(1): 18−24.
20. *Tolomiczenko G, Goering P.* Gender differences in legal involvement among homeless shelter users. Int J Law Psychiatry 2001; 24(6): 583−93.
21. *Hahn J, Kushel M, Bangsberg D Riley E, Moss AR.* BRIEF REPORT: The Aging of the Homeless Population: Fourteen-Year Trends in San Francisco. J Gen Intern Med 2006; 21(7): 775–8.
22. *Israel N, Toro PA, Ouellette N.* Changes in the composition of the homeless population: 1992-2002. Am J Community Psychol 2010; 46(1−2): 49−59.
23. *Žarković B, Petrović M, Timotijević M.* Without house, without home: Results of research of homelessness in Serbia. Beograd: Hausing Centar; 2012. (Serbian)
24. *Scott J.* Homelessness and mental illness. Br J Psychiatry 1993; 162: 314−24.
25. *Desai RA, Liu-Mares W, Dausey DJ, Rosenheck RA.* Suicidal ideation and suicide attempts in a sample of homeless people with mental illness. J Nerv Ment Dis 2003; 191(6): 365−71.
26. *Eynan R, Langley J, Tolomiczenko G, Rhodes AE, Links P, Wasylenki D*, et al. The association between homelessness and suicidal ideation and behaviors: results of a cross-sectional survey. Suicide Life Threat Behav 2002; 32(4): 418−27.
27. *Frankish CJ, Hwang SW, Quantz D.* Homelessness and health in Canada: research lessons and priorities. Can J Public Health 2005; 96(2): 23−9.
28. *Okuyemi KS, Caldwell AR, Thomas JL, Born W, Richter KP, Nollen N*, et al. Homelessness and smoking cessation: insights from focus groups. Nicotine Tob Res 2006; 8(2): 287−96.
29. *Szerlip MI, Szerlip HM.* Identification of cardiovascular risk factors in homeless adults. Am J Med Sci 2002; 324(5): 243−6.
30. *Kubisová D, Adámková V, Lánská V, Dlouhý P, Rambousková J, Andel M.* Higher prevalence of smoking and lower BMI, waist circumference, cholesterol and triacylglyceride levels in Prague's homeless compared to a majority of the Czech population. BMC Public Health 2007; 7:51.
31. *Djikanovic B, Marinkovic J, Jankovic J, Vujanac V, Simic S.* Gender differences in smoking experience and cessation: do wealth and education matter equally for women and men in Serbia? J Public Health (Oxf) 2011; 33(1): 31−8.
32. *Wiersma P, Epperson S, Terp S, Lacourse S, Finton B, Drenzek C*, et al. Episodic illness, chronic disease, and health care use among homeless persons in Metropolitan Atlanta, Georgia, 2007. South Med J 2010; 103(1): 18−24.
33. *Plumb JD.* Homelessness: care, prevention, and public policy. Ann Intern Med 1997; 126(12): 973−5.
34. *Schanzer B, Dominguez B, Shrout PE, Caton CL.* Schanzer Homelessness, Health Status, and Health Care Use. Am J Public Health 2007; 97(3): 464−9.

35. *Trabert G.* Health status and medical care accessibility of single, homeless persons. Gesundheitswesen 1997; 59(6): 378−86. (German)
36. *Hwang SW, Orav EJ, O'Connell JJ, Lebow JM, Brennan TA.* Causes of death in homeless adults in Boston. Ann Intern Med 1997; 126(8): 625−8.
37. *Lee TC, Hanlon JG, Ben-David J, Booth GL, Cantor WJ, Connelly PW,* et al. Risk factors for cardiovascular disease in homeless adults. Circulation 2005; 111(20): 2629−35.
38. *Snyder LD, Eisner MD.* Obstructive lung disease among the urban homeless. Chest 2004; 125(5): 1719-25.
39. *Lundy JW.* The burden of comorbidity among the homeless at a drop-in clinic. JAAPA 1999; 12(4): 32−4, 37−8, 41−2 passim.
40. *Sun S, Irestig R, Burström B, Beijer U, Burström K.* Health-related quality of life (EQ-5D) among homeless persons compared to a general population sample in Stockholm County, 2006. Scand J Public Health 2012; 40(2): 115−25.
41. *Salize HJ, Horst A, Dillmann-Lange C, Killmann U, Stern G, Wolf I,* et al. How do mentally ill homeless persons evaluate their quality of life? Psychiatr Prax 2001; 28(2): 75−80. (German)
42. *Sullivan G, Burnam A, Koegel P, Hollenberg J.* Quality of life of homeless persons with mental illness: results from the course-of-homelessness study. Psychiatr Serv 2000; 51(9): 1135−41.
43. *Wright NM, Tompkins CN.* How can health services effectively meet the health needs of homeless people? Br J Gen Pract 2006; 56(525): 286−93.
44. *Drulovic J, Pekmezovic T, Matejic B, Mesaros S, Manigoda M, Dujmovic I,* et al. Quality of life in patients with multiple sclerosis in Serbia. Acta Neurol Scand 2007; 115(3): 147−52.
45. *Votta E, Farrell S.* Predictors of Psychological Adjustment Among Homeless and Housed Female Youth. J Can Acad Child Adolesc Psychiatry 2009; 18(2): 126−32.
46. *Miner M.* The self-concept of homeless adolescents. J Youth Adolesc 1991; 20(5): 545−60.
47. *Lee Y, Choi K, Lee YK.* Association of Comorbidity with Depressive Symptoms in Community-Dwelling Older Persons. Gerontology 2001; 47(5): 254−62.
48. *Gallegos-Carrillo K, García-Peña C, Mudgal J, Romero X, Durán-Arenas L, Salmerón J.* Role of depressive symptoms and comorbid chronic disease on health-related quality of life among community-dwelling older adults. J Psychosom Res 2009; 66(2): 127−35.
49. *Egede LE.* Effect of Comorbid Chronic Diseases on Prevalence and Odds of Depression in Adults With Diabetes. Psychosom Med 2005; 67(1): 46−51.

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Transference patterns and working alliance during the early phase of psychodynamic psychotherapy

Transferni obrasci i radni savez tokom rane faze psihodinamičke psihoterapije

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Abstract

Background/Aim. Working alliance, as a collaborative part of the therapeutic relationship has been proven to be one of the most powerful therapeutic factors in psychotherapy in general, regardless many technical differences between numerous psychotherapeutic modalities. On the other hand, transference is the basic concept of psychodynamic psychotherapy, and, according to the psychoanalytic theory and practice, it forms a major part of the therapeutic relationship. The aim of our paper was to determine the differences between the groups of patients with low, middle, and high working alliance scores and the dropout group in transference patterns, sociodemographic and clinical parameters, during the early phase of psychodynamic psychotherapy. **Methods.** Our sample consisted of 61 non-psychotic patients, randomly selected by the method of consecutive admissions and treated with psychoanalytic psychotherapy in the outpatient clinical setting. The patients were prospectively followed during 5 initial sessions of the therapeutic process. The working alliance inventory and Core conflictual relationship theme method were used for the estimation of working alliance and transference patterns, respectively. According to the Working Alliance Inventory scores, four groups of patients were formed and than compared. **Results.** Our results show a significant difference between the groups of patients with low, middle, and high working alliance inventory scores and the dropout group on the variable – transference patterns in the therapeutic relationship. **Conclusion.** Disharmonious transference patterns are more frequent in patients who form poor quality working alliance in the early phase of psychotherapy, or early dropout psychotherapy. It is of great importance to recognize transference patterns of a patient at the beginning of the psychotherapeutic process, because of their potentially harmful influence on the quality of working alliance.

Key words:
psychotherapy; young adult; physician-patient relations; transference (psychology).

Apstrakt

Uvod/Cilj. Dokazano je da je radni savez, kao saradnički deo psihoterapijskog odnosa, jedan od najmoćnijih terapijskih faktora u psihoterapiji uopšte, bez obzira na mnoge razlike u tehnici koje postoje među brojnim psihoterapijskim pravcima. Sa druge strane, transfer je osnovni koncept psihodinamičke psihoterapije, i prema psihoanalitičkoj teoriji i praksi, čini veliki deo terapijskog odnosa. Cilj našeg rada bio je da utvrdimo razlike između grupa bolesnika sa niskim, srednjim i visokim skorom radnog saveza i grupe bolesnika koji su prekinuli terapiju, i to u odnosu na transferne obrasce u terapijskom odnosu, kao i sociodemografske i kliničke karakteristike. **Metode.** U ovu studiju bio je uključen 61 nepsihotični bolesnik, slučajno izabran metodom konsekutivnih prijema i tretiran psihoanalitičkom psihoterapijom u ambulantnim uslovima. Bolesnici su prospektivno praćeni tokom pet početnih seansi terapijskog procesa. Za procenu radnog saveza i transfernih obrazaca korišćeni su Upitnik radnog saveza i Metod jezgrovne konfliktne teme u odnosima. Na osnovu visine skora na Upitniku radnog saveza, formirane su i upoređivane četiri grupe bolesnika. **Rezultati.** Naši rezultati pokazuju da postoji značajna razlika između bolesnika sa niskim, srednjim i visokim skorom radnog saveza i grupe bolesnika koji su prekinuli terapiju, i to u odnosu na ponavljanje transfernih obrazaca u terapijskom odnosu. **Zaključak.** Disharmonični transferni obrasci češći su kod bolesnika koji u ranoj fazi psihoterapije, formiraju radni savez lošeg kvaliteta, ili rano prekidaju psihoterapiju. Veoma je značajno transferne obrasce bolesnika na početku psihoterapijskog procesa, zbog njihovog potencijalno štetnog uticaja na kvalitet radnog saveza.

Ključne reči:
psihoterapija; mlade osobe; lekar-bolesnik, odnosi; transfer (psihološki).

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Introduction

During past several decades, there has been growing interest in psychotherapy research. This is partly due to the fact that it has been established that research is one of the ways leading to the integration of more and more numerous psychotherapeutic modalities, because there has been success in research aimed at extracting common therapeutic factors as the ground basis for integration [1]. The therapeutic or working alliance (WA) has been proven to be one of the factors with the constant and the most potent predictive value for psychotherapy outcome, found out in many researches, on different types of psychotherapeutic treatments and patient's problems [2–4]. In psychotherapy research WA has been conceptualized as the collaborative relationship between a patient and a therapist, defined according to the Bordin's tripartite definition [5]. This definition points to the importance of the agreement between patient and therapist about the goals of the therapy, tasks leading to these goal's achievement, as well as emotional bond between the participants of psychotherapy process (a patient and a therapist). It refers mainly to the conscious dimension of the wholeness of a therapeutic relationship.

Transference is one of the cornerstones of the psychoanalytic theory. It is mainly unconscious part of a therapeutic relationship, covering all the unconscious feelings, whishes, attitudes and behaviors displaced (transferred) from the important persons in patient's past and present life, to the person of therapist in a therapeutic relationship [6]. In psychotherapy research, transference is operationalized through transference patterns, referring to the enduring relational patterns, repeated (enacted) in the therapeutic relationship [7].

Working alliance and transference patterns are intertwined in different proportions during a process of psychotherapy. Their interrelationship has not been investigated, until instruments for their assessment have been developed. There are still many unknowns about their mutual influences. It seems that the therapeutic relationship is "the servant of two cruel masters" – transference and working alliance, like in the famous Freud's metaphor about the Ego as a servant of two masters – Id and Superego. It is not clear – do patient's transference patterns manifest themselves in the early phase of psychotherapy.

The aim of our paper was to determine differences between groups of patients with low, middle, high working alliance scores and dropout group in transference patterns, socio-demographic and clinical parameters, during the early phase of psychodynamic psychotherapy.

Methods

Sample. For this study, 61 non-psychotic outpatients of the Clinic for Mental Health-Clinical Center Niš, referred from the Department for Psychiatric Diagnostics to the Department for Psychotherapy, with indication for psychodynamic psychotherapy, were selected by the method of consecutive admissions in the period from January 2009 to January 2012. Clinical diagnosis was established in accordance with the criteria of the International Classification of Mental Disorders – 10 (ICD 10) [7] and using Mini International Neuropsychiatric Interview (M.I.N.I. Version 5.0.0) [8]. For the assessment of global functioning, the Global Assessment of Functioning (GAF) [9] Scale was used. The patients were from the diagnostic groups – neurotic, stress related and somatoform disorders (F40-F48), depressive episode without psychotic features (F32.0-F32.2), and personality disorders (F60-F61). All the patients were 18 to 45 years old, they were informed about the research and signed informed consent form. Patients with mental retardation, dependency disease, organic mental disorders, imminent suicidality, serious somatic disease, or in need for hospitalization were excluded from the study. After the psychiatrist had done initial psychiatric assessment, the patients were assessed by the psychotherapist. For the establishment of psychodynamic diagnosis, semi-structured interview for the Operationalized Psychodynamic Diagnosis (OPD-2) [10] was used. The Relationship Anecdotes Paradigm Interview (RAP) [11] was a part of it directed toward collecting relationship episodes with important others. This is the semi structured interview, lasting about 20 minutes, describing relevant interactions with important persons from the patient's past and present. Six to 8 interactions are needed, describing 3 components of a transference pattern – dominant wish, attitude or need of the patient toward the other; answer of the other person to this wish; and reaction of the patient to this person's answer. These 3 elements constitute a relationship episode. The most dysfunctional repetitive relationship episode is used for extracting core conflictual relationship theme (CCRT) which is than scored using Category system CCRT-LU [12]. CCRT has been used in many researches as the measure of transference patterns. Scoring has been done by the practicing psychotherapist, immediately after the initial psychodynamic assessment. For every patient, the most dominant wish (W), reaction of other (RO) and reaction of self (RS) match with one category from CCRT-LU category system that best describes it. This category system consists of 119 descriptive categories and 13 clusters. Clusters A, B, C and D with belonging categories are harmonious, and clusters from E to M are disharmonious in relation to the feelings associated with them [13–16].

After the initial psychiatric and psychodynamic assessment, all of the patients started psychodynamic psychotherapy treatment with the same therapist. The therapy has been conducted according to the standard procedure for psychodynamic psychotherapy – once weekly sessions lasting 45 to 50 minutes, always at the same place and time. Therapy was not manualized and transference interpretations were not used. After each session, the therapist extracted relationship episodes including patient-therapist interactions and patient's spontaneous as well as elicited comments about this relationship. After the 4th session a patient and the therapist independently filled in the Working Alliance Inventory-short version (WAI S) for the patient and the therapist [13–16], revision from 1989 [17]. The therapist also scored relationship episodes of the therapeutic relationship using CCRT-LU categories. The patients have continued psychodynamic psychotherapy treatment after this research.

A total of 14 patients dropped out the treatment before the 5th session. They were assigned to the dropout or D group. The rest of the patients (47 of them) were assigned to the therapeutic or T group, which was divided into 3 subgroups in respect to the WAI S patient's score – T1 group of patients with low WAI S – 13 patients, T2 group with medium WAI S – 20 patients and T3 group with high WAI S – 14 patients. Cut off values between the groups were 25th and 75th percentiles of the WAI S scores (recommended by the author of WAI – Horvath AO). The groups were compared on socio-demographic, clinical variables and number of disharmonious categories of wish, reaction of self and reaction of others in relationships with important others and with the therapist. For input of data, graphs and tables we used a Microsoft Excel personal computer (PC) software. Quantitative statistical analysis was done by PC software SPSS 15.0. Mean values for numerical variables were compared using the Kruskal-Volis test. Attributive variables were compared using the Mantel-Hansel χ^2 test or Fisher's test when the frequency was lower than 5. Values $p < 0.05$ were considered statistically significant.

This research was approved by the Ethics Committee of Medical Faculty, University of Niš.

Results

Our investigation included 61 patients in the process of individual psychoanalytic psychotherapy. Socio-demographic characteristics – gender, age, education, employment, marital status and economic status are shown in Table 1.

Table 2 shows clinical characteristics – diagnostic groups and GAF of the patients in our sample.

The distribution of frequencies for socio-demographic characteristics and results of comparison of groups T1, T2, T3 and D by age, gender, occupation, marital status, employment and average monthly income *per* person of household are shown in Table 3. There was no significant difference between the groups compared by these socio-demographic variables.

The parameters of clinical diagnosis did not differ significantly in the groups T1, T2, T3 and D. The clinical characteristics and the results of group comparison are shown in Table 4.

The mean patients score for the whole T group on WAI S was 61.53 ± 6.99, and the mean therapist score was 67.38 ± 11.18. The mean value of WAI S for the T1 group was 52.92 ± 6.946, for the T2 group 68.65 ± 4.580 and for the T3 group 79.00 ± 3.211.

Table 1

Socio-demographic characteristics of the whole sample of patients

Age in years (mean ± stand.dev.)	27.39 ± 6.230
Gender (n, %)	
female	49 ± (80.3)
male	12 ± (19.7)
Education (n, %)	
attending high school	3 (4.9)
high school education	17 (27.9)
university student	21 (34.4)
bachelor degree	7 (11.5)
master degree	13 (21.3)
Marital status (n, %)	
not married	46 (75.4)
Married	15 (24.6)
Employment status (n, %)	
unemployed	44 (72.1)
Employed	17(27.9)
Average monthly income in Euro (E) (*per* person of household) (n, %)	
without income	1 (1.6)
under 50 E	5 (8.2)
under 80 E	24 (39.3)
under 100 E	17 (27.9)
under 150 E	10 (16.4)
under 200 E	3 (4.9)
over 200 E	1 (1.6)

Table 2

Clinical characteristics of the whole sample of patients

Prevalent diagnosis	n	%
Neurotic (F40-F48)	20	32.8
Depressive (F32.0-F32.2)	12	19.7
Personality disorder	29	47.5
With comorbid diagnosis	21	34.4
Without comorbid diagnosis	40	65.6
Total	61	100.00
GAF score, mean ± st. deviation	66.18	8.68

GAF – global assessment of functioning.

Table 3

Socio-demographic parameters in the groups of patients: distribution of frequencies and the results of group of patients comparison

Parameter	Number (%) of patients in the group				Results of group comparison			
	T1	T2	T3	D	Pearson χ^2	df	*p*	phi
Age (years)								
18–25	6 (46.2)	12 (60.0)	5 (35.7)	6 (42.9)				
26–34	6 (46.2)	4 (20.0)	7 (50.0)	5 (35.7)	4.701	6	0.583	0.278
35–45	1 (7.7)	4 (20)	2 (14.3)	3 (21.4)				
Gender								
female	9 (69.2)	15 (75.0)	13 (92.9	12 (85.7)	3.020	3	0.389	0.223
male	4(30.8)	5 (25.0)	1 (7.1)	2 (14.3)				
Education								
attending high school	0 (0.0)	2 (10.0)	0 (0.0)	1 (7.1)				
high school	2 (15.4)	3 (15.0)	7 (50.0)	5 (35.7)				
university student	7 (53.8)	8 (40.0)	3 (21.4)	3 (21.4)	15.112	12	0.235	0.498
bachelor degree	2 (15.4)	1 (5.0)	3 (21.4)	1 (7.1)				
master degree	2 (15.4)	6 (30.0)	1 (7.1)	4 (28.6)				
Marital status								
not married	10 (76.9)	14 (70.0)	11 (78.6)	11 (78.6)	0.483	3	0.923	0.089
married	3 (23.1)	6 (30.0)	3 (21.4)	3 (21.4)				
Employment								
unemployed	9 (69.2)	15 (75.0)	11 (78.6)	9 (64.3)				
employed	4 (30.8)	5 (25.0)	3 (21.4)	5 (35.7)	0.584	3	0.837	0.118
without	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)				
Average monthly income in Euro (per person of household)								
under 50 E	2 (15.4)	1 (5.0)	2 (14.3)	0 (0.0)				
under 80 E	2 (15.4)	8 (40.0)	7 (50.0)	7 (50.0)				
under 100 E	7 (53.8)	6 (30.0)	2 (14.3)	2 (14.3)	21.385	18	0.260	0.592
under 150 E	1 (7.7)	4 (20.0)	2 (14.3)	3 (21.4)				
under 200 E	1 (7.7)	0 (0.0)	0 (0.0)	2 (14.3)				
over 200 E	0 (0.0)	0 (0.0)	1 (7.0)	0 (0.0)				

T1 – the group with a low Working Alliance Inventory – short version (WAIS) score; T2 – the group with a medium WAIS score; T3 – the group with a high WAIS score; D – the dropout group.

Table 4

Clinical parameters in the groups of patients: distribution of frequencies and the results of group comparison

Parameter	Number (%) of patients in the group				Results of group comparison			
	T1*	T2*	T3*	D*	Pearson χ^2	df	*p*	phi
Diagnostic category								
neurotic disorder	4 (30.8)	8 (40.0)	3 (21.4)	5 (35.7)				
depressive disorder	0 (0.0)	4 (20.0)	5 (35.7)	3 (21.4)	6.988	6	0.322	0.338
personality disorder	9 (69.2)	8 (40.0)	6 (42.9)	6 (42.9)				
Presence of comorbidity	2 (15.4)	9 (45.0)	3 (21.4)	7 (50.0)	5.630	3	0.131	0.304
absent	11 (84.6)	11 (55.0)	11 (78.6)	7 (50.0)				
present								
GAF score	26.23	33.88	28.39	33.93	2.210	3	0.530	0.28
mean range	60.00	70.00	62.50	67.50				

***For explanation see under Table 3; GAF – global assessment of functioning.**

The results on CCRT-LU categorical system show that frequency of disharmonious wish is much higher in the patient-therapist relationship in the T1 group compared to the other groups (Table 5).

In the group D there was a statistically significant higher frequency of disharmonious categories of reaction of other in patient-therapist relationship. In the group T2 frequencies were lower than expected (Table 6).

The frequencies of CCRT-LU disharmonious categories of reaction of self were significantly higher in the group D, while at the same time, their frequencies in the groups T2 and T3 were lower than expected (χ^2 =13.476, df = 3, $p < 0.01$, phi = 0.470) (Table 7).

Table 5

Frequencies of conflictual relationship theme category system (CCRT-LU) disharmonious categories of wish (W) in patient-therapist relationship

CCRT-LU W patient-therapist relationship	Patient group				Total
	T1*	T2*	T3*	D*	
Harmonious					
count	7	18	13	13	51
expected count	10.9	16.7	11.7	11.7	51.0
% within group	53.8	90.0	92.9	92.9	83.6
Disharmonious					
count	6	2	1	1	10
expected count	2.1	3.3	2.3	2.3	10.0
% within group	46.2	10.0	7.1	7.1	16.4

***For explanation see under Table 3; $\chi^2 = 10.745$; df = 3; $p = 0.004$; phi = 0.420.**

Table 6

Frequencies of conflictual relationship theme category system (CCRT-LU) disharmonious categories of reaction of others (RO) in patient-therapist relationship

CCRT-LU RO patient-therapist relationship	Patient group				Total
	T1*	T2*	T3*	D*	
Harmonious					
count	11	19	12	8	50
expected count	10.7	16.4	11.5	11.5	50.0
% within group	84.6%	95.0%	85.7%	57.1%	82.0%
Disharmonious					
count	2	1	2	6	11
expected count	2.3	3.6	2.5	2.5	11.0
% within group	15.4%	5.0%	14.3%	42.9%	18.0%

***For explanation see under Table 3; $\chi^2 = 8.330$; df = 3; $p = 0.04$; phi = 0.370.**

Table 7

Frequencies of conflictual relationship theme category system (CCRT-LU) disharmonious categories of reaction of self in patient-therapist relationship

CCRT-LU RS patient-therapist relationship	Patient group				Total
	T1*	T2*	T3*	D*	
Harmonious					
count	6	13	11	2	32
expected count	6.8	10.5	7.3	7.3	32.0
% within group	46.2	65.0	78.6	14.3	52.5
Disharmonious					
count	7	7	3	12	29
expected count	6.2	9.5	6.7	6.7	29.0
% within group	53.8	35.0	21.4	85.7	47.5

***For explanation see under Table 3; $\chi^2 = 13.476$; df = 3; $p = 0.004$; phi = 0.470.**

Discussion

Our sample of patients consisted mainly of young patients in the post-adolescent age, the majority of them were students. This is in accordance with our psychotherapeutic clinical practice, as well as with the findings of other authors [18, 19], which also underlined the great need for psychotherapy in this population. Young people are also those who are the most interested in psychotherapy and, at the same time, the most susceptible to personal change and growth that psychotherapy enables. Post-adolescence is the developmental phase when individuality is finally shaped, and independence of personality achieved. We could state that psychotherapy is often "the treatment of choice" for these patients. At the same time, this developmental phase is also very vulnerable, because it makes visible all of the deficits and failures of previous developmental stages, so that psychiatric and psychotherapeutic treatment often becomes necessary.

A review of clinical parameters of our sample points to the fact that the most frequent diagnostic category was personality disorder, and also, that there was a high frequency of comorbidity. This finding is understandable having in mind that personality disorders prevalence is greatest in adolescents and young adults. It is also well-known that 50% of patients with personality disorder have the comorbid diagnosis on Axis I (clinical syndromes) [20–24].

Therapeutic or working alliance has been proven to be a critical feature of all successful or effective psychotherapies [25]. Our results show the difference in estimation of working alli-

ance between the patient and therapist. The patients consistently estimated alliance with higher scores than therapists. This finding conforms to the findings of many other authors [26–28], but in spite of this fact, it was not finally explained. It is possible to assume that discrepancy in scoring of working alliance could be consequence of different observing perspectives of patients and therapists.

Development of therapeutic alliance passes through several phases, but the most vulnerable one is the initial phase of psychotherapy, because of many influences on the part of a patient and the therapist which come into their emerging relationship [29]. Working alliance thus becomes a mirror of therapeutic relationship trends. We hypothesized that one of the most powerful influences on working alliance comes from the patterns of interpersonal relationships or transference patterns which patient brings to the therapy relationship. Transference patterns are enduring relational patterns, repeated or enacted in the therapeutic relationship. One of the first methods for the estimation of transference patterns was the Core Conflictual Relationship Theme Method by Luborsky [11]. It was later further developed and transformed in the model CCRT-LU [12]. It enables differentiation of 3 elements of the relationship that could make relationship dysfunctional. In our research, the Core conflictual relationship method – LU version, was the useful instrument for the assessment of interpersonal relationships and early detection of dysfunctional therapeutic relationship.

Our results show that disharmoniousness of every one of these elements – wish, reaction of other and reaction of self – was more frequent in the patients with lower level of working alliance, or with dropout of therapy. Early psychotherapy dropout is considered the most extreme manifestation of disturbance in working alliance.

Higher frequencies of disharmonious categories of dominant wish in the therapeutic relationship were evidenced in patients with low WAI S patient's score. This could be explained with the fact that the patients who form weak therapeutic alliance more frequently have unrealistic expectancies (wishes) from the therapist they meet, maybe even stronger if they already had insufficient or deficient relationships in their past. There could be a wish to "compensate" or repair this deficiency in idealized therapeutic relationship. These patients are not ready for true psychotherapeutic work and personal change, instead, they expect much more "corrective emotional experience".

The second element of dysfunctional relationship pattern enacted in therapeutic relationship is the way a patient experiences reaction of the therapist to his dominant wish. Our results show that patients with disharmonious experience on this dimension of relationship are prone to dropout of therapy. On the other hand, patients with good therapeutic alliance have no disharmonious experience to this extent. Disharmonious experience in the relationship with therapist could be a consequence of coloring of actual experience according to the experiences in the patient's past. Patients with interpersonal problems (especially with diagnosis of personality disorder), have very deep feeling of misunderstanding and maltreatment by the others in previous or actual relationships. Often, it is the sense that the other people do not accept their needs and wishes, or do not want to answer them appropriately. Often, it is possible to trace this experience to the earliest developmental phases and internalized object relations. Sometimes, as our previous results show, interpersonal disappointment of a patient arises from the inadequate wish, inappropriate in actual relationship.

Connecting previous experiences with the actual ones, and discussing with patient about their differences, could help a patient to understand the nature of his interpersonal problems. This technical tool in psychoanalytic psychotherapy, named transference interpretation, emphasizes interpersonal perspective in the "here and now" situation.

Our findings are similar to those of Beretta et al. [30] who used the CCRT method in their research showing that therapeutic alliance is connected with the patient's wish to be close to someone, to experience others as supporting and confident, but at the same time, they perceive the answers from others as negative, because their object representations are not confident and are hurting [30].

The final element of dysfunctional relationship pattern is the reaction of the self of the person after receiving answer from others to his dominant wish. Our results are similar in this dimension as in the previous one – disharmonious type of reaction of the self was more frequently present in the dropout group of patients, and less frequent in the groups with middle and high WAI S scores. Patients who react to interpersonal disappointment with disharmonious attitudes or behaviors are more prone to splitting of experience and relationships [31, 32].

The dropout group was prominent by its differences in the frequencies of disharmonious categories on dimensions – reaction of other and reaction of self, even without higher frequencies of disharmonious wish. It means that these patients do not have unrealistic expectations in therapeutic relationship, but they still experience the therapist (mostly on the basis of transference experience) as unaccepting, malicious, distant, unhelpful, etc. and than react to these experiences withdrawing from the relationship. It is considered a manifestation of bad early object relationship. We considered these results especially important because of the practical reasons – prevention of patient attrition from psychotherapy. Prevention becomes more important if we have in mind that these patients actually need therapy even more than the others, because of their cumulative interpersonal problems. Early detection of dysfunctional relationship patterns would be of decisive importance in this regard.

Our results lead to the conclusion that in the early phase of psychotherapy, with patients who have difficulties in interpersonal relationships, we need some adjustments in technique in order to prevent dropouts and poor working alliances. The results of Hoglend et al. [33], suggest the importance of transference work in this regard. In his study, Jarry [34] has investigated effectiveness of CCRT-based guided psychotherapy. In spite of the small sample size (6 patients) in this research, the results are promising suggesting possibility that psychotherapy can change dysfunctional relationship patterns even in short-term treatments.

For patients with prominent interpersonal dysfunctionality, treatments like this one could be a preparation for long-term psychotherapy.

In spite of the fact that our groups of patients did not differ significantly by clinical parameters, important limitation of our study concerns diagnostic heterogeneity of the sample of patients. For increasing of data generalizability, it would be important to conduct research on larger and diagnostically more homogenous samples of patients. Further research should also include more frequent working alliance assessments, because trends of working alliance increase or decrease could give more valuable data. Including observer's ratings, in order to control possible counter transference influences, would be also recommended for future research.

Conclusion

There is a significant difference between the groups of patients with low, middle, and high working alliance inventory scores and the dropout group on the variable – transference patterns in therapeutic relationship. Disharmonious transference patterns are more frequent in patients who form poor therapeutic alliance or early dropout psychotherapy. It is of great importance to recognize patient's transference patterns at the beginning of the psychotherapeutic process, because of their potentially harmful influence on the quality of working alliance. Early detection of a dysfunctional therapeutic relationship opens up the space for further improvements in therapeutic interventions and techniques in the early phase of psychotherapy.

R E F E R E N C E S

1. *Bordin ES*. Building therapeutic alliances: The base for integration. Paper presented at the Society for Psychotherapy Research. Berkley, CA: University of California Press; 1989.
2. *Horvath AO, Bedi RP*. The alliance. In: *Norcross JC*, editor. Psychotherapy relationships that work. New York: Oxford University Press; 2002.
3. *Horvath AO, Del Re AC, Fluckiger C, Symonds DB*. Alliance in individual psychotherapy. In: *Norcross JC* editor. Psychotherapy relationships that work. 2nd ed. New York: Oxford University Press; 2011.
4. *Martin DJ, Garske JP, Davis KM*. Relation of the therapeutic alliance with outcome and other variables: a meta-analytic review. J Consult Clin Psychol 2000; 68(3): 438−50.
5. *Bordin ES*. Theory and research on the therapeutic working alliance: New directions. In: *Horvath AO, Greenberg LS,* editors. The working alliance: theory, research, and practice New York: Wiley; 1994. p. 13–37.
6. *Erić Lj*. Psihoanaliza i psihoanalitička psihoterapija. In: *Erić Lj*, editors. Psihoterapija. Belgrade: Faculty of Medicine, University of Belgrade; 2001.
7. *World Health Organization*. The ICD 10 Classification od mental and behavioral disorders: clinical descriptions and diagnostic guidelines. Geneva: WHO; 1992.
8. *Sheehan DV, Lecubrier Y, Sheehan KH, Amorim P, Janavs J, Weiller E,* et al. The Mini International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM IV and ICD10. J Clin Psychiatry 1998; 59(Suppl 20): 22−33; quiz 34−57.
9. *Ramirez A, Ekselius L, Ramklint M*. Axis V - Global Assessment of Functioning scale (GAF), further evaluation of the self-report version. Eur Psychiatr 2008; 23(8): 575−9.
10. *OPD Task Force*. Operationalized Psychodynamic Diagnosis OPD-2- Manual of Diagnosis and Treatment Planning. USA, Toronto: Hogrefe and Huber; 2008.
11. *Luborsky L*. The relationship anecdotes paradigm (RAP) interview as a versatile source of narratives. In: *Luborsky L, Crits-Christoph P*, editors. Understanding Transference - The Core Conflictual Relationship Theme Method. 2nd ed. Washington DC: American Psychological Association; 1998.
12. *Albani C, Pokorny D, Blaser G, Kachele H*. Beziehungsmuster und Beziehungskonflikte-Theory, Klinik und Forschung. Gottingen: Vandenhoeck & Ruprecht; 2008.
13. *Horvath AO*. Working Alliance Inventory (WAI) Home Page. Available from: http://www.educ.sfu.ca/alliance/allianceA. [cited 1986].
14. *Horvath AO, Greenberg LS*. The working alliance: Theory, Research and Practice. New York: John Wiley and Sons; 1994.
15. *Horvath AO*. The therapeutic relationship: from transference to alliance. J Clin Psychol 2000; 56(2): 163−73.
16. *Horvath AO, Greenberg LS*. Development and validation of the Working Alliance Inventory. J Couns Psychol 1989; 36(2): 223−33.
17. *Tracey TJ, Kokotovic AM*. Factor structure of the Working Alliance Inventory. J Clin Consult Psychol 1989; 1: 207−10.
18. *Fazlagić A, Rakić-Bajić G*. Attitudes of students toward psychotherapy. PONS Med J 2011; 8(2): 48−52. (Serbian)
19. *Gavrilov-Jerković V*. Modern tendencies in psychotherapy: The specialisation of practice and knowledge integration. Psihologija 2003; 36(1): 7−38. (Serbian)
20. *Lenzenweger MF, Lane MC, Loranger AW, Kessler RC*. DSM-IV personality disorders in the National Comorbidity Survey Replication. Biol Psychiatry 2007; 62(6): 553−64.
21. *Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE*. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2005; 62(6): 593−602.
22. *Zimmerman M, Chelminski I, Young D*. The frequency of personality disorders in psychiatric patients. Psychiatr Clin North Am 2008; 31(3): 405−20.
23. *Krueger RF*. Continuity of axes I and II: toward a unified model of personality, personality disorders, and clinical disorders. J Pers Disord 2005; 19(3): 233−61.
24. *Durrett C, Westen D*. The structure of axis II disorders in adolescents: a cluster- and factor-analytic investigation of DSM-IV categories and criteria. J Pers Disord 2005; 19(4): 44061.
25. *Barber JP, Connolly MB, Crits-Christoph P, Gladis L, Siqueland L*. Alliance Predicts Patients' Outcome Beyond In-Treatment Change in Symptoms. Pers Disord: Theory, Res Treatment 2009; S(1): 80−9.
26. *Fitzpatrick MR, Iwakabe S, Stalikas A*. Perspective divergence in the working alliance. Psychother Res 2005; 15(1−2): 69−79.
27. *Tryon GS, Blackwell S, Hammel E*. A meta analytic examination of client therapist perspectives of the working alliance. Psychother Res 2007; 17(6): 629−42.
28. *Tryon GS, Blackwell SC, Hammel EF*. The magnitude of client and therapist working alliance ratings. Psychotherapy (Chic) 2008; 45(4): 546–51.
29. García Y, Botella L. Differential Analysis of Psychotherapy Outcome and Process:Correlates of Initial Disturbance (Anxiety vs. Depression). Int J Psychol Psychological Therapy 2011; 11(3): 347−58.

30. *Beretta V, Despland JN, Drapeau M, Michel L, Kramer U, Stigler M*, et al. Are relationship patterns with significant others reenacted with the therapist? A study of early transference reactions. J Nerv Ment Dis 2007; 195(5): 443−50.
31. *Barret MS, Chua WJ, Crits-Christoph P Gibbons MB, Casiano D, Thompson D*. Early withdrawal from mental health treatment: implications for psychotherapy. Psychotherapy 2008; 45(2): 247–67.
32. *Ardito RB, Rabellino D*. Therapeutic alliance and outcome of psychotherapy: historical excursus, measurements, and prospects for research. Front Psychol 2011; 2: 270.
33. *Høglend P, Hersoug AG, Bøgwald KP, Amlo S, Marble A, Sørbye Ø,* et al. Effects of Transference Work in the Context of Therapeutic Alliance and Quality of Object Relations. J Consult Clin Psychol 2011; 79(5): 697−706.
34. *Jarry JL*. Core conflictual relationship theme - guided psychotherapy: Initial effectiveness study of a 16-session manualized approach in a sample of six patients. Psychol Psychother 2010; 83(4): 385−94.

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Bleeding gastroduodenal ulcers in patients without *Helicobacter pylori* infection and without exposure to non-steroidal anti-inflammatory drugs

Krvareći gastroduodenalni ulkusi kod bolesnika bez *Helicobacter pylori* infekcije i bez upotrebe nesteroidnih antiinflamatornih lekova

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Abstract

Background/Aim. A high risk of bleeding in *Helicobacter pylori (H.pylori)*-negative, non-steroidal anti-inflammatory drugs (NSAID)-negative ulcers highlights the clinical importance of analysis of the changing trends of peptic ulcer disease. The aim of the study was to investigate the risk factors for ulcer bleeding in patients with non-*H. pylori* infection, and with no NSAIDs use. **Methods.** A prospective study included patients with endoscopically diagnosed ulcer disease. The patients were without *H. pylori* infection (verified by pathohistology and serology) and without exposure to NSAIDs and proton pump inhibitors (PPI) within 4 weeks before endoscopy. After endoscopy the patients were divided into 2 groups: the study group of 48 patients with bleeding ulcer and the control group of 47 patients with ulcer, but with no bleeding. Prior to endoscopy they had completed a questionnaire about demographics, risk factors and habits. The platelet function, von Willebrand factor (vWF) and blood groups were determined. Histopathological analysis of biopsy samples were performed with a modified Sydney system. The influence of bile reflux was analyzed by Bile reflux index (BRI). **Results.** Age, gender, tobacco and alcohol use did not affect the bleeding rate. The risk of bleeding did not depend on concomitant diseases (p = 0.509) and exposure to stress (p = 0.944). Aspirin was used by 16/48 (33.3%) patients with bleeding ulcer, as opposed to 7/47 (14.9%) patients who did not bleed (p = 0.036). Abnormal platelet function had 12/48 (25.0%) patients who bled, as opposed to 2/47 (4.3%) patients who did not bleed (p = 0.004). Patients with BRI < 14 bled in 79.2%, and did not bleed in 57.4% of the cases (p = 0.023). There was no statistical difference between groups in regards to blood groups and range of vWF. Antrum atrophy was found in 14/48 (29.2%) patients with bleeding ulcer and in only 5/47 (10.6%) patients who had ulcer without bleeding (p = 0.024). **Conclusion.** Abnormal platelet function, aspirin use and antrum atrophy were the risk factors for ulcer bleeding in non-*H. pylori*, non- NSAIDs ulcer disease.

Key words:
peptic ulcer hemorrhage; helicobacter pylori; anti-inflammatory agents, non-steroidal; risk factors.

Apstrakt

Uvod/Cilj. Visoki rizik od krvarenja *Helicobacter pylori* (*H. pylori*)-negativnih i sa nesteroidnim inflamatornim lekovima (NSAIL)-negativnih ulkusa potencirao je klinički značaj analiziranja novih tendencija peptičke ulkusne bolesti. Cilj ove studije bio je ispitati faktore rizika od nastanka krvarećeg ulkusa kod bolesnika bez *H. pylori* infekcije i bez upotrebe NSAIL. **Metode.** Prospektivna studija obuhvatala je bolesnike sa endoskopski dijagnostikovanom ulkusnom bolešću. Bolesnici su bili bez *H. pylori* infekcije (potvrđeno patohistološki i serološki) i bez upotrebe NSAIL i inhibitora protonske pumpe (IPP) tokom četiri nedelje pre endoskopije. Posle endoskopije bolesnici su bili podeljeni u dve grupe: studijsku grupu od 48 bolesnika sa krvarećim ulkusom i kontrolnu grupu od 47 bolesnika sa ulkusom bez krvarenja. Pre endoskopije bolesnici su popunjavali upitnik o demografskim podacima, faktorima rizika i navikama. Funkcija trombocita, von Willebrand faktor (vWF) i krvne grupe ispitivane su, takođe. Patohistološki su analizirani biopsijski

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uzorci po modifikovanom Sydnejskom sistemu. Uticaj bilijarnog refluksa analiziran je pomoću bilijarnog refluksnog indeksa (BRI). **Rezultati.** Godine, pol, pušenje i upotreba alkohola nisu imali uticaj na stopu krvarenja. Rizik od krvarenja nije zavisio od udruženih bolesti (p = 0,509), niti od izloženosti stresu (p = 0,944). Aspirin je uzimalo 16/48 (33,3%) bolesnika sa krvarećim ulkusom, u poredeđenju sa 7/47 (14.9%) bolesnika koji nisu krvarili (p = 0,036). Abnormalnu funkciju trombocita imalo je 12/48 (25,0%) bolesnika sa krvarenjem, u poređenju sa 2/47 (4,3%) bolesnika koji nisu krvarili (p = 0,004). Bolesnici sa BRI < 14 krvarili su u 79,2%, a nisu krvarili u 57,4% slučajeva (p = 0,023). Nije bilo statistički značajne razlike između dve grupe u odnosu na krvne grupe i nivo vWF. Antralna atrofija nađena je kod 14/48 (29,2%) bolesnika sa krvarećim ulkusom i kod 5/47 (10,6%) bolesnika koji su imali ulkus bez krvarenja (p = 0,024). **Zaključak.** Abnormalna funkcija trombocita, upotreba aspirina i antralna atrofija jesu faktori rizika od krvarenja kod *H. pylori*-negativne i NSAIL-negativne ulkusne bolesti.

Ključne reči:
peptički ulkus, krvarenje; helicobacter pylori; antiinflamatorici, nesteroidni; faktori rizika.

Introduction

Peptic ulcer disease has a multifactorial pathogenesis. The discovery of *Helicobacter pylori (H. pylori)* infection has dramatically changed the understanding and management of this clinical entity. In the 1990s a number of reports from around the world defined that *H. pylori* infection was present in more than 90% of patients with duodenal, and in about 85% of those with gastric ulcers [1]. On the other hand, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is the other major cause of peptic ulcers. Several authors have found that NSAIDs were used by 25–75% of patients with *H. pylori*-negative duodenal ulcers (DU) or that NSAIDs are the most frequent identifiable causes of non-infected DU [2].

However, the epidemiology of peptic ulcer has significantly changed in the past decades because of the huge effort made to eradicate *H. pylori* infection [3]. The proportion of peptic ulcers which is unrelated to NSAIDs and *H. pylori* appears to be increasing. In North America, there is good evidence that 20–40% of peptic ulcers are not associated with *H. pylori* or NSAIDs [3–5], whereas in other parts of the world, the proportion of *H. pylori*-negative ulcers remains much lower (less than 4%) [5]. A study from Northern Italy reported the prevalence of only 8% [6, 7]. In Europe, three studies from Scotland, Denmark and Italy showed a prevalence of *H. pylori*-negative duodenal ulcer of 10–15% [1]. Reports from different parts of Asia show a wide variation of its prevalence. The prevalence of *H. pylori*-negative ulcer ranges from 3% in Japan to 29% in Singapore and Pakistan. Recent reports show that the prevalence of *H. pylori*-negative ulcers will also depend upon the background prevalence of *H. pylori* in general population as *H. pylori*-negative ulcers will not exist if everyone has the infection [2, 5]. A meta-analysis of seven randomized double blind trials in North America found that 20% of patients with *H. pylori*-associated ulcers had ulcer recurrence within 6 months, despite successful *H. pylori* eradication and no reported NSAID use [3, 8].

Ulcers attributed to the use of aspirin have risen in number, because aspirin is widely used for the prevention of thrombotic events 9. Also, the proportion of patients taking low-dose aspirin in combination with other antiaggregants, such as clopidogrel is increasingly high [10].

About 25% of patients with idiopathic ulcers also have reactive gastritis, which might be related to bile reflux or prior NSAID use or *H. pylori* infection, so it might be that mucosa does not recover fully and remains vulnerable to other injuries.

Other etiological factors in *H. pylori*-negative, NSAID-negative ulcers are poorly defined, but may include a genetic predisposition, altered acid secretion, rapid gastric emptying, defective mucosal defense mechanisms, psychological stress, smoking, drinking alcohol or gender [11]. A number of studies have demonstrated a relationship between ABO blood group and hemostasis. Indeed, a higher rate of bleeding complications has been described in patients belonging to the group O [12, 13] and blood group O individuals are consistently over-represented in patients with inherited bleeding disorders [12, 14]. Some authors mentioned that individuals with blood group O have a higher risk of bleeding disorders due to low levels of the circulating plasma protein, von Willebrand factor (vWf) [15, 16]. In a large twin study, Orstavik et al. [17] found that 66% of the total variation in plasma vWf levels was genetically determined and 30% of this genetic component was explained by ABO blood group. Plasma levels of vWf and other coagulation factors influence risk of hemorrhage. Also, vWf is involved in platelet adhesion and subsequent platelet aggregation during primary haemostasis [18, 19]. Another reason for ulcer bleeding is probably the platelet dysfunction. It may be acquired, inherited or induced by platelet inhibiting agents, such as acetyl salicylic acid.

H. pylori-negative ulcers have been shown to have a higher incidence of mortality and recurrent bleeding [20]. Many recent reports suggest that the pathogenesis of bleeding duodenal ulcer is different from that of non-complicated ulcer disease and that other risk factors different from *H. pylori* are perhaps responsible for a relatively high proportion of bleeding ulcers [2]. Study of Hung et al. [21] showed that the incidence of *H. pylori*-negative idiopathic bleeding ulcers has increased in recent years. According to that study, *H. pylori* negative ulcers account for around 16% of bleeding peptic ulcers.

The aim of this study, was to investigate the risk factors for bleeding in *H. pylori*-negative, NSAID-negative peptic ulcers, and to examine the factors that contribute to complicated ulcers, and also to make new efforts to improve the prevention of these conditions.

Methods

The study was conducted in the Clinical Center of Montenegro from January 2010 to January 2012. A prospective study included 95 consecutive patients with endoscopically diagnosed peptic ulcer disease who were without *H. pylori* infection and without exposure to NSAIDs. We also excluded patients who were taking proton pump inhibitors (PPI), anticoagulant drugs and antiplatelet drugs except aspirin. *H. pylori* status was verified by histology (two biopsy specimens taken from the stomach antrum and two from the corpus) and serology (antibodies to *H. pylori*). In cases of bleeding ulcer we performed second look endoscopy within 72 hours from the initial endoscopy and then took biopsies for histopathology analyses. Blood samples were taken from all the patients within 24 hours in order to perform serological analyses in regard to the presence of antibodies to *H. pylori* [22]. Enzyme-linked immunoassay test (ELISA) for the quantitative detection in serum of anti-*H. pylori* IgG antibodies was performed using a commercial test kit, according to instructions of the manufacturer. The results were expressed as: reactive-positive (IgG level > 20 IU/mL); grey zone-equivocal (IgG level: 15–20 IU/mL) and non reactive-negative (IgG level < 15 IU/mL). We included in the study only patients with IgG level < 15 IU/mL (non-reactive). The patients who positively responded to the questions about NSAIDs, PPI, anticoagulant and antiplatelet drugs (except aspirin) use less than 4 weeks before endoscopy were excluded from the study. The patients were divided into two groups. The study group consisted of 48 patients with bleeding ulcer, while the control group of 47 patients also with ulcer, but without sings of bleeding.

Before endoscopy all the patients filled out the questionnaire on demographic data, habits (alcohol consumption and smoking), concomitant diseases, exposure to stress during last year, as well as on taking aspirin during the past 4 weeks. We also analyzed information related to the treatment of *H. pylori* infection in the past. They also responded to questions related to previous treatment of the diseases of upper gastrointestinal tract (gastritis, gastric ulcer and duodenal ulcer) diagnosed by endoscopy over a period of 6 months prior to inclusion in this study.

All the patients were analyzed according to gender and age. The patients who were current smokers for at least 6 months were considered as smokers. Consuming more than 10 g of alcohol/day by women, and more than 20 g/day by men was considered as medically relevant. The impact of concomitant diseases in ulcer disease was determined by the use of individual index of co-existent diseases (ICED). The values of ICED are 0–3, and reflect the impact of concomitant disease to the severity of ulcer disease. The ICED score of 0 indicates that associated diseases have no impact, score 1 indicates a mild impact, score 2 moderate and score 3 serious impact on the occurrence of ulcers [23]. Exposure to a stressful situation was graded by "Holmes and Rahe stress scale". The patients answered whether in the past year they had been exposed to some of the possibly stressful situations. By adding points for all the exposure situations, the total number (score) indicates the impact of stress on health, or occurrence of the disease. Therefore, the score value of 300 and more indicates a risk of disease, score value of 150–299 means that the risk of disease is intermediate (reduced by 30%) and the score value of 150 or less indicates a very low risk of disease [24]. Blood groups were determined by the principle of hemagglutination. The possible impact of duodenogastric biliary reflux was assessed by calculating bile reflux index (BRI) according to Sobala et al. [25]. This index is calculated by the following formula: BRI = (7 × E) + (3 × IM) + (4 × CI) – (6 × Hp), where E is edema in the lamina propria, IM – intratestinal metaplasia, CI – chronic inflammation and Hp represents colonization of the stomach with *H. pylori*. The pathologists were grading each parameter from antral biopsy specimens from 0 to 3. A value of BRI above 14 indicates significant duodenogastric reflux (bile acid level grater than 1 mmol/L, which is the upper limit of normal biliary reflux) with 70% sensitivity and 85%, specificity. Von Willebrand factor plays an important role in both primary hemostasis by the formation of the hemostatic plug (due to its function in platelet adhesion) and in aggregation. We used a Dade Behring vWF Ag test kit intended for *in vitro* diagnostic use with Dade Behring coagulation analyzers for the quantitative determination of vWF Ag in human plasma by immunoturbidimetry. The normal plasma vWF level in adult population is usually in the range of 50–160%. Detection of platelet dysfunction in citrated human whole blood was done by the PFA - 100 system. This system consists of an instrument and two different test cartridges in which the process of platelet adhesion and aggregation following a vascular injury is simulated *in vitro*. The collagen/epinephrine (Col/EPI) test cartridge is the primary cartridge used to detect platelet dysfunction induced by intrinsic platelet defects, vWF, or exposure to platelet inhibiting agents. A reference range for Col/EPI is 84–160. The collagen/ADP (Col/ADP) test cartridge is used to indicate if an abnormal result obtained with the Col/EPI test cartridge may have been caused by the effect of aminosalicylic acid (ASA) or medication containing ASA. A reference range for Col/ADP is 68–121 s. The platelet function test is marked as normal if both Col / EPI and Col / ADP are normal, and when any of them or both abnormal, test is marked as abnormal.

Endoscopy procedure and histopathology examination of mucosal biopsy samples

All endoscopies were done by an Olympus endoscope (GIF TYPE Q 165) and biopsies were taken with standard forceps. Ulcer was defined as any visual loss of mucosal integrity, of a diameter greater than 5 mm, and bleeding stigmata were classified according to the modified Forrest classification [26]. According to that classification all ulcers are divided into those who have not bled, those that have bled and those with bleeding during endoscopy. In cases when ulcer bleeding required endoscopic hemostasis, it was done using either injection technique with diluted epinephrine or by mechanical device (hemoclipping). From any ulcer and surrounding mucosas biopsies were taken to exclude the existence of the diseases which required different therapeutic approaches, such as malignant ulcers, Crohn's disease, lymphoma, etc.At the same time, two biopsies from the antrum and two from the corpus were taken in order to histopathologically confirm the absence

of *H. pylori* infection, and to additionally analyze the type of gastritis. The modified Sydney system for classification of gastritis was used for interpretation of biopsy samples (Hematoxylin and Eosin staining) [27]. Modified staining by Giemsa was used for *H. pylori* determination.

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by Ethical Committee, Clinical Center of Montenegro (No 03/01-688/2, from January 28, 2010). All the patients provided a written informed consent.

All data were analyzed using SPSS, version 16.0 (SPSS Inc, Chicago, IL, USA). Values are expressed as means or percentages as appropriate. The χ^2-test was used to analyze the difference between the two groups. Univariate and multivariate logistic regression was used to analyze the risk [odds ratio (OR) and 95% confidence interval (CI)] of developing bleeding ulcer in patients with peptic ulcer disease. The differences were considered significant at the level of $p < 0.05$.

Results

There were 48 patients with bleeding ulcer in the study group and 47 patients with ulcer with no signs of bleeding in the control gruop.

The demographic characteristics, habits and medical histories with the results for the patients in the study and the control group are shown in Table 1. Gender did not affect bleeding. A higher incidence of bleeding peptic ulcer was found in men but with no statistical significance ($p = 0.358$). There was no difference in bleeding ulcer in relation to age groups. There was higher incidence of bleeding ulcers among older patients, but without statistical significance ($p = 0.350$). Bleeding was not affected by smoking. Alcohol consumption was more frequent among patients who bled, but the difference was not significant ($p = 0.115$). Risk of bleeding from ulcer did not depend on concomitant diseases. We had similar findings related to exposure to stress with no significant difference between the investigated groups ($p = 0.944$). The previous treatment of *H. pylori* infection had no effect on new bleeding ulcers. Of the patients covered by the study, 44.8% of those who were previously treated for *H. pylori* infection bled, as opposed to 55.2% of the patients who were also previously treated, but now had ulcer without signs of bleeding ($p = 0.461$). The previous treatment of gastric or duodenal ulcer, did not affect future ulcer bleeding. The history of gastritis was significantly different among the investigated groups ($p = 0.038$). The logistic regression showed that the medical history of gastritis had a protective effect on future ulcer bleeding with relative risk for ulcer bleeding 5 times less than in patients with no previous history of gastritis ($p = 0.017$). Logistic regression model (univariate and multivariate) is shown in Table 2.

Table 1

Demographic sharacteristics, habits and medical history of patients

Characteristics of the patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number	48 (50.5)	47 (49.5)	
Gender			
men	30 (62.5)	25 (53.2)	0.358
Age, years ≥ 50	33 (68.8)	28 (59.6)	0.351
Medical history			
gastritis	10 (20.8)	19 (40.4)	0.038*
gastric ulcer	5 (10.4)	6 (12.8)	0.72
duodenal ulcer	13 (27.1)	7 (14.9)	0.145
Smoking			
yes	22 (45.8)	22 (46.8)	0.924
Alcohol user			
yes	24 (50.0)	16 (34.0)	0.115
Exposure to stress [24]			
< 150	15 (31.3)	15 (31.9)	0.944
150–299	22 (45.8)	20 (42.6)	
> 300	11 (22.9)	12 (25.5)	
Concomitant diseases			
yes	33 (68.7)	36 (76.6)	0.391
Previous treament of *H. pylori*			
yes	13 (44.8)	16 (55.2)	0.461

***statistically significant difference**

Table 2

Logistic regression model for bleeding ulcers

Characteristics of the patients	Univarate Logistic Regression		Multivariate Logistic Regression	
	Exp B (95% CI)	p	Exp B (95% CI)	p
Aspirin use	2.857 (1.048–7.786)	0.040*	1.758 (0.423–7.295)	0.437
Previous gastritis	0.388 (0.156–0.962)	0.041*	0.197 (0.052–0.746)	0.017*
Platelet dysfunction	7.500 (1.576–35.683)	0.011*	20.703 (1.724–48.643)	0.017*
Bile reflux index ≥ 14	0.355 (0.144–0.878)	0.025*	0.144 (0.040–0.514)	0.003*
Antrum atrophy	3.459 (1.132–10.566)	0.029*	9.075 (1.768–46.576)	0.008*

***statistically significant difference**

The influence of aspirin and platelet function on ulcer bleeding can be seen in Table 3. Aspirin consumption was a significant predictive factor for bleeding in univariate logistic regression, but with no significance in multivariate regression model (Table 2). The platelet function was abnormal in 12 (25.0%) patients with bleeding peptic ulcer, as opposed to 2 (4.3%) who had abnormal function of platelet and did not bleed (χ^2 test; $p = 0.004$). At the same time, relative risk for ulcer bleeding in the patients with platelet dysfunction was very high: 20.7 ($p = 0.017$) (Table 2).

Table 3

Effects of aspirin and platelet function on bleeding ulcer

Use of aspirin and platelet function	Study group n (%)	Control group n (%)	χ^2-test (p)
Drug aspirin use	16 (33.3)	7 (14.9)	0.036*
Platelet function abnormal	12 (25.0)	2 (4.3)	0.004*

***statistically significant difference**

The effects of blood groups and serum levels of vWF on ulcer bleeding are presented in Table 4. Most of the patients with bleeding ulcer had blood group O (45.8%), while blood group A was the most frequent among the patients without ulcer bleeding (40.4%). There was no significant difference between the study and control patients regarding blood groups ($p = 0.268$). The serum level of vWF did not affect the bleeding rate, since the most of the patients in both groups had normal level of vWF and similar proportion in both groups had lower level of vWF ($p = 0.935$).

Histopathological analysis of gastric mucosa and its impact on ulcer bleeding are shown in Table 5. Of all the parameters of histopathological analysis of gastritis, only atrophy of the antrum was significant predictive factor for ulcer bleeding (χ^2; $p = 0.024$). Relative risk for ulcer bleeding among patients with gastric antrum atrophy was 9.075 ($p = 0.008$). The presence of significant duodenogastric bile reflux seemed to be protective against bleeding since almost half of patients in the control group (42.6%) and only 20.8% from the study group had BRI >14 (χ^2-test; $p = 0.023$). Relative risk for ulcer bleeding was almost 7 times less in patients with high value of BRI ($p = 0.003$) (Table 2).

Table 4

The influence of blood groups and serum levels of von Willebrand factor (vWF) on ulcer bleading

Characteristics of patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number	48 (50.5)	47 (49.5)	
Blood groups			
A	12 (25.0)	19 (40.4)	
B	8 (16.7)	9 (19.1)	0.268
AB	6 (12.5)	6 (12.8)	
0	22 (45.8)	13 (27.7)	
(vWF)			
normal	29 (60.4)	28 (59.6)	
lower	5 (10.4)	6 (12.8)	0.935
higher	14 (29.2)	13 (27.7)	

Table 5

The influence of histopathological characteristics on ulcer bleeding

Characteristics of the patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number of patients	48 (50.5)	47 (49.5)	0.05
Antrum histology (present)			
intestinal metaplasia	13 (27.1)	18 (38.3)	0.244
inflammation	46 (95.8)	46 (97.9)	0.570
atrophy	14 (29.2)	5 (10.6)	0.024*
activity	34 (70.8)	25 (53.2)	0.076
Corpus histology (present)			
intestinal metaplasia	10 (20.8)	12 (25.5)	0.587
inflammation	46 (95.8)	44 (93.6)	0.629
atrophy	13 (27.1)	13 (27.7)	0.950
activity	24 (50.0)	30 (63.8)	0.174
Biliary reflux index > 14	10 (20.8)	20 (42.6)	0.023*

***statistically significant difference**

Discussion

The prevalence of *H. pylori* infection is changing and proportion of ulcers that are *H. pylori*-negative and NSAID-negative seems to be increasing. Some authors observe that these "idiopathic" peptic ulcers seem to be more resistant to standard therapy, may be associated with more frequent complications and these that relapse may require long-term maintenance therapy [1, 2, 11, 28]. However, as antisecretory medications are often less effective in controlling gastric pH in *H. pylori*-negative patients, it is recommended to prescribe full doses of these drugs as maintenance therapy in this scenario [2].

The incidence of bleeding ulcers is between 32 and 51 *per* 100 000 *per* year [29]. Bleeding represents about 70% of all ulcer disease complications with the highest morbidity and mortality [30].

It seems that the prevalence of *H. pylori* infection is lower among patients with bleeding ulcer than in patients with ulcer disease without bleeding. Hung et al. [20] show that among 638 patients with bleeding ulcers there was 18.8% *H. pylori* negative patients.

One explanation for the increase in bleeding rate among *H. pylori*–negative, NSAIL-negative ulcers is stress-related ulcerogenesis due to other medical conditions [3, 31]. Between 5% and 20% of patients with gastric or duodenal ulcer lack an identifiable organic etiology and data available in studies published worldwide suggest that psychosocial factors play a significant role [31–33]. Wong et al. [34] have shown that exposure to a stressful situation plays an important role in the pathogenesis of *H. pylori* negative ulcers, with a high risk of rebleeding and mortality. Even though we could not find that exposure to stress is related to bleeding peptic ulcers, we observed that almost two thirds of patients with bleeding ulcer (68.7%) had the score higher than 150, graded by "Holmes and Rahe stress scale".

Patients with non-NSAID and non-*H. pylori* ulcers are often older, sicker and more frequently experience bleeding episodes while in hospital [28]. Xia et al. [35] have shown that 17% of duodenal ulcers were *H. pylori*- and NSAID-negative and revealed that the presence of concomitant diseases was an independent predictor for those ulcers. Some other studies gave the same results [36–38]. Na et al. [39] compared geriatric (older than 65) and non-geriatric patients with peptic ulcer bleeding and found that there was no difference in the incidence of *H. pylori* negative ulcers between compared groups. In the same time, geriatric patients with bleeding ulcers had much higher rate of cardiovascular and pulmonary concomitant diseases compared to the group of non-geriatric patients.

Our results are not in accordance with these findings especially in regard to concomitant diseases where we found that even greater percentage of patients with other diseases did not have ulcer bleeding. At the same time, patients older than 50 were more frequent in our study group than in the control group, again with no significant difference, probably because of a small number of patients included in the present study.

The relationship between blood group antigens and peptic ulcer disease, especially with upper gastrointestinal bleeding was widely evaluated in the past and one of the studies found that the blood group O had higher frequency in the group with bleeding ulcer than in the control group compared to other blood groups [40]. The reason for bleeding is due to the level of the circulating plasma protein von Willebrand factor [15]. It seems that vWf levels are 25% higher in non-O compared to group O individuals [16]. An interesting finding is that the rebleeding rate between patients with different blood groups was similar [40]. At the same time, some other studies find determination of blood groups not being a useful tool to determine the individual risk for gastroduodenal ulcer and ulcer bleeding [41, 42]. In our study we found that the most patients in the bleeding group had blood group O and blood group A in the control group, but there was no significant difference between the two groups in regard to blood groups.

Although a number of studies have demonstrated the influence of ABO blood group on the levels of von Willebrand factor, the nature of this association and its clinical importance is still largely unknown. A deficiency of vWF is responsible for a hemorrhagic diathesis [12]. In our study low level of vWf did not affect ulcer bleeding.

Platelet aggregation can be inhibited by aspirin. Thrombocytopathy or impaired platelet function leads to the commonest gastrointestinal complication – bleeding [43, 44]. Kang et al. [44] found that the most important risk factor for bleeding peptic ulcers in *H. pylori* negative patients was a history of aspirin and/or antiplatelet agent use. We have also shown that abnormal platelet function was a significant risk factor for ulcer bleeding. In the present study, one third of patients who were taking aspirin had bleeding ulcer, and only 14.9% in the group of patients who were taking aspirin and had ulcer disease without signs of bleeding. Regular or periodical use of aspirin, other antiplatelet agents and/or anticoagulant drugs is an important reason for ulcer bleeding.

After *H. pylori* cure, gastric acid hypersecretion is not a risk factor for bleeding from duodenal ulcer, neither among patients with previous bleeding episodes. However, duodenal ulcer recurrence with bleeding may occasionally occur in patients cured of *H. pylori*, even if acid output is normal [45]. Kang et al. [44] found that patients with bleeding peptic ulcer had a higher proportion history of peptic ulcer disease. In our study, neither previous treatment of *H. pylori* infection, nor previous ulcers existence of any localization had any significant effect on new bleeding ulcer. Mc Coll [5] reported that ulcers recurring after *H. pylori* eradication are most probably equivalent to ulcers in patients without evidence of current or previous infection and should be managed in the same way. In our study we had 44.8% of patients with bleeding ulcer who were previously treated for *H. pylori* infection, as well as 55.2% of those with ulcers without bleeding and with previous *H. pylori* infection treatment. Eradication of *H. pylori* infection did not seem to protect our patients from developing bleeding ulcer disease. It is interesting that patients with previously treated gastritis had a less risk of ulcer bleeding in our study.

More studies founded that the long-standing mucosal inflammation caused by *H. pylori* infection can weaken the gastric barrier against acid or bile reflux [3]. Bile and duodenal contents have chronic noxious effects on both stomach and esophagus. Long-term exposure, proximal reflux of duodenal juice can damage unprotected mucosa and can cause dysplasia, intestinal metaplasia and ulcers. The bile reflux index is derived from observed changes in tissue histology and is, thus, an important tool that can reflect mucosal changes caused by the bile [46]. In the present study we showed that the possibility for ulcer bleeding was less if BRI was higher. It seems that the alkaline content of bile may have a protective effect on bleeding.

Kemppainen et al. [47] showed that antrum atrophy was associated with a higher risk of bleeding ulcer. While *H. pylori* infection is associated with chronic active antral gastritis, a lower prevalence of *H. pylori* is found in patients with atrophic gastritis and intestinal metaplasia. This study also showed that atrophy of the antrum was a significant risk factor for ulcer bleeding.

Conclusion

The etiopathogenesis of non-*H. pylori* and non-NSAID ulcers and ulcer bleeding in that setting has not been established yet. Our results suggest that different factors were involved in the etiopathogenesis of bleeding in *H. pylori*-negative, NSAID-negative peptic ulcers. Relative risk for bleeding from ulcer was significantly higher among aspirin users, patients with abnormal platelet function and patients with antrum atrophy. A high bile reflux index and previous treatment of gastritis had a protective effect on ulcer bleeding among patients with non-*H. pylori* and non-NSAID ulcers.

R E F E R E N C E S

1. *Arroyo MT, Forne M, de Argila CM, Feu F, Arenas J, de la Vega J*, et al. The prevalence of peptic ulcer not related to Helicobacter pylori or non-steroidal anti-inflammatory drug use in negligible in Southern Europe. Helicobacter 2004; 9(3): 249−54.
2. *Gisbert J P, Calvet X*. Helicobacter pylori-negative duodenal ulcer disease. Aliment Pharmacol Ther 2009; 30(8): 791−815.
3. *Chow KL, Sung JY*. Is the prevalence of idiopathic ulcers really on the increase? Nat Clin Pract Gastroenterol Hepatol 2007; 4(4): 176−7.
4. *Jyotheeswaran S, Shah AN, Jin HO, Potter GD, Ona FV, Chey WY*. Prevalence of Helicobacter pylori in peptic ulcer patients in greater Rochester, NY: is empirical triple therapy justified? Am J Gastroenterol 1998; 93(4): 574−8.
5. *McColl EL*. How I manage H.pylori-negative, NSAID/Asprin-negative peptic ulcers. Am J Gastroenterol 2009; 104(1): 190−3.
6. *Goenka MK, Majumder S, Sethy PK, Chakraborty M*. Helicobacter pylori negative, non-steroidal anti-inflammatory drug-negative peptic ulcers in India. Indian J Gastroenterol 2011; 30(1): 33−7.
7. *Meucci G, Di Battista R, Abbiati C, Benassi R, Bierti L, Bortoli A*, et al. Prevalence and risk factors of Helicobacter pylori-negative peptic ulcer. J Clin Gastroenterol 2000; 31: 42−7.
8. *Laine L, Hopkins RJ, Girardi LS*. Has the impact of Helicobacter pylori therapy on ulcer recurrence in the United States been overstated? A meta-analysis of rigorously designed trials. Am J Gastroenterol 1998; 93(9): 1409−15.
9. *Schlansky B, Hwang JH*. Prevention of nonsteroidal anti-inflammatory drug-iduced gastropathy. J Gastroenterol 2009; 44(19): 44−52.
10. *Nardulli G, Lanas A*. Risk of gastrointerstinal bleeding with aspirin and platelet antiaggregants. Gastroenterol Hepatol 2009; 32(1): 36−43.
11. *Quan C, Talley NJ*. Management of peptic ulcer disease not related to Helicobacter pylori or NSAIDs. Am J Gastroenterol 2002; 97(12): 2950−61.
12. *Franchini M, Capra F, Targher G, Montagnana M, Lippi G*. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. Thromb J 2007; 5: 14.
13. *Evans D, Horwich L, McConnell R, Bullen M*. Influence of the ABO blood groups and secretor status on bleeding and on perforation of duodenal ulcer. Gut 1968; 9(3): 319–22.
14. *Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR*. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood 1987; 69(6): 1691–5.
15. *Corcoran PA, McGuane DE, McGrath AM, Burke CM, Byrne MF*. Blood group O and vWf expression may be involved in development of peptic ulcer disease secondary to Helicobacter pylori infection. Med Hypotheses 2009; 73(3): 338-9.
16. *Jenkins PV, O'Donnell JS*. ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? Transfusion 2006; 46(10): 1836−44.
17. *Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W*. Factor VIII and factor IX in a twin population: evidence for a major effect of ABO locus on factor VIII level. Am J Hum Genet 1985; 37(1): 89–101.
18. *Matsui T, Titani K, Mizuochi T*. Structures of the asparagine-linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B, and H(O) structures. J Biol Chem 1992; 267(13): 8723–31.
19. *Sadler JE*. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 1998; 67: 395−424.
20. *Wong GL, Wong VW, Chan Y, Ching JY, Au K, Hui AJ*, et al. High incidence of mortality and recurrent bleeding in patients with Helicobacter pylori-negative idiopathic bleeding ulcers. Gastroenterology 2009; 137(2): 525−31.
21. *Hung LC, Ching JY, Sung JJ, To KF, Hui AJ, Wong VW*, et al. Long-term outcome of Helicobacter pylori-negative idiopathic bleeding ulcers:a prospective cohort study. Gastroenterology 2005; 128(7): 1845−50.
22. *Tseng-Shing C, Fen-Yau Li, Full-Young C, Shou-Dong L*. Immunoglobulin G Antibody against Helicobacter pylori: Clinical Implications of Levels Found in Serum. Clin Diagn Lab Immunol 2002; 9(5): 1044−8.
23. *Greenfield S, Apolone G, McNeil BJ, Cleary PD*. The importance of co-existent disease in the occurrence of postoperative complications and one-year recovery in patients undergoing total hip replacement. Comorbidity and outcomes after hip replacement. Med Care 1993; 31(2):141–54.
24. *Holmes TH, Rahe RH*. The Social Readjustment Rating Scale. J Psychosom Res 1967; 11(2): 213−8.
25. *Sobala GM, O'Connor HJ, Dewar EP, King RF, Axon AT, Dixon MF*. Bile reflux and intestinal metaplasia in gastric mucosa. J Clin Pathol 1993; 46(3): 235−40.

26. *Forrest JA, Finlayson ND, Shearman DJ*. Endoscopy in gastrointestinal bleeding. Lancet. 1974; 2(7877): 394–7.
27. *Dixon MF, Genta RM, Yardley JH, Correa P*. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996; 20(10): 1161−81.
28. *Chow DK, Sung JJ*. Non-NSAID non-H. pylori ulcer disease. Best Pract Res Clin Gastroenterol 2009; 23(1): 3−9.
29. *van Leerdam ME, Tytgat GN*. Review article: Helicobacter pylori infection in peptic ulcer haemorrhage. Aliment Pharmacol Ther 2002; 16(Suppl 1): 66−78.
30. *Sánchez-Delgado J, Gené E, Suárez D, García-Iglesias P, Brullet E, Gallach M*, et al. Has H.pylori prevalence in bleeding peptic ulcer been underestimated? A meta-regression. Am J Gastroenterol 2011; 106(3): 398−405.
31. *Chen TS, Chang FY*. Clinical characteristics of Helicobacter pylori-negative duodenal ulcer disease. Hepatogastroenterology 2008; 55(86−87): 1615−8.
32. *Jones MP*. The role of psychosocial factors in peptic ulcer disease: beyond Helicobacter pylori and NSAIDs. J Psychosom Res 2006; 60(4): 407−12.
33. *Aoyama N, Shinoda Y, Matsushima Y, Shirasaka D, Kinoshita Y, Kasuga M*, et al. Helicobacter pylori-negative peptic ulcer in Japan: which contributes most to peptic ulcer development, Helicobacter pylori, NSAIDs or stress? J Gastroenterol 2000; 35(Suppl 12): 33−7.
34. *Wong GL, Wong VW, Chan Y, Ching JY, Au K, Hui AJ*, et al. High incidence of mortality and recurrent bleeding in patients with Helicobacter pylori-negative idiopathic bleeding ulcers. Gastroenterology 2009; 137(2): 525−31.
35. *Xia HH, Wong BC, Wong KW, Wong SY, Wong WM, Lai KC*, et al. Clinical and endoscopic characteristics of non-Helicobacter pylori, non-NSAID duodenal ulcers:a long-term prospective study. Aliment Pharmacol Ther 2001; 15(12): 1875−82.
36. *Cadiot G*. What role today for Helicobacter pylori in peptic ulcer? Gastroenterol Clin Biol 2003; 27(3): 409−14.
37. *Pawlik T, Konturek PC, Konturek JW, Konturek SJ, Brzozowski T, Cześnikiewicz M*, et al. Impact of Helicobacter pylori and nonsteroidal anti-inflammatory drugs on gastric ulcerogenesis in experimental animals and in humans. Eur J Pharmacol 2002; 449(1−2): 1−15.
38. *Moriya M, Uehara A, Okumura T, Miyamoto M, Kohgo Y*. Stress-induced haemorrhagic gastric ulcer after successful Helicobacter pylori eradication: two case reports. J Med Case Rep 2011; 5: 252.
39. *Na YJ, Shim KN, Kang MJ, Jung JM, Kim SE, Jung SA*,et al. Comparison of clinical characteristics and outcomes between geriatric and non-geriatric patients in peptic ulcer bleeding. Korean J Gastroenterol 2009; 53(5): 297−304.
40. *Bayan K, Tüzün Y, Yilmaz S, Dursun M, Canoruc F*. Clarifying the relationship between ABO/Rhesus blood group antigens and upper gastrointestinal bleeding. Dig Dis Sci 2009; 54(5): 1029−34.
41. *Keller R, Dinkel KC, Christl SU, Fischbach W*. Interrelation between ABH blood group O, Lewis(B) blood group antigen, Helicobacter pylori infection, and occurrence of peptic ulcer. Z Gastroenterol 2002; 40(5): 273−6.
42. *Kuyvenhoven JP, Veenendaal RA, Vandenbroucke JP*. Peptic ulcer bleeding: interaction between non-steroidal anti-inflammatory drugs, Helicobacter pylori infection and the ABO blood group system. Scand J Gastroenterol 1999; 34(11): 1082−6.
43. *McCarthy DM*. Ulcers, Helicobacter pylori infection, platelets and gastrointestinal complications of non-steroidal anti-inflammatory drugs: what are the connections? Eur J Surg Suppl 2002; (587): 89−99.
44. *Kang JM, Kim N, Lee BH, Park HK, Jo HJ, Shin CM*, et al. Risk factors for peptic ulcer bleeding in terms of Helicobacter pylori, NSAIDs, and antiplatelet agents. Scand J Gastroenterol 2011; 46(11): 1295−301.
45. *Capurso G, Annibale B, Osborn J, D'Ambra G, Martino G, Lahner E*, et al. Occurrence and relapse of bleeding from duodenal ulcer:respective roles of acid secretion and Helicobacter pylori infection. Aliment Pharmacol Ther 2001; 15(6): 821−9.
46. *Kuran S, Parlak E, Aydog G, Kacar S, Sasmaz N, Ozden A*, et. al. Bile reflux index after therapeutic biliary procedures. BMC Gastroenterol 2008; 8: 1−4.
47. *Kemppainen H, Räihä I, Kujari H, Sourander L*. Characteristics of Helicobacter pylori-negative and-positive peptic ulcer disease. Age Ageing 1998; 27(4): 427−31.

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Exercise-induced bronchoconstriction and non-specific airway hyperreactivity in patients suffering from bronchial asthma

Bronhokonstrikcija izazvana naporom i nespecifična hiperreaktivnost disajnih puteva kod obolelih od bronhijalne astme

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Abstract

Background/Aim. Physical activity is a common stimulus of asthmatic symptoms manifestation. Airway hyperreactivity is a predisposing cause of exercise induced bronchial obstruction, diagnosed by histamine inhalation. The aim of this study was to determine the relation between the amounts of histamine needed to induce non-specific airway hyperreactivity and exercise-induced bronchial obstruction. **Methods.** This randomized cross-over study included 160 male patients (age 19–27 years) suffering from bronchial asthma who showed positive results as the reaction after the histamine bronchial provocation test. Histamine concentrations were in a range of 0.03 to 4 mg/mL. Each patient participated in the exercise stress test conducted on a conveyor belt. The results of the exercise stress test were considered positive if the FEV1 level dropped by at least 15% from its initial value, 5–10 minutes after the test. **Results.** All the patients showed positive results as the reaction after the histamine bronchial provocation test, while 50 of them showed positive results after the exercise-induced stress test. There was a statistically highly significant difference in administrated histamine concentrations between the group of patients that had positive results on exercise stress test and those who did not (1mg/mL *vs* 0.5mg/mL; U = 1678; $p < 0.01$). Also, there was a statistically significant difference concerning the frequency of the positive results regarding histamine concentration after induced stress test ($\chi 2 = 10.885$; $p = 0.001$). Among the patients with positive results, there was a statistically highly significant number of patients with bronchial obstruction induced by less than 2 mg/mL of histamine ($p < 0.01$). A statisticlly significant relation between the amount of histamine needed to induce bronchial obstruction and the results of the exercise stress test ($p < 0.01$) was also observed after the testing. **Conclusion.** In the group of patients with positive results after the exercise-induced stress test, there were significantly more patients with positive results to non-specific bronchial provocation test with lower histamine concentrations. Histamine concentrations needed to induce non-specific hyperreactivity of asthmatic airway were shown to be related to the reactivity to physical effort.

Key words:
asthma; histamine; diagnosis; exercise test; respiratory function tests.

Apstrakt

Uvod/Cilj. Fizički napor je čest stimulus-uzročnik ispoljavanja astmatičnih simptoma. Hiperreaktivnost disajnih puteva je predisponirajući faktor za nastanak bronhoopstrukcije na napor i ona se dokazuje inhalacijom histamina ili sličnih materija. Cilj ove studije bio je da se ispita odnos između doze histamina potrebne za izazivanje nespecifične hiperreaktivnosti disajnih puteva i bronhoopstrukcije izazvane naporom. **Metode.** Ova studija preseka obuhvatila je 160 astmatičara, muškaraca, starih 19–27 godina, koji su imali pozitivan bronhoprovokacijski test na histamin. Koncentracija histamina kretala se od 0,03 do 4 mg/mL. Svim bolesnicima urađen je test opterećenja na pokretnoj traci. Test opterećenja smatran je pozitivnim ukoliko bi 5–10 minuta po završetku testa došlo do pada FEV1 za najmanje 15% od početnih vrednosti. **Rezultati.** Svi ispitanici imali su pozitivan histaminski test, a njih 50 i pozitivan test opterećenja. Postoji statistički visokoznačajna razlika u datim koncentracijama histamina između bolesnika koji su imali pozitivan test opterećenja i drugih kod kojih je test opterećenja bio negativan (1 mg/mL *vs* 0,5 mg/mL; U = 1678; $p < 0,01$). U grupi bolesnika sa pozitivnim testom opterećenja, postoji statistički visoko značajno više onih

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kod kojih je bronhoopstrukcija izazvana histaminom u dozi < 2 mg/mL ($p < 0,01$). Postoji statistički visokoznačajna povezanost doze histamina potrebne da se izazove bronhokonstrikcija i nalaza testa opterećenja ($p < 0,01$). **Zaključak.** U našoj grupi bolesnika koji su imali pozitivan test opterećenja bilo je značajno više bolesnika sa pozitivnim nespecifičnim bronhoprovokacijskim testom pri nižim koncentracijama histamina. Ovo ukazuje da postoji povezanost doze histamina potrebne da se izazove bronhokonstrikcija sa reaktivnošću na fizički napor.

Ključne reči:
astma; histamin; dijagnoza; vežbanje, testovi; respiratorna funkcija, testovi.

Introduction

The main pathophysiological characteristic of bronchial asthma is a reversible bronchial obstruction. Bronchial obstruction is caused by inflammation, which is, to some extent, constantly present in asthmatic airway mucosa. Chronic inflammation causes airway hyperreactivity, which leads to bronchial obstruction, *ie* asthmatic symptom manifestation [1].

Each of these processes (inflammation, hyperreactivity and bronchial obstruction) is variable and can be amplified by certain factors [1, 2]. Thus, the stimulus (inhaled or, in some cases, produced by the body itself), will trigger the manifestation of bronchial obstruction. There are many substances and states which can induce an asthma attack. The most common stimuli are inhaled allergens, airway infections and increased physical activity, which can induce an asthma attack by direct or indirect stimulation of bronchoconstriction receptors that narrow down the airways [2].

Exercise is a common cause of manifestation and aggravation of bronchial asthma because it triggers an asthma attack *via* indirect stimulation. In most cases, asthmatic symptoms occur 5–10 minutes after exposure to physical effort and resolve after resting for 30 minutes, or by using short-acting bronchodilators. Apart from this early reaction to physical effort, in rare cases, there can also be a late reaction to physical activity, even 6–8 hours after the exercise [3]. Considering its specific nature and the pathophysiological mechanism of formation, exercise-induced bronchospasm is sometimes considered as a separate entity, as "exercise-induced bronchoconstriction" (EIB) [4].

It is estimated that the prevalence of EIB in general population varies from 7% to 20%. [3, 5], while in asthmatic population, more than 90% of patients show the symptoms of EIB. In certain cases, especially in children and young people, exercise can be the only cause of asthma attacks and coughing during physical effort, as the only symptom of bronchial asthma [5, 6]. The manifestation of EIB is tightly related to the level of bronchial reactivity. This is also confirmed by the fact that the majority of patients with moderate asthma attacks and minimal reactivity increase, subjected to uniform physical effort, do not experience any clinically significant bronchoconstriction [3, 7, 8].

During exercise, the respiratory tract gets partially inflamed (this is confirmed by bronchoalveolar lavage cell structure, as well as through the reduction in proinflammatory effect on people who use inhalatory corticosteroids). A potential proinflammatory effect of exercise on respiratory system increases chronic inflammation that induces the process of bronchial obstruction in asthmatics [8–10].

Directly induced airway hyperreactivity is caused by vasoactive amines that affect the H_1 receptors in the airway smooth muscles. In laboratory conditions, this hyperreactivity is induced by inhaling bronhoconstrictory substances, such as histamine or methacholine [11]. Because of asthmatic airway chronic inflammation, hyperreactivity induced in this way is more intense and more easily trigged (by using lower concentration of bronhoconstrictory substances).

Considering that inhaling higher concentrations of histamine will induce bronchoconstriction even in healthy people, histamine is considered as a non-specific stimulus, and the reactivity which it induces as a non-specific reactivity. High sensitivity and simplicity of non-specific reactivity tests to histamine and methacholine makes them challenge tests in asthma diagnostication, *ie* airway hyperreactivity [11, 12].

Unlike the non-specific hyperreactivity tests, airway exercise reactivity tests, conducted in laboratory conditions, have higher specificity, but significantly smaller sensitivity [13, 14].

The aim of this study was to determine the relation between non-specific hyperreactivity to histamine and EIB in asthmatics.

Methods

This cross-sectional study included 160 male examinees, age 19–27 years, with positive results after the induced histamine bronchial provocation test. The study was performed in the Lung Functional Diagnostics Department, Military Medical Academy in Belgrade. All the examinees had the diagnosis of bronchial asthma. However, they did not take any long-acting bronchodilators and anti-inflammatory medicines during the last 48 hours before the tests. Short-acting bronchodilators had not been taken during the last 8 hours before the tests. Spirometry was initially undertaken on each patient and all of them showed normal spirometry results. After the spirometry test, non-specific bronchoprovocation test to histamine was conducted on each examinee, in which they were required to inhale growing dosages of histamine through inhalers in a 1- minute period. Histamine concentrations were from 0.03 to up to 4 mg/mL. The test was considered positive if the FEV1 level was reduced by at least 20% from its initial value after histamine inhaling.

The next day, after the control spirometry had been conducted with positive results, each examinee participated in the exercise stress test. The tests were terminated when heart rate was increased by 80% percent from the age-predicted maximum heart rate (formula: 220 – age). Exercise stress test was considered positive if in the first 5 minutes

after the test the FEV1 level was reduced by at least 15% percent from its initial value [3].

Spirometry test and exercise stress test were conducted on pneumoscrin and a Jaeger conveyor belt.

Statistic data processing was performed with probability distributions tests (such as Kolmogorov–Smirnov test), non-parametric significance tests, such as Mann-Whitney and chi-squared test (goodness-of-fit test or chi-squared test for independence), as well as the binary dependent variable test (logistic regression).

Results

Positive results at the exercise stress test were obtained in 50 (31.3%) of the patients. In 105 (65.6%) patients bronchial obstruction was induced with less than 2 mg/mL of histamine inhaled. The median histamine concentration needed to induce bronchial obstruction in the group of patients with positive result on the exercise stress test was 1 mg/mL, and in the group with negative results it was 0.5 mg/mL (U = 1678; $p < 0.01$) (Figure 1).

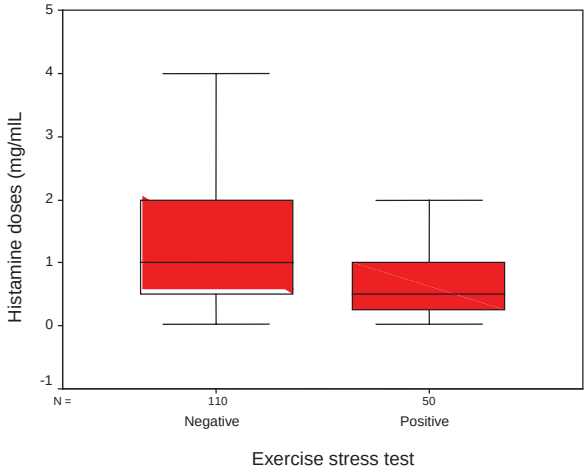

Fig. 1 – Histamine dosages needed to induce non-specific bronchial hyperreactivity in relation to the results of the exercise stress test.

There was a statistically significant difference in the frequency of positive exercise stress test findings in relation to histamine concentration ($\chi 2 = 10.885$; $p = 0.001$) (Figure 2). Namely, 42 (84%) out of 50 patients, with positive results on the exercise stress test, also showed a positive non-specific hyperreactivity to less than 2 mg/mL of histamine, while 47 (85%) patients who demonstrated non-specific hyperreactivity induced with histamine dosages of 2–4 mg/mL showed negative results after the exercise stress test. In the group with positive results after the exercise stress test there was a statistically significantly higher frequency of patients with non-specific hyperreactivity induced with less than 2 mg/mL of histamine ($\chi 2 = 23.120$; $p < 0.01$).

Also, a statistically significant relation was identified between the histamine dosages needed to induce bronchoconstriction and the results of exercise stress test ($p < 0.01$).

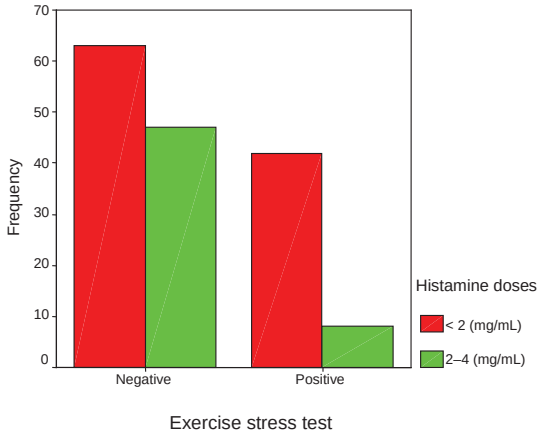

Fig. 2 – Exercise stress test results in relation to administrated histamine dosages.

Discussion

Triggering asthma attack was shown to be a complex process which included an interaction between multiple factors and the processes in the respiratory tract.

Speaking of exercise as a factor which could trigger asthma, the key factor was the ventilation volume increase, which was necessary to satisfy the increased need for oxygen.

The main bronchoconstrictive stimuli of EIB were: increased ventilation and inhalation of increased volume of cold air, change in airway surface liquid osmolality (the layer coating the airway mucosal surface), as well as increased disposition of allergens into respiratory tract. The above mentioned changes could have been categorized into two groups, based on two theories of EIB origin: heating and hyperosmolarity theory.

The theory is based on the assumption that temperature variations in the respiratory tract (sudden cooling at the beginning of exercise and subsequent warming during exercise) are the main reasons for the bronchoconstricting substances release and the airway smooth muscle irritation.

According to the second, hyperosmolarity theory, hyperventilation that takes place during exercise, dries the airway tract, thus increasing the mineral concentration of the liquid surface layer which cools the airway mucus tissue. The increase in osmolarity dries out the water from mast cells and eosinophils, which releases vasoactive amines (histamine, prostaglandins and leukotrienes) that directly irritate nerve endings and increase blood vessels permeability leading to edema and bronchospasm [3].

The mechanisms used to explain the above mentioned theories were not sufficient enough to cause bronchospasm all by themselves, because increased physical effort did not induce such dramatic changes in a healthy person's respiratory tract. Asthmatics, however, were more prone to bronchoconstriction due to the chronic respiratory tract inflammation, because, in many cases, physical effort could not induce it by itself. Healthy persons who took deep breaths during the exercise even experienced lower level of airway reactivity to methacholine. In other words, the exercise combined with deep breathing demonstrated some sort of protective role

against bronchoconstriction. This type of bronchodilatative condition after exercise probably occurred due to the reduction in parasympathetic-induced bronchoconstriction [7].

Apart from the above mentioned factors, other relevant factors which can cause EIB were present: the increase in inflammatory and bronchoconstrictive mediators, especially LTC 4 and LTD 4 histamine and interleukine-IL-8; activation of TH2 lymphocytes with the increase in T cell expression CD25 and B cell expression CD23; eosinophilic influx and airway activation [3].

The goal of pharmacodynamic or bronchoprovocation tests, conducted in laboratory conditions, was to induce a process similar to real asthma attack in the lungs. This included bronchoprovocation tests with histamine, as well as exercise. They had an important role in the bronchial asthma diagnostics. Considering their somewhat different pathophysiologic mechanisms of action, they enabled more complete and precise insight into bronchial obstruction origin in asthmatics [11, 12].

By inhaling certain dosages and monitoring the respiratory tract response, it was possible not only to determine the reactivity but also to quantify the response value. Non-specificity of this kind of bronchial asthma test was reflected by its positive response in other inflammatory and allergic respiratory tract diseases, such as allergic rhinitis, chronic bronchitis and bronchiectasis [11].

Our goal was to determine whether there was or there was not a non-specific reactivity to histamine in asthmatics. We concluded that each examinee showed positive result (because they had experienced the expected FEV1 level reduction from its initial value with histamine concentration of up to 4 mg/mL). Due to different extent of respiratory tract inflammation, as well as the number of other factors, the examinees reacted to different dosages of histamine, which confirmed earlier data about the variability of inflammation and lack of stable correlation with the symptoms of bronchial asthma [7].

By participating in the exercise stress test, the group of examinees with positive results on histamine test helped us to ascertain that the test results were more frequently positive in the group of examinees that reacted to lower levels of histamine. In other words, 84% of examinees with positive exercise stress test results also showed positive results on the bronchoprovocation test with histamine dosages of up to 2 mg/mL. These results clearly speak in favor similarity in the origin of mechanisms of these two types of reactivity, *ie* the same preconditions needed for their formation.

On the other hand, a medium histamine concentration in the examinees that were positive only to histamine test was lower than the concentration in the examinees that had shown positive results on both tests, which, on the other hand, indicated a difference between them. The above mentioned characteristics of the reactivity that manifested them through our research were also noted by Dor et al. [15].

Our study proved that bronchial response in asthmatics is related to the intensity of non-specific hyperreactivity to histamine.

Many other authors studying these questions came to similar results, thus confirm the previous statement about pathophysiologic processes in asthma [8–10].

Conclusion

In the group of examinees with positive results on the exercise stress test, there were more those who reacted to a lower histamine concentration, while the frequency of those with positive results on the exercise stress test was lower in the patients with positive bronchoprovocation test with a higher histamine concentration.

Thus, histamine concentrations needed to induce non-specific hyperreactivity of asthmatic respiratory tract, as a factor of their tendency to asthma attacks in the presence of a trigger, were related to the reactivity to physical effort.

REFERENCES

1. GINA Report. Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma [updated 2012 Decembar]. Available from: www.ginasthma.org/documents/4
2. *Souza AC, Pereira CA*. Bronchial provocation tests using methacholine, cycle ergo meter exercise and free running in children with intermittent asthma. J Pediatr (Rio J) 2005; 81(1): 65−72. (Portuguese)
3. *Hildebrand K*. Exercise-induced bronchoconstriction. Pneumonol Alergol Pol 2011; 79(1): 39−47. (Polish)
4. *Randolph C*. Exercise-induced bronchospasm in children. Clin Rev Allergy Immunol 2008; 34(2): 205−16.
5. *Anderson SD*. Indirect challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest 2010; 138(2 Suppl): 25S−30S.
6. *Maurer M, Simonett D, Brutsche MH*. Challenge of exercise-induced asthma and exercise-induced bronchoconstriction. Expert Rev Respir Med 2009; 3(1): 13−9.
7. *Scichilone N, Morici G, Zangla D, Chimenti L, Davì E, Reitano S*, et al. Effects of exercise training on airway responsiveness and airway cells in healthy subjects J Appl Physiol (1985) 2010; 109(2): 288−94.
8. *Brannan JD, Turton JA*. The inflammatory basis of exercise-induced bronchoconstriction. Phys Sportsmed 2010; 38(4): 67−73.
9. *Moreira A, Delgado L, Haahtela T, Fonseca J, Moreira P, Lopes C,* et al. Physical training does not increase allergic inflammation in asthmatic children. Eur Respir J 2008; 32(6): 1570−5.
10. *Denguezli M, Ben Chiekh I, Ben Saad H, Zaouali-Ajina M, Tabka Z, Abdelkrim Z*. One-year endurance training: effects on lung function and airway inflammation. J Sports Sci 2008; 26(12): 1351−9.
11. *Cockcroft D, Davis B*. Direct and indirect challenges in the clinical assessment of asthma. Ann Allergy Asthma Immunol 2009; 103(5): 363−9; quiz 369−72, 400.
12. *Anderson SD, Brannan JD*. Bronchial provocation testing: the future. Curr Opin Allergy Clin Immunol 2011;11(1): 46−52.
13. *Anderson SD*. Provocative challenges to help diagnose and monitor asthma: exercise, methacholine, adenosine, and mannitol. Curr Opin Pulm Med 2008;14(1): 39−45.
14. *Cockcroft DW, Davis BE*. Diagnostic and therapeutic value of airway challenges in asthma. Curr Allergy Asthma Rep 2009; 9(3): 247−53.
15. *Dor A, Liebhart J, Małolepszy J*. Comparison of exercise and histamine provocation tests in patients with bronchial asthma. Pneumonol Alergol Pol 1999: 67(1−2): 22−7.

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GENERAL REVIEW

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Hippocampus – Why is it studied so frequently?

Hipokampus – zašto se toliko proučava?

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Key words:
hippocampus; anatomy; neurophysiology; memory.

Ključne reči:
hipokampus; anatomija; neurofiziologija; pamćenje.

Introduction

From the very beginnings of brain research, the hippocampus has been the focus of attention for anatomists. Nowadays, its complexity and clinical importance have attracted the interest of a vast number of researchers of different profiles.

Work on hippocampal tissue facilitated some important neurophysiological discoveries: identification of excitatory and inhibitory synapses, transmitters and receptors, discovery of long-term potentiation and long-term depression, role of oscillations in neuronal networks, underlying mechanisms of epileptogenesis and of memory disorders [1].

The Neuron Theory and the hippocampus

When Theodor Schwann and Matthias Schleiden (1839) proclaimed that a cell is a basic functional unit of all living things, they did not believe this applicable to the nervous system [2]. That was going to change after Camillo Golgi in 1873 introduced the black visualisation of neurons by silver impregnation. Suddenly, nerve cell and the full extent of its "protoplasmic processes", later named "dendrites" by Wilhelm His (1889) and "axons" by Albert von Kölliker (1896), became highly visible in sharp contrast[3]. Wilhelm von Waldeyer-Hartz, who was aware of the findings of His and August Forel on individuality of the nerve cell function, after being introduced to Golgi and Santiago Ramon y Cajal images of the hippocampal nerve cells, understood the nature of the organization of the nervous system, coined the name "neuron" and promoted the Neuron Theory in 1891 [4–6].

On the hippocampus nomenclature

According to the *Terminologia Anatomica* (1998), hippocampus is the name for practically the entire protrusion on the medial wall of the temporal horn of the lateral ventricle. The name hippocampus to this structure was given by the Bolognese anatomist Giulio Cesare Aranzio-Arantius in 1564 which crossectional appearance resembled a seahorse [1] to him. Winslow in 1732, used the term *cornu arietis* (ram`s horn) for the appearance of the hippocampal section, which De Garengeot in 1742 turned to *cornu Ammonis* after the Egyptian god Ammon who was depicted with a human body and the head of a ram with the horn[7]. The hippocampus proper is the more commonly used name for Ammon's horn. The name *cornu Ammonis* survived as the acronim *CA* for the subdivisions of the hippocampus proprius as it was proposed by Lorente de No [8] in 1934 and is still in use.

On the hippocampal anatomy

Anatomists are equivocal about the structures involved within hippocampus, yet, it is generally accepted that the term hippocampus by itself is not quite adequate. Various criteria have been used to define the hippocampus, such as: the anatomical findings, the cell types and connectivity, the number of cortical layers, but most frequently the combination of listed, related to authors.

The contemporary terms in use are the hippocampal region [9–11], the hippocampal system [12], the hippocampus with joined structures [13] or the hippocampal formation [14, 15] and all of them include further subdivisions. Macroanatomically, hippocampus appears as bilaminar, with the lamina of the gyrus dentatus rolled up inside the lamina of the hippocampus proprius.

Laminar organization and cytoarchitectonic characteristics

We consider as useful the division of the hippocampus into the three layered and the six layered areas. The hippo-

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campus proper with its four subfields: CA1, CA2, CA3 and CA4 [8], dentate gyrus and the subiculum are the simplest part of the cortex [16] sharing the characteristic three-layered appearance of the so-called allocortex [13]. The six layered appearance that characterizes the neocortex[17] consists of presubiculum, parasubiculum and entorhinal cortex [14] (Figure 1), comprising perirhinal and postrhinal cortices.

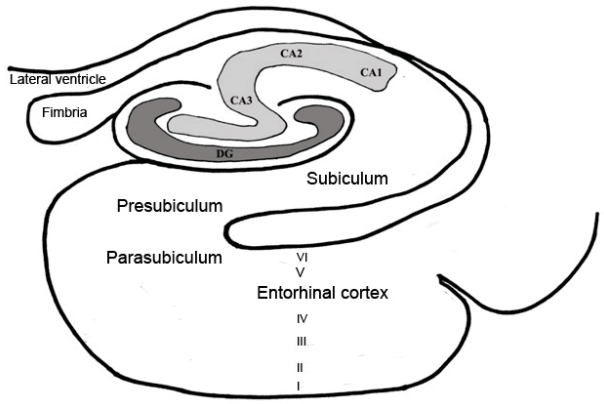

Fig. 1 – Structures of the human hippocampal formation.
DG – dentate gyrus;
CA – cornu ammonis.

There are two major groups of neurons within the hippocampal formation: the principal cells (Figure 2), which are the main source for extrahippocampal connections, and a range of interneurons, which are mainly local-circuit neurons [18].

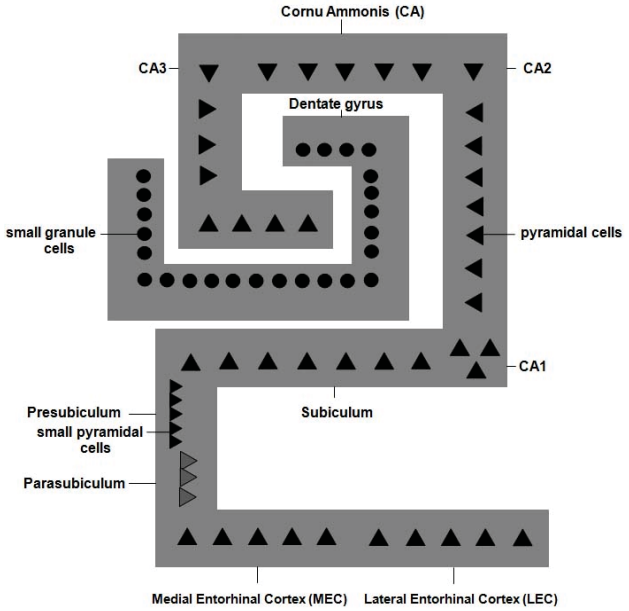

Fig. 2 – Principal cells within the hippocampal formation.

The three layered division

Dentate gyrus

Dentate gyrus has three layers: the molecular, the granular and the polymorphic cell layer. The first one of the three, the molecular layer is continuous with that of the hippocampus proprius. It lies closest to the hippocampal fissure, and was considered cell free for exception few interneurons [13]. Recently, six interneuron types and two GABA negative types were differentiated within dentate molecular layer in the rabbit hippocampus [19]. The principal, granular layer, contains small granule cells with axons which form the mossy fiber pathway in the overlaying molecular layer. The granule cell is the only cell type that gives axons to innervate the CA3 region of the hippocampus proprius. The contact of the dentate mossy fibers with the spines of pyramidal cells occurs in the stratum lucidum of CA3 region [20, 21]. The axons of the dentate gyrus granule cells (mossy fibers) are excitatory glutamatergic but also contain GABA and the opiate peptide dynorphin [22, 23]. Beside the granule cells, in the granular layer are also excitatory mossy cells and inhibitory interneurons. Mossy cells have extensive ramification reminding to moss, and their axons innervate contralateral dentate gyrus. There are also various interneurons which connections remain within the gyrus dentatus [24]. The third dentate gyrus layer is the polymorphic cell layer [14] (also called the hilus [25]).

Cornu ammonis (hippocampus proprius)

The hippocampus proprius has three layers: stratum oriens, stratum pyramidale, and the molecular zone [13]. The well-defined pyramidal cell layer formed by excitatory pyramidal neurons is the principal layer of the hippocampus proper. It consists of large pyramidal cells tightly packed in CA1, and less tightly packed in CA2 and CA3 fields. The rest of the tissue consists of axons and dendrites and various types of interneurons which inhibit the pyramidal cells. CA1 field continues from the subiculum. CA2 is a narrow zone, between CA1 and CA3 field and CA3 continues into the hilus of the dentate gyrus. The hilar part in rats, and not in humans, is defined by Lorente de No [8] as CA4. In primates, the hilus is dominated by pyramidal cells and the assignment of these neurons to a specific hippocampal subfield remains controversial. All pyramidal neurons within the human dentate hilus can be designated as the CA3 hilar neurons [26].

Further, hippocampal three layers could be subdivided into six sublayers [13]. Starting under the ependyma of the ventricular surface these sublayers are: the alveus, the stratum oriens, the stratum pyramidale, the stratum lucidum, the stratum radiatum and the stratum lacunoso-moleculare. The alveus contains powerful efferent axons of the hippocampal and subicular pyramidal neurons which are passing toward fimbria and fornix and also afferent fibers mainly from the septum [13]. The stratum oriens, a layer between the alveus and the pyramidal cell bodies, contains basal dendrites of pyramidal cells, inhibitory basket interneurons and commissural fibers from the contralateral hippocampus, as well as afferents from the septum. These contralateral hippocampal connections are better developed in rodents than in primates. The stratum lucidum is exclusively interposed between the pyramidal cell bodies and the stratum radiatum, and can be seen in the CA3 field as a narrow cell-free zone occupied by the mossy fibers from the dentate granule cells which have contact with the proximal dendrites of pyramidal cells in the

field CA3. The stratum lucidum is not as prominent in humans as it is in primates. The stratum radiatum contains apical dendrites of pyramidal cells which interconnect with Schaffer colaterals (fibers from CA3 to CA1), fibers from septal nuclei, and commissural fibers. The stratum lacunosum is a thin layer containing Schaffer colaterals and perforant fibers from upper layers of entorhinal cortex. The stratum lacunoso-moleculare contains perforant fibers originating from entorhinal cortex which form synapses on the distal apical dendrites of pyramidal cells [11, 17, 27].

Subiculum

The border between CA1 and subiculum is characterised by the abrupt discontinuation of pyramidal cell layers of the CA1 which is replaced by a wide molecular layer of subiculum. The principal cell layer of the subiculum contains large pyramidal neurons among which smaller interneurons are present. At the border with presubiculum a significant decrease in the size of pyramidal cells is visible [10].

The six layered division

Presubiculum

The presubiculum should be considered an anatomical transition zone between the three-layered and six-layered areas because its six layers are not so well defined [28]. The most characteristic cells of the presubiculum are densely packed small, darkly Nissl-stained pyramidal cells located in the external cell layer [14].

Parasubiculum

The cells of the parasubiculum are densely packed, lightly stained pyramidal neurons which, by the use of distinctive staining, Timm sulfide silver method [29], differentiate the parasubiculum from both, presubiculum and entorhinal cortex.

Entorhinal cortex

The cells of the entorhinal cortex serve as the main interface between the hippocampus and other parts of the brain. There are four cellular (II, III, V and VI) and two acellular or plexiform (I and IV) layers. Layer II contains stellate cells grouped in clusters which caudally tend to merge. In layer III pyramidal cells are predominant. Pyramidal cells in layer V vary from grouped large, darkly stained neurons to rather loose arranged smaller pyramidal cells altogether with polymorphic cells. Cells in layer VI are heterogenous in size and shape. Columnar organization at caudal levels is present in layers V and VI [14]. The entorhinal cortex is the most heavily damaged in Alzheimer's disease and is the site of early onset of the disease [30].

The neuronal connections of the hippocampus

The quality of some nerve fiber staining can be augmented by fiber degeneration. However, several hippocampal fiber systems contain thinner axons as compared to other parts of the central nervous system [31]. This, probably, was the reason why the first attempts of Marchi and Algeri in 1886 only stained the thicker degenerating fibers [1]. Improvement was achieved by applying silver impregnation methods to degenerating hippocampal fibers [32] altogether with the electron microscopy [33] and the fiber tracing methods based on intra-axonal transport of injected radioactive amino acids [34].

Intrinsic hippocampal connections

Gathered data on intrinsic hippocampal neuronal connections showed that each part of hippocampal formation gives fibers to neighboring regions but does not always get reciprocal connections [35]. That is not the case with neocortical areas which are generally reciprocally interconnected [36]. Unidirectional intrahippocampal connections could be shown by the so-called "trisynaptic circuit" (Figure 3) [37].

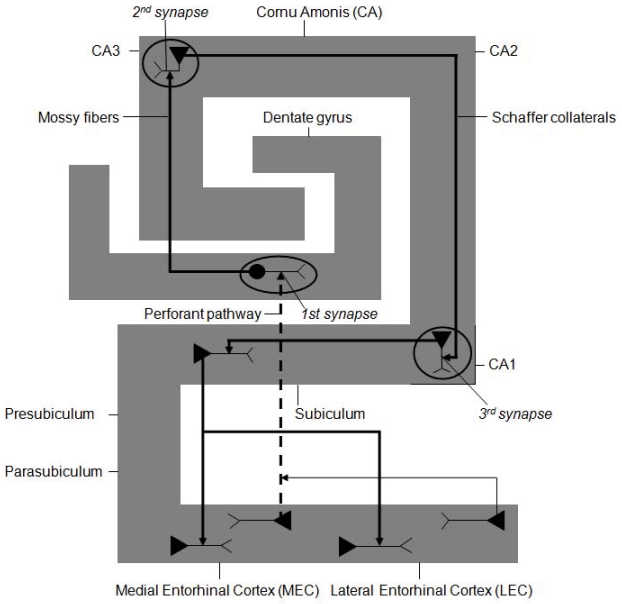

Fig. 3 – Mostly unidirectional intrahippocampal "trisynaptic circuit".

The excitatory glutamatergic [37] "trisynaptic circuit" arises in layer II of the entorhinal cortex [38], its axons perforate the subiculum forming the "perforant path". The majority of the perforant path fibers reach the molecular layer of the gyrus dentatus where they have synapse (the first synapse of the trisynaptic circuit) with the dendrites of the granular cells [39]. The gyrus dentatus does not project back to the entorhinal cortex. Similarly, the axons of the dentate gyrus granule cells called "mossy fibers" have synapse (the second synapse of the "trisynaptic circuit") with the apical dendrites of CA3 pyramidal cells and do not project back to the granule cells. The CA3 pyramidal cells axons emit the so-called "Schaffer collaterals" [1] to have synapse (the third synapse of the trisynaptic circuit) with the apical dendrites of CA1 pyramidal cells which also do not project back to the CA3 pyramidal cells. The same unidirectionality principle for intrahippocampal connections was confirmed for the CA1-subiculum and CA1-entorhinal cortex connections. The subiculum, further, sends axons to the presubiculum, parasubiculum and into the deep layers of the entorhinal cortex.

The CA1 and the subiculum with the projections to the entorhinal cortex are closing the circuit from the entorhinal cortex *via* the dentate gyrus, the hippocampus proprius, and back to the entorhinal cortex [1, 13, 14]**.** Thus, it could be that to some extent this entorhinal cortex-entorhinal cortex loop compensates the unidirectional type of neuronal connections between the hippocampal structures. Duvernoy [13] introduced the name "polysynaptic pathway", based on the findings that the subiculum also takes part in the intrinsic hippocampal circuitry. The polysynaptic pathway thus, is the chain of at least four synapses connecting the entorhinal area (presubiculum, parasubiculum and entorhinal cortex), the gyrus dentatus, the cornu ammonis fields, and the subiculum. It is accepted that there is also the direct intrahippocampal pathway originating from layer III of the entorhinal cortex and projecting directly to the CA1 pyramidal neurons by a different pathway from that of the perforant path [40].

Extrinsic hippocampal connections

The hippocampal formation is connected with numerous subcortical and cortical structures (Figure 4) [15].

from the amygdala. The afferents from the septal nuclei mainly release acetylcholine having a modulatory excitatory effect on the hippocampal pyramidal cells. Adrenergic and serotoninergic afferents from the locus ceruleus and the raphe nuclei have modulatory effects on the hippocampal long term potentiation which is related to the attention and motivation that both influence learning and memory functions [16].

Outputs (efferents)

The hippocampal formation efferents reach subcortical, as well as cortical areas. There are two main outways from where hippocampal efferent fibers leave the hippocampal region: rostral, toward subcortical and caudal, and toward cortical areas [17].

Rostral fibers, raising mainly from the subiculum [41] and adjacent areas, gather and through the fimbria of fornix reach the mammillary bodies, continuing *via* the mamillothalamic tract into the anterior thalamic nucleus, and further to the cingulate cortex. Some of fibers reach the nucleus accumbens, the ventromedial thalamus and the amygdala indicating that the hippocampus is also connected with neuronal groups related to emotions and motivation [15].

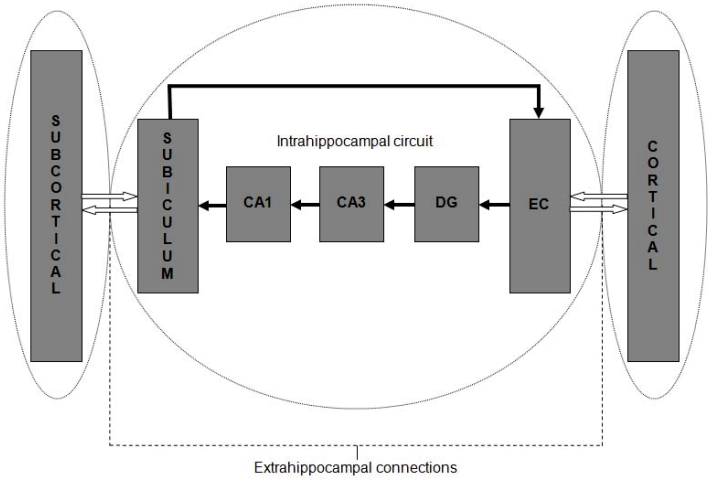

Fig. 4 – Intrahippocampal and extrahippocampal connections.
EC – entorhinal cortex; DG – dentate gyrus; CA – cornu ammonis; ← Intrahippocampal circuit

Inputs (afferents)

The main afferents to the hippocampal formation enter *via* the entorhinal area [15]. The neocortical inputs to the entorhinal cortex of the rat comprise two groups: those that terminate in the superficial layers (I–III) and those that terminate in the deep layers (IV–VI) [14].

The entorhinal area recevies the majority of the afferents from the parahippocampal gyrus and from the perirhinal cortex which receive information from association neocortical centers (the visual, auditory and somatosensory) and from the polysensory association areas which integrate various sensory data. Inputs are coming also from the cingulate gyrus, the insula and the prefrontal cortex. Important inputs to the hippocampal formation are from the septal nuclei, from the brainstem monoaminergic neurons (locus ceruleus and raphe nuclei), from the hypothalamus, from the thalamic nuclei and

Caudally, three parallel efferent pathways are reaching primarily the temporal and frontal association areas. One direct and one indirect bands, originate from the entorhinal cortex and project to the temporal lobe, medial and orbital parts of the prefrontal cortex and to the polysensory areas in the superior temporal gyrus. Indirect projections coming from the entorhinal cortex *via* parahippocampal gyrus and the perirhinal cortex terminate in the same areas. The third pathway reaches these areas directly from the CA1 and the subiculum [15].

The neurotransmitters of the hippocampus

Glutamate has been stressed as the main neurotransmitter in the mammal brain [42, 43]. Excitatory neurotransmitters have been localized in principal neurons of the neocortex [44]. On the other side, GABA plays the major inhibitory role in the brain [45, 46] mainly localized in interneurons [24].

The granule and pyramidal cells of the hippocampus are excitatory glutamatergic, whereas the interneurons are GABAergic [47]. In comparison with glutamate and GABA, other neurotransmitters are present at far fewer synapses in the hippocampal region [46, 48]. In particular, the hippocampus proprius and gyrus dentatus contain neurons producing substance P, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin, corticotropin-releasing factor (CRF), and neuropeptide Y [13], which all are involved in local inhibitory or excitatory circuits [49–52]. Enkephalin and glutamate containing hippocampal afferent fibers arise from the adjacent entorhinal cortex [53]. The gyrus dentatus granular neurons also may produce enkephalins and dynorphins [54].

On the human hippocampus functions

Various theories based on anatomical and clinical correlations have been proposed for the human hippocampus role, such as roles in olfactory function, emotions, attention and memory [1, 55]. Furthermore, many functions of the hippocampus have been recognized and based on the addressed clinical impairment.

The hippocampus and emotional behavior

At present, emotional behavior is mostly attributed to the amygdala whose central nucleus is believed to modulate the autonomic reactions produced by emotions and whose basolateral nucleus projections to the dorsomedial thalamic nucleus and further to the prefrontal cortex are thought to regulate an individual`s behavior. The hippocampal involvement in emotional behavior remained related to pain; the polysynaptic pathway gives fibers to the anterior cingulate cortex where end the spinoreticulothalamic pathways involved in the perception of some aspects of pain [13].

The hippocampus and memory

Bekhterev (1900) was the first who pointed out the importance of the subicular complex in memory processes, when he studied the damage of the temporal lobe [1].

The case of a patient is famous who in 1950 underwent bilateral resection of the hippocampus and surrounding areas in order to control his otherwise intractable grand mal epilepsy. After the surgery, the patient could recall memories from early life but could not acquire long-term memory for new facts or events (anterograde amnesia). Subsequent detailed neuropsychological assessment of that patient revealed severity of memory impairment and furthermore an array of cognitive and mnemonic functions unaffected by bilateral lesion [56].

It is believed that the hippocampus (in particular CA3 region) is the site where new declarative (episodic, explicit or declarative memory that can be described in words [57]) events and facts (short-term memories) are processed and encoded into memory trace and as such transferred to the other parts of the brain where they turn into long-term memories [58, 59]. An isolated damage of the hippocampal formation usually does not abolish the recall of older memories, although it prevents the learning of new material [15]. There is a general acquired agreement that the hippocampus is not involved in the memory process of acquiring motor (procedural, implicit or non-declarative memory) skills and procedures [1, 57].

There are two types of the hippocampal electroencephalographic (EEG) activities registered in animals. While exploring their environment theta 5 Hz–10 Hz frequencies were recorded. In a period of quiet wakefulness sharp-wave activity of large amplitude replaced theta frequencies. It was speculated that during theta EEG pattern the hippocampus is acquiring a new information while during sharp-wave (quiet) and slow-wave sleep activities the hippocampus is transfering this information elsewhere in the brain [25].

Duvernoy [13] considers that the hippocampus is implicated in all aspects of the declarative memory, i.e., the semantic memory, which involves memory of facts and concepts, the episodic memory, which permits conscious recollection of events and the relations between them, and the spatial memory, which involves spatial location recognition.

Based on the huge amount of data, Stark [60] elaborated four conclusions related to the human hippocampus involvement in memory along with the adjacent cortical structures in the parahippocampal gyrus: the hippocampus is critically involved in memory for facts and events, this involvement is time limited, the hippocampus is not involved in immediate or working memory process and is not involved in implicit or non-declarative long-term memory process, the hippocampus is not involved in non-mnemonic aspects of cognition including spatial processing.

Certain differences in opinion related to the role of the hippocampus in spatial location recognition, i.e. spatial processing could be better understood having in mind the case of second patient. Unlike the first patient (above described), the second one has what is likely complete loss of the hippocampal region [59], thus he could retrieve spatial information and navigate in a spatial environment learned long before the onset of his amnesia but apparently has not learned any spatial information about his environment after the onset of his amnesia. His performance dropped from 83% correct in his childhood environment to 0% correct in his current environment on the navigation tasks. With complete hippocampal loss, spatial processing therefore appears normal despite a complete inability to acquire new spatial information [61, 62].

The hippocampal involvement in stress

The findings that the density of mineralocorticoid receptors in the hippocampus is the highest in the brain [63, 64] linked the hippocampus with stress, having in mind that increased corticosterone is released from the adrenals during stressful situations [65, 66]. Chronically elevated levels of cortisol induce atrophy of the hippocampus in Cushing`s disease [67]. Certain volume decrease also was found in the hippocampus, putamen and caudate nucleus in posttraumatic stress disorder patients [68].

Plasticity of hippocampal neurons

Plasticity represents the capacity of cell to change in response to various stimuli or injury. In relation to neuronal cells it is related to the well-known synaptic activity as well to anatomical changes. Different laboratories identified axonal sprouting and synaptogenesis in the hippocampus after lesions

of the fimbria. Synaptogenesis was manifested in new multiple synaptic buttons appearance at dendritic spines [69, 70].

Further efforts were directed to explore regenerative capacity of transplanted neurons to give axons toward neuronal target cells of recipient tissue. It was shown that transplanted adrenergic and cholinergic neurons could extend axons and reinervate the hippocampus with followed restoration of maze learning ability [71, 72]. Further, the transplanted hippocampal tissue showed electrophysiological properties typical of hippocampal neurons when grafted into the cerebellum [73].

Conclusion

There is no simple answer to the question why hippocampus is studied so frequently. It attracted early investigators by its distinct, relatively simple and well-structured form as compared to other parts of pallium. However, certain anatomical and neurobiological features of the hippocampus such as: single cell layer and strictly laminated inputs, predominantly unidirectional connections toward different cortical regions, numerous contacts with target neuronal dendrites, highly plastic synapses, tissue suitable for transplantation studies, neurons that can be successfully grown in culture, acute or cultured slices surviving for prolonged periods *in vitro* are of contemporary interest especially having in mind its proven role in memory process.

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R E F E R E N C E S

1. *Andersen P, Morris R, Amaral D, Bliss T, O'keefe J.* Historical Perspective: Proposed Functions, Biological Characteristics, and Neurobiological Models of the Hippocampus. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press. 2007. p. 9−36.
2. *Squire L, Berg D, Bloom F, Lac DS, Ghosh A, Spitzer N.* Fundamental neuroscience. 3rd ed. San Diego: University Press; 2008.
3. *Sabbatini R.* Neurons and Synapses. The History of its Discovery. Brain & Mind Magazine 17, 2003. Available from: en.wikipedia.org/wiki/Neuron [cited April-July 2003].
4. *Guillery RW.* Observations of synaptic structures: origins of the neuron doctrine and its current status. Philos Trans R Soc Lond B Biol Sci 2005; 360(1458): 1281−307.
5. *De Carlos JA, Borrell J.* A historical reflection of the contributions of Cajal and Golgi to the foundations of neuroscience. Brain Res Rev 2007; 55(1): 8−16.
6. *Akert K.* August Forel - Cofounder of the Neuron Theory (1848–1931). Brain Pathol 1993; 3(4): 425−30.
7. *Pearce JM.* Ammon's horn and the hippocampus. J Neurol Neurosurg Psychiatry 2001; 71(3): 351.
8. *Lorente de Nó R.* Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. J Psychol Neurol 1934; 46: 113–77.
9. *Rakic P, Nowakowski RS.* The time of origin of neurons in the hippocampal region of the rhesus monkey. J Comp Neurol 1981; 196(1): 99−128.
10. *Witter M, Amaral D.* Hippocampal Formation. In: *Paxinos G,* editor. The Rat Nervous System. 3rd. Sidney: Elsevier Academic Press; 2004. p. 635−704.
11. *Paxinos G.* The Rat Nervous System. San Diego: Elsevier Academic Press; 2004.
12. *Lakocević MB, Malobabić SP, Ocić GG.* Functional morphology of the hippocampal region in higher primates. Srp Arh Celok Lek 2001; 129(11−12): 313−8. (Serbian)
13. *Duvernoy H.* The Human Hippocampus. 3rd ed. Berlin Heidelberg, New York: Springer-Verlag; 2005.
14. *Amaral D, Lavenex P.* Hippocampal Neuroanatomy. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press. 2007. p. 37−114.
15. *Brodal P.* The Central Nervous System - Structure and Function. 4th ed. Oxford: University Press; 2010.
16. *El FH, Kubikova E, Benuska J.* The microscopical structure of the hippocampus in the rat. Bratisl Lek Listy 2008; 109(3): 106−10.
17. *O'keefe J, Nadel L.* The Hippocampus as a Cognitive Map. Oxford: University Press; 1978.
18. *Buhl E, Whittington M.* Local circuits. Oxford: University Press; 2007.
19. *Sancho-Bielsa FJ, Navarro-López JD, Alonso-Llosa G, Molowny A, Ponsoda X, Yajeya J,* et al. Neurons of the dentate molecular layer in the rabbit hippocampus. PLoS One 2012; 7(11): e48470.
20. *Blackstad TW, Kjaerheim A.* Special axo-dendritic synapses in the hippocampal cortex: electron and light microscopic studies on the layer of mossy fibers. J Comp Neurol 1961; 117: 133−59.
21. *Gaarskjaer FB.* The organization and development of the hippocampal mossy fiber system. Brain Res 1986; 396(4): 335−57.
22. *Walker MC, Ruiz A, Kullmann DM.* Do mossy fibers release GABA. Epilepsia 2002; 43 (Suppl 5): 196−202.
23. *McGinty JF, Henriksen SJ, Goldstein A, Terenius L, Bloom FE.* Dynorphin is contained within hippocampal mossy fibers: immunochemical alterations after kainic acid administration and colchicine-induced neurotoxicity. Proc Natl Acad Sci USA 1983; 80(2): 589−93.
24. *Freund TF, Buzsáki G.* Interneurons of the hippocampus. Hippocampus 1996; 6(4): 347−470.
25. *Jonhnston D, Amaral D.* Hippocampus. In: *Shepherd G,* editor. The synaptic organization of the brain. Oxford: Oxford University Press; 1998. p. 417−458.
26. *Lim C, Blume HW, Madsen JR, Saper CB.* Connections of the hippocampal formation in humans: I. The mossy fiber pathway. J Comp Neurol 1997; 385(3): 325−51.
27. *Standring S.* Gray`s Anatomy: The Anatomical Basis of Clinical Practice. 40th ed. Edinburgh, New York: Chuchill Livingstone Elsevier; 2008.
28. *Spruston N, McBain C.* Structural and functional properties of hippocampal neurons. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press; 2007. p. 133−201.
29. *Haug FM.* Sulphide silver pattern and cytoarchitectonics of parahippocampal areas in the rat. Adv Anat Embryol Cell Biol 1976; 52: 7−73.
30. *Afifi A, Bergman R.* Functional Neuroanatomy. New York, NY: McGraw-Hill; 2005.
31. *Shepherd GM.* General and variable features of varicosity spacing along unmyelinated axons in the hippocampus and cerebellum. Proc Nat Acad Sci 2002; 99(9): 6340−5.
32. *Nauta W, Gygax P.* Silver impregnation of degenerating axons in the central nervous system: a modified technic. Stain Technol 1954; 29(2): 91−3.

33. *Hamlyn L.* An electron microscope study of pyramidal neurons in the Ammon's horn of the rabbit. J Anat 1963; 97: 189−201.
34. *Grafstein B.* Transneuronal transfer of radioactivity in the central nervous system. Science 1971; 172(3979): 177−9.
35. *Hjorth-Simonsen A.* Some intrinsic connections of the hippocampus in the rat: an experimental analysis. J Comp Neurol 1973; 147(2): 145−61.
36. *Felleman DJ, Van ED.* Distributed hierarchical processing in the primate cerebral cortex. Cereb Cortex 1991; 1(1): 1−47.
37. *Andersen P, Bliss TV, Lomo T, Olsen LI, Skrede KK.* Lamellar organization of hippocampal excitatory pathways. Acta Physiol Scand 1969; 76(1): 4−5.
38. *Amaral D, Insausti R.* Hippocampal formation. In: *Praxinos G*, editor. The human nervous system. San Diego: Elsevier Academic Press; 1990. p. 711−55.
39. *Cerbone A, Patacchioli FR, Sadile AG.* A neurogenetic and morphogenetic approach to hippocampal functions based on individual differences and neurobehavioral covariations. Behav Brain Res 1993; 55(1): 1−16.
40. *Du F, Whetsell WO, Abou-Khalil B, Blumenkopf B, Lothman EW, Schwarcz R.* Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. Epilepsy Res 1993; 16(3): 223−33.
41. *Swanson LW, Cowan WM.* Hippocampo-hypothalamic connections: origin in subicular cortex, not ammon's horn. Science 1975; 189(4199): 303−4.
42. *Curtis DR, Phillis JW, Watkins JC.* Chemical excitation of spinal neurones. Nature 1959; 183(4661): 611−2.
43. *Fonnum F.* Glutamate: a neurotransmitter in mammalian brain. J Neurochem 1984; 42(1): 1−11.
44. *Storm-Mathisen J, Leknes AK, Bore AT, Vaaland JL, Edminson P, Haug FM,* et al. First visualization of glutamate and GABA in neurones by immunocytochemistry. Nature 1983; 301(5900): 517−20.
45. *Krnjević K, Schwartz S.* The action of gamma-aminobutyric acid on cortical neurones. Exp Brain Res 1967; 3(4): 320−36.
46. *Seress L, Ribak CE.* GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. Exp Brain Res 1983; 50(2−3): 173−82.
47. *Taupin P.* The Hippocampus: Neurotransmission and Plasticity in the Nervous System. New York: Nova Science Publishers, Inc. 2007.
48. *Kullmann D.* Synaptic Function. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press; 2007. p. 203–42
49. *Amaral DG, Campbell MJ.* Transmitter systems in the primate dentate gyrus. Hum Neurobiol 1986; 5(3): 169−80.
50. *Köhler C, Eriksson L, Davies S, Chan-Palay V.* Neuropeptide Y innervation of the hippocampal region in the rat and monkey brain. J Comp Neurol 1986; 244(3): 384−400.
51. *Nunzi M, Milan F, Polato P, Gorio A.* GABAergic neurons and coexistence of GABA and neuropeptides in the hippocampal microcircuitry. In: *Nunzi M, Milan F, Polato P, Gorio A*, editors. Ion Channels In Neural Membranes. New York: Liss; 1986. p. 333–45.
52. *Chan-Palay V.* Somatostatin immunoreactive neurons in the human hippocampus and cortex shown by immunogold/silver intensification on vibratome sections: coexistence with neuropeptide Y neurons, and effects in Alzheimer-type dementia. J Comp Neurol 1987; 260(2): 201−23.
53. *Haines DE.* Synopsis of Functional Components, Tracts, Pathways, and Systems. In: *Haines DE*, editor. Neuroanatomy. 6th ed. Baltimore: Williams and Wilkins. 2004.
54. *Siggins GR, Henriksen SJ, Chavkin C, Gruol D.* Opioid peptides and epileptogenesis in the limbic system: cellular mechanisms. Adv Neurol 1986; 44: 501−12.
55. *Morris R.* Theories of Hippocampal Function . In: : *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press; 2007. p. 581−713.
56. *Scoville W, Milner B.* Loss of recent memory after bilateral hippocampal lesions. J Neurosurg Psychiatry 1957; 20: 11−21.
57. *Zola-Morgan S, Squire LR, Amaral DG.* Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 1986; 6(10): 2950−67.
58. *Martinez J, Harvey S, Martinez A, Barea-Rodriguez E.* Information Processing. In: *Ramachandran VS,* editor. Encyclopedia of the human brain. San Diego: Elsevier Academic Press; 2002. p. 557−70.
59. *Hendelman W.* Hippocampal formation. In: *Hendelman W*, editor. Atlas of Functional Neuroanatomy. 2nd ed. New York: CRC Press, Taylor & Francis Group; 2006. p. 210–1.
60. *Stark C.* Functional role of the human hippocampus. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors. The Hippocampus Book. Oxford: University Press. 2007. p. 549−79.
61. *Stefanacci L, Buffalo EA, Schmolck H, Squire LR.* Profound amnesia after damage to the medial temporal lobe: A neuroanatomical and neuropsychological profile of patient E. P. J Neurosci 2000; 20(18): 7024−36.
62. *Teng E, Squire LR.* Memory for places learned long ago is intact afterhippocampal damage. Nature 1999; 400(6745): 675−7.
63. *McEwen BS, Weiss JM, Schwartz LS.* Selective retention of corticosterone by limbic structures in rat brain. Nature 1968; 220(5170): 911−2.
64. *McEwen BS, Stephenson BS, Krey LC.* Radioimmunoassay of brian tissue and cell nuclear corticosterone. J Neurosci Methods 1980; 3(1): 57−65.
65. *Lupien SJ, Lepage M.* Stress, memory, and the hippocampus: can't live with it, can't live without it. Behav Brain Res 2001; 127(1−2): 137−58.
66. *Morris R.* Stress and the Hippocampus. In: *Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J,* editors.The Hippocampus Book. Oxford: University Press; 2007. p. 581−713.
67. *Starkman MN, Gebarski SS, Berent S, Schteingart DE.* Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 1992; 32(9): 756−65.
68. *Filipovic BR, Djurovic B, Marinkovic S, Stijak L, Aksic M, Nikolic V,* et al. Volume changes of corpus striatum, thalamus, hippocampus and lateral ventricles in posttraumatic stress disorder (PTSD) patients suffering from headaches and without therapy. Cent Eur Neurosurg 2011; 72(3): 133−7.
69. *Goldwitz D, Cotman CW.* Induction of extensive fimbrial branching in the adult rat brain. Nature 1978; 275(5675): 64−7.
70. *Raisman G, Field PM.* Synapse formation in the adult brain after lesions and after transplantation of embryonic tissue. J Exp Biol 1990; 153: 277−87.
71. *Bjorklund A, Johansson B, Stenevi U, Svendgaard NA.* Re-establishment of functional connections by regenerating central adrenergic and cholinergic axons. Nature 1975; 253(5491): 446–8.
72. *Björklund A, Stenevi U, Svendgaard N.* Growth of transplanted monoaminergic neurones into the adult hippocampus along the perforant path. Nature 1976; 262(5571): 787−90.
73. *Hounsgaard J, Yarom Y.* Intrinsic control of electroresponsive properties of transplanted mammalian brain neurons. Brain Res 1985; 335(2): 372−6.

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CASE REPORTS

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Human case of fasciolosis in Serbia treated with triclabendazole

Humana fascioloza u Srbiji lečena triklabendazolom

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Abstract

Introduction. The number of humans infected by *Fasciola hepatica* is increasing worldwide. Humans can become accidental hosts by ingesting drinking water or plants contaminated with *metacercariae*. **Case report.** We reported a case of a 68-year-old Serbian woman, in which the diagnosis of acute fasciolosis had been established after serious diagnostic concerns. Based on clinical picture (episodic right upper quadrant abdominal pain, febrility and generalized body pain) and biochemical analyses (high eosinophilia and high activity of alkaline phosphatase), she was appointed as suspected to the acute fasciolosis. Stool and duodenal aspirate exams were negative for *Fasciola ova*. In the absence of adequate serologic diagnostic for fasciolosis in Serbia, the diagnosis was confirmed using enzyme immunoassays and immunoblot at the Institute for Tropical Diseases in Hamburg, Germany. Soon after triclabendazole was administered, the symptoms disappeared and biochemical values returned to normal. **Conclusion.** The diagnosis of human fasciolosis may be problematic and delayed, especially in non endemic areas, because physicians rarely encounter this disease and a long list of other diseases must be considered in the differential diagnosis. The syndrome of eosinophilia, fever, and right upper quadrant abdominal pain suggest acute fasciolosis. Unclear source does not rule out fasciolosis.

Key words:
fasciola hepatica; liver diseases, parasitic; humans; diagnosis; anthelmintics; treatment outcome.

Apstrakt

Uvod. Broj ljudi zaraženih parazitom *Fasciola hepatica* je u porastu širom sveta. Čovek postaje slučajan domaćin unošenjem infektivnih oblika, metacerkarija, kontaminiranom vodom ili biljkama. **Prikaz bolesnika.** U radu je prikazana 68-godišnja bolesnica iz Srbije, kod koje je nakon dijagnostičkog lutanja postavljena dijagnoza akutne fascioloze. Na osnovu kliničke slike (bol pod desnim rebarnim lukom, febrilnost, generalizovani bol), visoke eozinofilije i povišene aktivnosti alkalne fosfataze u serumu posumnjalo se na akutnu fasciolozu. U fecesu i duodenalnom aspiratu nisu nađena jaja *Fasciola hepatica*. U nedostatku imunološke dijagnostike za fasciolozu u Srbiji, dijagnoza je postavljena na osnovu prisustva specifičnih antitela imunoenzimskim testom i potvrđena imunoblot metodom u Institutu za tropske bolesti u Hamburgu, Nemačka. Lečenje je sprovedeno primenom triklabendazola, posle čega su se simptomi povukli, a biohemijske vrednosti vratile u normalu. **Zaključak.** Humana fascioloza može biti teška za dijagnostiku naročito u neendemskim područjima, gde kliničari retko pomisle na nju i gde je duga lista bolesti koje treba isključiti u diferencijalnoj dijagnostici. Sindrom eozinofilije, febrilnost i bolovi ispod desnog rebarnog luka sugerišu akutnu fasciolozu. Nejasan izvor infekcije ne isključuje fasciolozu.

Ključne reči:
fasciola hepatica; jetra, parazitne bolesti; ljudi; dijagnoza; antihelmintici; lečenje, ishod.

Introduction

Fasciolosis is a zoonotic infection caused by *Fasciola* (*F.*) *hepatica* and *gigantica*. Human fasciolosis (HF) is endemic in some parts of South America, Africa, Eastern Asia and Europe [1, 2]. Cases of human infection by *F. hepatica* are not uncommon in European countries [3] and can be predominantly found in France, Portugal, Spain and the former USSR [1], where fasciolosis is highly endemic in domestic animals.

Humans can become accidental hosts of this parasite by ingesting contaminated drinking water or plants in endemic area. Infective *metacercariae* excyst in the duodenum and larvae penetrate the wall of the small intestine, the peritoneal cavity, the liver capsule, and pass through the liver tissue into the biliary tract. High prevalence of HF does not neces-

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sarily occur in areas where fasciolosis is a major veterinary problem [1, 3]. Animal fasciolosis is enzootic to some parts of former Yugoslavia, with no recent reports related to it. Survival of *F. hepatica* in this area is related to numerous animal hosts, the presence of *Lymnaea truncatula* snails as the original intermediate host, and also suitable environmental and climatic factors. Recent studies indicate that sheep and cattle are the main reservoir of *F. hepatica* in the territory of the former Yugoslavia [4, 5].

The last patient with fasciolosis was treated more than 20 years ago at the Clinic for Infectious and Tropical Diseases in Belgrade. Human fasciolosis is a disease whose incidence is difficult to determine in Serbia because it is not a subject to mandatory reporting, it was not described in our literature in the last several decades, there were no oral presentations at medical meetings, diagnostics market is not developed and there are no effective drugs. There are also no requirements for the procurement and registration of the specific drugs for fasciolosis, so we can conclude that it is rarely diagnosed and cured.

Case report

A 68-year-old woman was admitted at the Clinic for Infectious and Tropical Diseases in Belgrade on March 10, 2005. The patient was a pensioner from Belgrade, who spent the last few months in a village near Trebinje in Herzegovina. Her last travell, out of borders of former Yugoslavia, was in Canada, two and a half years ago. She had a medical history of benign breast node which was surgicaly removed in 1984. The patient had been in a good health until the end of November 2004, when she developed episodic right upper quadrant (RUQ) abdominal pain which spread to the right shoulder, occasional bloating, fever (39–40°C), shaking chills, occasional dizziness, weakness and fatigue. This condition persisted and she was admitted at the Department of Hematology of Clinical Hospital Center "Zvezdara", Belgrade (from January 10 to February 9, 2005) with suspicion to eosinophilic leukemia. Detailed clinical, laboratory and radiographic tests were performed. The laboratory tests revealed mild normocytic normochromic anemia, high eosinophilia, high serum activity of alkaline phosphatase (ALP), which maintained during all hospitalizations, with normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Abdominal ultrasound (US) showed the inhomogeneous right liver lobe. Computed tomography (CT) scan showed hypodense changes (total diameter up to 5 cm) in the right liver lobe below the diaphragm predominantly localized posteriorly and centrally that are partly flown together. After application of contrast most of the changes remained hypodense and only edges were imbibed by getting a grape look appearance. The left liver lobe was homogeneous. Two enlarged lymph nodes (up to 2 cm in size) were evident in the retroperitoneum.

The diagnosis of malignancy was initially suspected and for additional examination she was transfered to the Department of Surgery (from February 9 to February 14, 2005). Because the diagnosis of malignancy was initially considered, laparoscopic liver biopsy under visual control was performed at the Department of surgery on February 14, 2005, and before obtaining the histological diagnosis the patient was released. Two fragments of the liver tissue were obtained by biopsy. The first specimen showed less liver tissue and largely fibrous tissue infiltrated by lymphocytes and numerous eosinophils. The second specimen showed liver tissue with fibrosis and small cells infiltration of the portal fields. Some portal areas had a strong, almost serious proliferation of connective tissue with bile ducts and blood vessels. One part of the specimen showed nodules with strong fibrosis and septa that form pseudo-tubules similar to inactive cirrhosis. There was also a fibrous tissue containing bile ducts and numerous blood vessels with thickened walls. There were no signs of malignancy. A conclusion was that it was focal nodular hyperplasia. After receiving a histopathological finding the patient was advised to continue testing at the Clinic for Infectious and Tropical Diseases because of her subjective complaints, fever and eosinophilia, the existence of non-specific histopathological lesions in the liver and the history of travelling to tropical areas in the past.

Upon admission to our Clinic, her temperature was 37.8°C. Abdominal exam showed localized tenderness over RUQ and a palpable liver border on the right costal margin. The rest of the physical exam was unremarkable. On admission, hemoglobin level was 118 g/L, eritrocyte count was 3.96×10^{12}/L, the white-cell count was 13.5×10^{9}/L with 43.7% eosinophils, the platelet count was 243×10^{9}/L. The erythrocyte sedimentation rate (ESR) was 60/90 mm/h and serum biochemical analysis revealed the following values: Fe 7.1 μmol/L, ALP 174 U/l; ALT 16 U/l; AST 14 U/l L and alpha amylase 49 U/L. Total bilirubin was 7 μmol/L, fibrinogen value was 5.6 g/L, C-reactive protein (CRP) 10 mg/L. Antibodies to *Brucella* spp, HCV, HBV were not found. The results of tumor markers (CEA, CA 19.9, AFP) were within normal range. Laboratory was controlled weekly (AST 20 U/L, ALT 26 U/L, than AST 57 U/L, ALT 68 U/L). The highest value for CRP was 15 mg/L, and fibrinogen 6.6 g/L.

In view of the persistent fever, hepatomegaly, and eosinophilia serologic tests for a variety of parasites were requested. Serology was negative for *Toxoplasma gondii*, *Trichinella spiralis*, *Echinococcus*, *Leishmania*, *Entamoeba histolytica, Schistosoma*, but *Toxocara* (*T.*) *canis* (1.27) and *Cysticercus* (1.21) enzyme immunoassays (EIA) were mildly positive.

Chest radiographs showed no abnormalities. Abdominal US on March 16 showed homogeneous liver of normal size, with no visible focal changes. Two enlarged periportal hilar lymph nodes (diameter 18 mm and 17 mm) with hypoechoic appearance were detected.

The liver-spleen scintiscan on March 21, 2005, showed mild hepatomegaly with track-like zones of hypofixation of radiofarmaci (RF, radioactive colloids) in the right posterior liver lobe. Liver and spleen scintigraphy showed the enlarged liver, with straight upper edges. The contours of the upper part of the right hepatic lobe were irregular and uneven. The distribution of RF was non-homogeneous with the appearance of zones of weaker binding of RF. Track-like zones of hypofixation of RF in the right posterior liver lobe were observed resembling two oval recesses of the top of the right lobe.

It was unclear whether these changes were focal zones or there was an extrahepatic origin of these recesses. The spleen was mildly enlarged with tiny, oval discreet zones of lower levels binding of RF. Because ultrasound did not show focal liver lesions, radiologist recommended further investigation (CT and scintigraphic control).

Abdominal CT (on April 4, 2005) demonstrated multiple hypodense foci with subcapsular location on both sides of the liver, partially merged (Figure 1). Repeated US on April 11, 2005 showed irregular oval hyperechoic solitary liver lesion, in the upper right lobe of the liver, near the confluence of the right hepatic vein, 52 × 42 mm in size, without a hypoechoic halo (Figure 2).

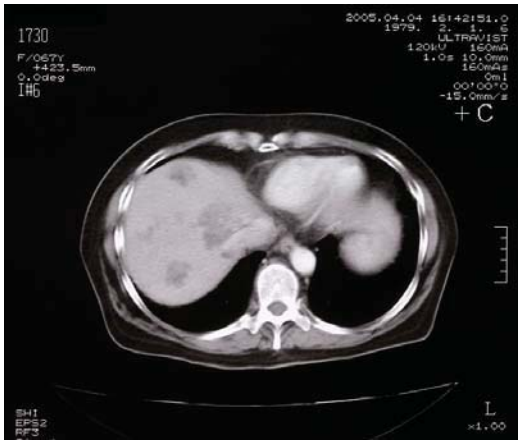

Fig. 1 – Abdominal computed tomography (CT) scan shows multiple hypodense foci with subcapsular location.

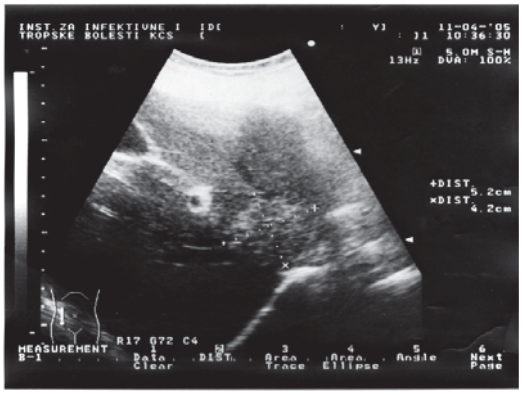

Fig. 2 – Irregular oval hyperechoic solitary liver lesion (52 × 42 mm in size), in the upper part of the right liver lobe.

A detailed re-evaluation with repeated investigation of liver biopsy (April 23, 2005) revealed a histopathological picture suggesting parasitic infection. In a small part of the obtained material were hepatocytes, and in the rest of the sample there was young granulation tissue infiltrated by eosinophils and other inflammatory cells. Portal fields were normal in size, easy to moderately infiltrated, mainly by eosinophils, with a small number of other inflammatory cells. Glycogenated nuclei were seen in the parenchyma. Inflammatory cells of the same type as those found in portal fields were seen in the sinusoids.

Repeated stool and duodenal aspirate examinations were negative for *Fasciola* eggs. Since the serology for *T. canis* was not affirmative, along with the fact that *Toxocara* generally gives changes in central nervous system and eyes, this type of affection of the liver and bile ducts with high eosinophilia resembled acute fasciolosis. Because acute fasciolosis was highly suggested and serological diagnosis for fasciola was not available in Serbia, the patient's serum was sent to the Institute for Tropical Diseases in Hamburg, Germany. Serology to *Wuchereria bancrofti*, *Schistosoma mansoni*, *Dirofilaria immitis* and *Strongyloides stercoralis* was negative but the EIA was positive for *Fasciola*-specific IgG antibodies. The final diagnosis of fasciolosis was confirmed by immunoblot testing.

After the diagnosis was confirmed, triclabendazole (Egaten®, Novartis) was administered at the dose of 10 mg/kg for 1 day.

Control abdominal ultrasound on May 4, 2005, was unchanged. Before the patient was discharged on May 5, 2005, ESR was 52/84 mm/h, leukocytes 10.4 × 10^9/L with eosinophilia 34%, platelets 227 × 10^9/L, AST 15 U/L, ALT 17 U/L, ALP 125 U/L.

The patient appeared clinically well two weeks after the treatment. At a 6 month follow-up examination, her eosinophil count, hematological and biochemical results were normal (ESR l5 mm/hour, CRP 6 mg/L, leukocytes 7,2 × 10^9/L, eosinophilia l2%).

Discussion

We reported a case of acute HF of uncertain origin of infection. Although the beginning of the disease coincides with the patient's several months stay in Herzegovina, considering the fact that there were no reports of animal fasciolosis in this area, we could not be sure if the infection was positively acquired there. A source of infection was unclear, too. It could be due to the consumption of improperly washed fresh vegetables since the patient used to buy them supplied in the green market with no defined product sourcing or she could have been infected by chewing grass contaminated with *metacercaria* or by contaminated water. This is consistent with data about human infection in areas where people do not have a history of eating watercress [2]. The experimental results suggest that humans who consume raw dishes prepared from fresh livers infected with immature flukes could become infected with *F. hepatica* [6], but our patient did not have these data in her history. It fits into the explanation of Mas-Coma et al. [1] about the existence of isolated autochthonous, nonconstant cases [7].

Infection with *F. hepatica* has a variable clinical presentation depending on the stage of the disease. The syndrome of eosinophilia, fever, and right abdominal upper quadrant pain, without jaundice, hypodense liver lesions on CT, and an appropriate exposure history (history of eating fresh vegetables that may be improperly washed) suggests acute fasciolosis. A negative history does not rule out fasciolosis. High ESR, anaemia and leukocytosis with high eosinophilia (may be up to 70%) are frequent findings in infected individuals in the early phase of the disease [2]. Clearly, febrile diseases and other para-

sitic infections causing eosinophilia and/or similar symptoms should be ruled out. Hypereosinophilic syndrome was rarely diagnosed as *Toxocara*, *Strongyliodes* and *Fasciola* [8]. In this case, the clinical presentation and liver problem indicate that HF was probably the unique diagnosis. Because of the endemicity of toxocariosis and cysticercosis in Serbia, positive serology for both infections was probably due to anamnestic antibody response or a nonspecific antibody response. Aminotranspherase levels are usually in normal range or are only minimally elevated, and bilirubin levels are typically in normal range, as was the case with our patient. Saba et al. [9] reported 28 patients with acute fasciolosis who predominantly had epigastric pain, fatigue, fever and RUQ abdominal pain and elevated eosinophilia, ALT level and acute-phase reactant in laboratory findings. Eosinophilia in fasciolosis is striking and almost always present [9, 10]. According to results of Haseeb et al. [11] high activity of ALP, as well as significantly high eosinophilia and low hemoglobin, are the most significant laboratory features of HF, as it was in our case.

Hepatic lesions are produced by migration of juvenile *Fasciola* through liver in the invasive stage. Histologically, they correspond to microabscesses and tunnel-like areas of parenchymal necrosis [12]. Characteristic parenchymal lesions are clearly demonstrated by imaging procedures. Imaging techniques used for diagnosis of fasciolosis include radiology, radioisotope scanning, US, CT scan or MRI that may show the tunnels caused by the migrating young flukes or the flukes in the biliary passages (chronic infection) [7]. The first abdominal CT finding in our patient showing hypodense clustered lesions in a periphery of the right lobe of the liver and enlarged retroperitoneal lymph nodes was compatible with hepatic phase of fasciolosis.

Han et al. [13] reported the characteristic radiological features in 5 patients with hepatic fasciolosis that involve cluster of microabscesses arranged in track-like hypodense lesions with subcapsular location, and a very slow evolution of the lesion. Marcos et al. [14] further reported hepatomegaly as a common finding on abdominal CT scan in patients with fasciolosis. First abdominal CT finding in our patient was compatible with hepatic phase of fasciolosis, but radiologists were not familiar with findings of this disease. Typical findings on US, which should be absolutely performed as initial assessment procedure, facilitate the diagnosis.

Magnetic resonance imaging (MRI) reflects the extent of the lesions better than CT in the earlier stage. In the late parenchymal phase, the extent of the lesions on the specimens is well correlated with both CT scan and MRI [15]. Periportal lymph node enlargement or lymphadenopathy is helpful in the diagnosis [16], which was the case in our patient.

The biliary phase is usually asymptomatic, and only intermittent cholangitis may be the prominent sign, but it is rarely reported that it can lead to extrahepatic obstruction and cholestasis [17].

The diagnosis of fasciolosis is complex and requires application of both direct an indirect methods of diagnostics. Diagnosis of *F. hepatica* infection has traditionally relied on detecting the presence of eggs in fecal samples or bile specimens, but this method is unreliable and complicated. Negative stool examinations do not rule out fasciolosis. A period of at least 3 to 4 months is necessary for *F. hepatica* flukes to attain sexual maturity in humans [18]. Our patient was admitted to our Clinic with a 3-month history of illnes. This can be the reason why the stool examination was negative. Human is generally believed to be a non-suitable host and the possibility of hepatic infections by flukes which are unable to attain maturity, cannot be disregarded [19].

At present, the routine diagnosis of HF is based on the detection of antifluke antibodies in the serum. Specific antibodies to *Fasciola* may be detectable within 2 to 4 weeks after infection, which is 5 to 7 weeks before eggs appear in stool. Immunological techniques present the advantages of being applicable during all phases of the disease, but especially during the acute phase. This is important in areas where HF is rare [1], such as in Serbia. Although, early diagnosis of fasciolosis is performed mainly on serum assay [10], for rare parasitic infection, such as fasciolosis, there is a lack of registered diagnostic tests in Serbia. The current test of choice for immunodiagnostic of human *F. hepatica* infection is EIA [20] combined with confirmation of positives by immunoblot [14] with a sensitivity of 100% and a specificity of 97.8% [21]. Since signs and symptoms of fasciolosis may be confused with a wide variety of disorders, including hepatobiliary and extrahepatobiliary diseases (hepatitis, cholecystis, cholangitis, liver abscess, brucellosis, leishmaniosis, schistosomiosis and primary and secondary hepatobiliary malignancies) [16], diagnosis and treatment are often problematic and delayed, which was the case in our patient.

Many drugs have been used to treat fasciolosis with variable success [14, 22–24]. The first-line treatment of HF is with a single oral dose (10 mg/kg) of triclabendazole which is highly effective against mature and immature flukes, safe, and easy to use [2, 14, 25]. Treatment should be repeated when a single dose fails to cure the infection [10]. A single dose of triclabendazole was successful and without side effects in our patient. Albendazole, widely used in animal fasciolosis, is ineffective against human infections [22], as it was the case in the presented patient.

Conclusion

Human fasciolosis can be very difficult to diagnose, because it sometimes appears with atypical and severe clinical presentation. Problematic and delayed diagnosing is especially risky in nonendemic areas where clinicians are not familiar with this disease. The syndrome of eosinophilia, fever, and RUQ abdominal pain without jaundice; liver damage, which is manifested by a high activity of liver enzymes and hypodense liver lesions on CT, including an appropriate exposure history, suggest acute fasciolosis. Unclear history does not rule out fasciolosis. Fortunately, with the exact diagnosis simple therapy with triclabendazole is extremely effective.

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REFERENCES

1. *Mas-Coma S, Bargues MD, Valero MA.* Fascioliosis and other plantborne trematode zoonoses. Int J Parasitol 2005; 35(11–12): 1255–78.
2. *Soliman MFM.* Epidemiological review of human and animal fascioliosis in Egypt. J Infect Developing Countries 2008; 2(3): 182–9.
3. *Arjona R, Riancho JA, Aguado JM, Salesa R, González-Macías J.* Fasciolosis in developed countries: a review of classic and aberrant forms of the disease. Medicine 1995; 74(1): 13–23.
4. *Rajkovic-Janje R, Bosnic S, Kosier N, Husnjak M.* Invasion with endoparasites in sheep, goats and bovine cattle and its significance. Praxis Veterinaria 2000; 48(1–2): 41–5 (Croatian)
5. *Milanovic V, Nitovski A, Kulisic Z, Mirilovic M. Fasciola hepatica* findings in cattle of Jablanica region (Serbia) Veterinarski glasnik 2007; 61(1–2): 89–97. (Serbian)
6. *Taira N, Yoshifuji H, Boray JC.* Zoonotic potential of infection with *Fasciola* spp. by consumption of freshly prepared raw liver containing immature flukes. Int J Parasitol 1997; 27(7): 775–9.
7. *Esteban JG*, *Bargues MD.* Epidemiology of human fascioliosis: a review and proposed new classification. Bull WHO 1999; (77): 340–6.
8. *Ehrhardt S, Burchard GD.* Eosinophilia in returning travelers and migrants. Dtsch Arztebl Int 2008; 105(46): 801–7.
9. *Saba R, Korkmaz M, Inan D, Mamikoglu L, Turhan O, Gunseren F et al.* Human fascioliosis. Clin Microbiol Infect 2004; (10): 385–7.
10. *Dauchy FA, Vincendeau P, Lifermann F.* Eight cases of fascioliosis: clinical and microbiological features. Med Mal Infect 2006; 36(1): 42–6.
11. *Haseeb AN, El-Shazly AM, Arafa MA, Morsy AT.* Clinical, laboratory and ultrasonography features of proven human fascioliosis. J Egypt Soc Parasitol 2003; 33(2): 397–412.
12. *Pagola Serrano MA, Vega A, Ortega E*, *Gonzales A.* Computed tomography of hepatic fascioliosis. J Comput Assist Tomog 1987; 11(2):269–72.
13. *Han JK, Choi BI, Jae Min Cho, Chung KB, Han MC, Kim CW.* Radiological findings of human fascioliosis. Abdom Imaging 1993; 18(3): 261–4.
14. *Marcos LA, Tagle M, Terashima A, Bussalleu A, Ramirez C, Carrasco C*, et al. Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fasciolosis. Am J Trop Med Hyg 2008; 78(2): 222–7.
15. *Taheri MS, Aminzade Z, Shokohi Sh, Birang Sh, Aghazade K.* Case Report. Hepatobiliary fascioliosis: clinical and radiological features. Iranian J Parasitol 2007; 2(4): 48–55.
16. *Kabaalioglu A, Ceken K, Alimoglu E, Saba R, Cubuk M, Arslan G,* et al. Hepatobiliary fascioliosis: sonographic and CT findings in 87 patients during the initial phase and long-term follow-up. AJR Am J Roentgenol 2007; 189(4): 824–8.
17. *Moghadami M, Mardani M.* Fasciola hepatica: A cause of obstructive jaundice in an elderly man from Iran. Saudi J Gastroenterology 2008; 14(4): 208–10.
18. *Espino AM, Díaz A, Pérez A, Finlay CM.* Dynamics of antigenemia and coproantigens during a human Fasciola hepatica outbreak. J Clin Microbiol 1998; 36(9): 2723–6.
19. *Acosta-Ferreira W, Vercelli-Retta J, Falconi LM.* Fasciola hepatica human infection. Histopathological study of sixteen cases. Virchows Arch A Path Anat Histol 1979; 383(3): 319–27.
20. *Hernandez RJ, Blasco CA, Valdivieso BMF, Arribas MMI, Paciencia AM, Rodríguez SE,* et al. Familial outbreaks of fascioliosis: usefulness of serologic investigation by enzyme immunoassay. Rev Clin Esp 1998; (198): 33–5.
21. *Mansour WA, Kaddah MA, Sjaker ZA, al Assal FM, Derbala MF.* A monoclonal antibody diagnoses active *fasciola* infection in humans. J Egypt Soc Parasitol 1998; 28(3): 711−27.
22. *World Health Organization.* WHO/CDS/NTD/PCT/2007.1. Report of the WHO Informal Meeting on use of triclabendazole in fasciolosis control. WHO headquarters, Geneva, Switzerland: World Health Organization; 2006.
23. *Rossignol JF, Abaza H, Friedman H.* Successful treatment of human fascioliosis with nitazoxanide. Trans R Soc Trop Med Hyg 1998; 92(1): 103–4.
24. *Hien TT, Truong NT, Minh NH, Dat HD, Dung NT, Hue NT,* et al. A randomized controlled pilot study of artesunate versus triclabendazole for human fascioliosis in central Vietnam. Am J Trop Med Hyg 2008; 78(3): 388–92.
25. *Keiser J, Engels D, Buscher G, Utzinger J.* Triclabendazole for the treatment of fascioliosis and paragonimiosis. Expert Opin Investig Drugs 2005; 14(12): 1513–26.

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CASE REPORT

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Switch to hypomania induced by repetitive transcranial magnetic stimulation and partial sleep deprivation added to antidepressant: A case report

Hipomanija indukovana primenom repetitivne transkranijalne magnetne stimulacije i parcijalne deprivacije spavanja kod bolesnika na terapiji antidepresivima

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Abstract

Introduction. Bipolar depression is often unrecognized and difficult to treat because of two opposite problems: treatment resistance and risk of manic switch. **Case report.** A 53-year-old female was suffering from unipolar depressive disorder since the age of 36. During a recent major depressive episode pervasive feelings of sadness, lost of interest in activities, severe insomnia and highly expressed somatic anxiety dominated 7 months. After unsuccessful tries with two different antidepressants of adequate doses and duration, slow rate repetitive trascranial magnetic stimulation (rTMS) was started, but the patient stayed at the fixed dose of antidepressant. Partial sleep deprivation (PSD) was additionaly applied twice during these 2 weeks with the idea to boost up, or enhance rTMS treatment response. At the last two rTMS sessions depression obviously meliorated, but the patient also expressed symptoms of hypomania. The therapy of rTMS was stopped, hypomanic symptoms gradually vanished and two weeks after the rTMS treatment the patient was euthymic. Antidepressant was kept on. In a follow-up period of 2 years the diagnose of bipolar affective disorder was definitely established. **Conclusion.** This case report shows that a combination of slow rate rTMS and partial sleep deprivation in the patient at the fixed dose of antidepressants, have strong synergistic effect even with potential to induce hypomanic switch, that is the first description in the literature to our knowledge.

Key words:
bipolar disorder; diagnosis, differential; depression; antidepressive agents; sleep deprivation; transcranial magnetic stimulation.

Apstrakt

Uvod. Bipolarna depresija često se ne prepoznaje, a teškoće u njenom lečenju odnose se na dva suprotna problema: terapijsku rezistenciju i rizik od maničnog preokreta. **Prikaz bolesnika.** Bolesnica stara 53 godine, lečena je od unipolarnog depresivnog poremećaja od svoje 36. godine. Tokom nedavne epizode velike depresije tokom sedam meseci dominirala su stalna osećanja potišenosti, gubitak interesovanja za uobičajene aktivnosti, teška nesanica i izrazita somatska anksioznost. Posle dva neuspela terapijska pokušaja sa različitim antidepresivima primenjenim u odgovarajućim dozama i dovoljno dugo, započeta je terapija niskofrekventnom repetitivnom transkranijalnom magnetnom stimulacijom (rTMS). Tokom rTMS terapije bolesnica je nastavila da prima nepromenjenu dozu antidepresiva. Parcijalna deprivacija spavanja (PSD) dodatno je primenjena u dva navrata tokom ove dve nedelje sa idejom da će potencirati ili pospešiti terapijski odgovor. Tokom poslednje dve rTMS sesije, simptomi depresije bili su značajno smanjeni, ali je bolesnica ispoljavala i simptome hipomanije. Terapija rTMS je prekinuta i hipomanični simptomi su postepeno prestali. U roku od dve nedelje posle rTMS terapije bolesnica je bila eutimična. Terapija antidepersivom je nastavljena. U periodu praćenja u naredne dve godine definitivno je postavljena dijagnoza bipolarnog afektivnog poremećaja. **Zaključak.** U ovom prikazu bolesnice, pokazalo se da istovremena primena rTMS i deprivacije spavanja kod bolesnika sa nepromenjenom dozom antidepresiva ima snažan sinergistički efekat, čak sa mogućnošću da izazove manični preokret, što je ujedno, prema našim saznanjima, prvi do sada objavljeni slučaj hipomanije indukovane ovom kombinacijom antidepresivnih terapija.

Ključne reči:
psihoze, manično-depresione; dijagnoza, diferencijalna; depresioni poremećaji; antidepresivi; spavanje, deprivacija; transkranijalna magnetna stimulacija.

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Introduction

Bipolar depression is a common and severe psychiatric disorder with high risk of suicide, but after many high quality surveys and expert consensus in practical guidelines, it is still often unrecognized and mismanaged. The main difficulties in therapy of bipolar depression regard two opposite issues: treatment resistance and risk of hypomanic/manic switch.

Antidepressant-associated manic switch was reported to be higher in bipolar type I disorder than unipolar depression [1] or bipolar type II, and antidepressant induced hypomania/mania during the treatment of unipolar depression is considered as a sign of latent bipolar disorder [2].

Transcranial magnetic stimulation (TMS) is relatively novel method of non-invasive stimulation of brain cortex and has been explored in neurology, psychiatry and neuroscince.

A number of randomized controlled trials have demonstrated effecacy and safety of rTMS in major depression [3–5], but there are only a few rTMS trials in bipolar depression where its efficacy and saftey has not yet been established. Available data, until now, are controversial.

In most rTMS studies on major depression high frequencies of stimulation (≥ 5 Hz) of the left dorso-lateral prefrontal cortex (DLPFC) and only a few trials used low frequent (≤ 1 Hz) rTMS of the right DLPFC [4, 5] suggesting its efficacy and even better safety, compared to high frequence rTMS.

Swich to hypomania/mania may occur also with therapeutic sleep deprivation [6]. A recently published review [7] including 53 rTMS randomized controlled trials in unipolar and bipolar depression found that switching occurance is similar as with antidepressant pharmacotherapy.

Several positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) studies already found changes in cerebral blood flow and glucosa metabolism in prefrontal regions [8, 9] after sleep deprivation, similar to changes observed after rTMS treatment in depressive patients [10], that means possible synergistic effect of this two antidepressant treatments.

Case report

A 53-year-old female was suffering from major depressive disorder, according to the Diagnostic and Statistic Manuel of Mental Disorders – IV Edition, Text Revision (DSM- IV-TR) [11] criteria. The patient was highly educated, employed, married, had two adult children, and no history of other psychiatric or somatic disorder. In family history for psychiatric disorders data were not completely reliable, because her father had a long period of alchohol abuse and aggressive behavior, but denied psychiatric treatment. First major depressive episode was diagnosed when the patient was 36 and after short antidepressant treatment she remitted and had not been regularly monitored by the psychiatrist until the age of 48. In the next 5-year period the patient had 3 major depressive episodes. The major depressive episode (before rTMS treatment was used) started 7 months ago with pervasive feelings of sadness, lost of interest in family and activities, severe insomnia and highly expressed somatic anxiety. During that episode the patient was treated as outpatient.

Baseline Hamilton Depresssion Rating Scale -17 items (HDRS-17) score was 27. Antidepressant treatment started with sertraline (gradually titrated to the dose of 200 mg/day) for about 8 weeks. Because of poor antidepressant response with 25 points at HDRS-17, sertraline was tapered off and the patients was treated with venlafaxine (up to 225 mg/day) without significant improvement and after additional 9 weeks the patients had quite severe depressive symptoms with 28 points at HDRS-17.

With the written informed consent we started rTMS treatment. The institutional review board, following the Declaration of Helsinki (1975), approved the experimental procedures.

TMS was delivered by a Magstim Magnetic Stimulator with a figure 8-shaped coil.

rTMS was then delivered at 110% rot test motor treshold (RMT) intensity on the frontal scalp area overlying the right dorsolateral prefrontal cortex (DLPFC), localized according to previous reports 5 cm in front of the best spot for inducing MEPs from the abductor policis brevis (APB) muscle [12].

The patient received 10 sessions of 1 Hz right prefrontal rTMS at 110% of RMT intensity, over a 2-week period (5 days/week). Each daily session of rTMS consisted of 5 trains 60 stimuli (300 stimuli/daily), with inter-train pauses of 3 minutes, lasted approximately 20 min. The total number of stimuli the patient received during a 2-week treatment was 3,000.

Twice during a 2-week period late partial sleep deprivation (PSD) was applied; the patient went to bed as usual, woked-up at 01.30 h and stayed awake approximately the next 20 h. We chose late PSD instead of total sleep deprivation (TSD) because its proven efficacy similar to TSD (13) and abbreviated procedure seemed more likely acceptable for patients compliance. The patient was naive to sleep deprrination as well as rTMS. A family member was monitored the patient`s complience.

During the rTMS treatment the patient stayed at fixed dose of venlafaxine (225 mg/day).

At the last two rTMS sessions depression obviously meliorated (11 points at HDRS), but the patient also expressed symptoms of hypomania – became talkative, cheerful, optimistic, self-confident, and hyperactive at times and had a quite unrealistic plans. Young Mania Rating Scale (YMRS) score was 13 (symptoms adequate to meet DSM-IV-TR mild hypomania criteria).

rTMS was stopped after 10 sessions, as it was scheduled, because hypomania started at the end of treatment, and patient was carefully monitored. One week after the rTMS treatment the patient came to outpatient clinic with milder hypomania symptoms, not so hyperactive anymore, but still euphoric and at moments showed inappropriate friendly attitude toward doctor and nurses (8 points at YMRS). Two weeks after rTMS treatment patient was

definitely euthimic (4 points at YMRS, 8 points at HDRS-17) and then was decided to keep on with antidepressant treatment (225 mg venlafaxine/day), and be careful in monitoring a possible bipolar affective disorder. Remission was sustained in the next 8 months, when the patient was admitted to the hospital with severe depressive symptoms. Fifty mg/day of amytriptiline was added to venlafaxine 225 mg/day, without significant response. When amytriptiline dose was increased to 75 mg/day, the patient switched to mania, first time in her life. Antidepressants were stopped and the treatment with mood stabilizer started (lithium carbonatis 900 mg/day). After a 2-year follow-up, the patient stayed euthimic.

Discussion

This case report shows that a combination of slow rate rTMS boosted with partial sleep deprivation has strong, possibly synergistic antidepressant effect, with the potential to induce hypomanic switch in the patient at the fixed dose of antidepressants.

Our patient previously did not respond to high doses of sertraline and venlafaxine that justified the diagnose of treatment resistant depression. During the rTMS treatment the patient stayed at the fixed dose of medication, but it was not likely that venlafaxine caused switch to hypomania (although switch might be attributed to a long-term use of antidepressants, which may destabilize the illness). It is also not likely that after 9 weeks at the fixed dose of venlafaxine without mood improvement, venlafaxine caused manic switch; moreover, the patient became euthimic when rTMS was stopped, but venlafaxine was kept on, during the observation period. Thus, it is more likely that the low-frequent rTMS and partial sleep deprivation had strong synergistic antidepressant effect that result in hypomania in misdiagnosed bipolar spectrum disorder patient treated all the time with antidepressant monotherapy.

Interestingly, during the first major depressive episode in her life the patient was also treated with amytriptiline monotherapy and with 125 mg/day had a significant improvement, without switch to hypomania/mania. Hypomanic switch during rTMS combined with SD and followed by manic switch that occurred in a follow-up period, after the last major depressive episode, could be similar to reports of new onset of bipolar disorder during rTMS in patients previosely thought to have unipolar illness [14].

Bipolar affective disorder symptoms onset peaks in much younger age and it happens rarely that a patient is first time diagnosed as bipolar at the age of 53. Clinicians also know this sometimes happens considering the fact that many patients use to come on treatment only during depression phase and almost never come during hypomania when their subjective feeling is `high`, insight is poor and they often deny being ill at all. Family members are also happy to see a patient cheerful and active after severe suffering during depression phase, and sometimes hypomania episodes are so long and frequent that they see a patient as sick only during depression and as `normal – that is he/she – during hypomania. Clinical experience often shows that proper diagnoses of bipolar affective disorder have long delays, sometimes more than 10 years [15].

Nedjat and Folkerts [16] reported transient hypomanic symptoms during high frequency rTMS of the left PFC in 3 of 50 healthy volunteers.

Most of rTMS studies reporting on switch to hypomania/mania used high frequencies of stimulation. Until now, to our knowledge, only a few studies using low frequencies of stimulation in depression reported switch to hypomania/mania [17–19].

Ella et al. [17] reported two cases of manic switch during slow (1 Hz) rTMS treatment of the right DLPFC in resistant major depression, but the number of stimuli *per* session they used (1,200 stimuli/day) was much higher than we used (300 stimuli/day), and was applied during 3 weeks (15 sessions); in both cases switch occurred few days after the last sessions, that was similar to our case.

One study used bilateral rTMS (high over the left DLPFC and low over the right DLPFC) to enhance antidepressant outcome but a patient switched to mania on day 7 of stimulation [18].

Fitzgerald et al. [19] in a double-blind, parallel design study reported one switch to mania with stimulation of 1Hz, 100% RMT, 300 stimuli/session (in a group stimulated with 10 Hz was no switch).

Sakkas et al. [20] in their study protocol used more aggressive stimulation with 20Hz, 110% RMT, 1600 stimuli/ session, two sessions/day and reported one case of hypomania and one case of mania (one of them was in age of 55 first time experienced manic symptoms related to rTMS, similarly to our patient). This report definitely shows that mania-induced potential of rTMS correlates with the intensity of rTMS, but our protocol with less intensive stimulation also resulted in switch to hypomania.

Conclusion

Our case report shows that a combination of rTMS and partial sleep deprivation added to antidepressant may have strong antidepressive synergistic effect even with potential to induce hypomanic switch.

In the treatment of resistant depression clinicians always should be aware of possible unrecognized bipolar disorder, or, in other words, that bipolar depression is often a treatment-resistant depression.

Further controlled studies should give more precise safety guidelines and optimal tretment strategies in cases of hypomania/mania induced by rTMS; even a bit flexible individual strategies should still be the base of treatment of every particular patient with bipolar depression.

Acknowledgment

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REFERENCES

1. *Bond DJ, Noronha MM, Kauer-Sant'Anna M, Lam RW, Yatham LN*. Antidepressant-associated mood elevations in bipolar II disorder compared with bipolar I disorder and major depressive disorder: a systematic review and meta-analysis. J Clin Psychiatry 2008; 69(10): 1589−601.
2. *Akiskal HS, Hantouche EG, Allilaire JF, Sechter D, Bourgeois ML, Azorin JM,* et al. Validating antidepressant-associated hypomania (bipolar III): a systematic comparison with spontaneous hypomania (bipolar II). J Affect Disord 2003; 73(1−2): 65−74.
3. *Padberg F, George MS*. Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression. Exp Neurol 2009; 219(1): 2−13.
4. *Fitzgerald PB, Huntsman S, Gunewardene R, Kulkarni J, Daskalakis ZJ*. A randomized trial of low-frequency right-prefrontal-cortex transcranial magnetic stimulation as augmentation in treatment-resistant major depression. Int J Neuropsychopharmacol 2006; 9(6): 655−66.
5. *Krstic J, Diligenski V, Ilic TV*. Low-frequency repetitive transcranial magnetic stimulation conjugated with partial sleep deprivation in treatment of major depression-pilot study. Clin Neurophysiology 2010; 121(4): e9−e10.
6. *Colombo C Benedetti F, Barbini B, Campori E, Smeraldi E*. Rate of switch from depression into mania after therapeutic sleep deprivation in bipolar depression. Psychiatry Res 1999; 86(3): 267−70.
7. *Xia G, Gajwani P, Muzina DJ, Kemp DE, Gao K, Ganocy SJ*, et al. Treatment emergent mania in unipolar and bipolar depression: focus on repetitive transcranial magnetic stimulation. Int J Neuropsychopharmacol 2008; 11(1): 119−30.
8. *Wu JC, Gillin JC, Buchsbaum MS, Schachat C, Darnall LA, Keator DB,* et al. Sleep deprivation PET correlations of Hamilton symptom improvement ratings with changes in relative glucose metabolism in patients with depression. J Affect Disord 2008; 107(1−3): 181−6.
9. *Smith DF, Jakobsen S*. Molecular tools for assessing human depression by positron emission tomography. Eur Neuropsychopharmacol 2009; 19(9): 611−28.
10. *Kimbrell TA, Dunn RT, George MS, Danielson AL, Willis MW, Repella JD*, et al. Left prefrontal-repetitive transcranial magnetic stimulation (rTMS) and regional cerebral glucose metabolism in normal volunteers. Psychiatry Res 2002; 115(3): 101−13.
11. *American Psychiatric Association*. Diagnostic and statistic manuel of mental disorders. 4th ed. Text revision. Washington, DC: American Psychiatric Association; 2000.
12. *Pascual-Leone A, Rubio B, Pallardo F, Catala MD*. Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. Lancet 1996; 348(9022): 233−7.
13. *Giedke H, Schwarzler F*. Therapeutic response of sleep deprivation in depression. Sleep Med Rew 2002; 6(5): 361−77.
14. *Rachid F, Bertschy G*. Safety and efficacy of repetitive transcranial magnetic stimulation in the treatment of depression: a critical appraisalof the last 10 years. Neurophysiol Clinique 2006; 36(3):157−83.
15. *Hirschfeld RM, Lewis L, Vornik LA*. Perceptions and impact of bipolar disorder: how far have we really come? Results of the national depressive and manic-depressive association 2000 survey of individuals with bipolar disorder. J Clin Psychiatry 2003; 64(2): 161−74.
16. *Nedjat S, Folkerts HW*. Induction of a reversible state of hypomania by rapid-rate transcranial magnetic stimulation over the left prefrontal lobe. J ECT 1999; 15(2): 166−8.
17. *Ella R, Zwanzger P, Stampfer R, Preuss UW, Muller-Siecheneder F, Moller HJ,* et al. Switch to mania after slow rTMS of the right prefrontal cortex. J Clin Psychiatry 2002; 63(3): 249.
18. *Hausmann A, Kramer-Reinstadler K, Lechner-Schoner T, Walpoth M, Rupp CI, Hinterhuber H*, et al. Can bilateral prefrontal repetitive transcranial magnetic stimulation (rTMS) induce mania? A case report. J Clin Psychiatry 2004; 65(11): 1575−6.
19. *Fitzgerald PB, Brown TL, Marston NA, Daskalakis ZJ, De CA, Kulkarni J*. Transcranial magnetic stimulation in the treatment of depression: a double-blind, placebo-controlled trial. Arch Gen Psychiatry 2003; 60(10): 1002−8.
20. *Sakkas P, Mihalopoulou P, Mourtzouhou P, Psarros C, Masdrakis V, Politis A*, et al. Induction of mania by rTMS: report of two cases. Eur Psychiatry 2003; 18(4): 196−8.

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CASE REPORT

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Renal dysplasia with the ipsilateral ectopic ureter mimicking abscess of the prostate

Renalna displazija sa ipsilateralnim ektopičnim ureterom koji oponaša apsces prostate

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Abstract

Introduction. In males the ectopic ureter usualy drains into the prostate (50%). During ureteric developement a thin membrane (Chawalla's membrane) separates the lumen of the ureter and the urogenital sinus at the point where the ureter joins the urogenital sinus. This membrane ruptures allowing urin to drain from the ureter to the urogenital sinus. The authors reported a case of renal dysplasia associated with ipsilateral uretral ectopia mimicking prostatic abscess. **Case report.** A subfebrile (37.3°C), 23-year-old patient, otherwise healthy, presented with persistent ascending perineal pain non-responsive to antibiotics and analgetics. Digitorectal examination (DRE) showed asymmetric prostate with a soft, tender, buldging left lobe suggestive of prostatic abscess. The diagnosis was suspected using transrectal ultrasonography (TRUS), but the picture of the anechoic tubular structure in the left lobe of the prostate with a proximal undefined extraprostatic extension and a caudal intraprostatic blind end was incoclusive for the definitive diagnosis of prostatic abscess. Magnetic resonance imaging (MRI) was ordered and definitive diagnosis of renal dysplasia associated with the ipsilateral ectopic ureter filled with inflamed content mimicking prostatic abscess was made. Transurethral incision/minimal resection of the distal, blindly closed end of left ectopic ureter was done. Endoscopic surgical treatment was sufficient for relief of clinical symptoms. The patient's recovery was uneventful. **Conclusion.** To the best of our knowledge, a case of renal dysplasia with the ipsilateral ectopic ureter mimicking prostate abscess has not been reported so far. Cystic pelvic malformations in males may result from too craniall sprouting of the ureteral bud, with delayed absorption and ectopic opening of the distal end of the ureter.

Key words:
abnormalities; kidney diseases; ureteral diseases; prostatitis; abscess; diagnosis, differential; urologic surgical procedures; treatment outcome.

Apstrakt

Uvod. Ektopični ureter kod muškaraca obično se drenira u prostatu (50%). Tokom razvoja uretera tanka membrana (Chawallia membrana) razdvaja lumen uretera i urogenitalni sinus na nivou spoja uretera i urogenitalog sinusa. Rupturom ove membrane dolazi do drenaže urina iz uretera u urogenitalni sinus. U radu je prikazan bolesnik sa renalnom displazijom i ektopijom ipsilateralnog uretera koji je otkriven u okviru diferecijalnodijagnostičke pretrage suspektnog apscesa prostate. **Prikaz bolesnika.** Supfebrilan (37,3°C) bolesnik star 23 godine, inače zdrav, žalio se na konstantan, intenzivirajuci bol u perineumu koji nije prolazio na antibiotsku i analgetsku terapiju. Digitorektalnim pregledom (DRP) nađena je simetrična prostata sa mekim, osetljivim, izdignutim levim lobusom što je izazvalo sumnju na postojanje apscesa prostate. Načinjena je transrektalna ultrasonografija (TRUS), ali slika tubularne formacija u levom lobusu prostate sa proksimalno nedefinisanom ekstraprostatičnom ekstenzijom i kaudalnim slepo zatvorenim intraprostatičkim krajem bila je inkonkluzivna za definitivnu dijagnozu apscesa prostate. Načinjeno je snimanje magnetnom rezonancom (MRI) kada je i postavljena definitivna dijagnoza renalne displazije sa ipsilateralnim ektopičnim ureterom, ispunjenim inflamiranim sadržajem koji daje lažnu sliku apscesa prostate. Načinjena je transuretralna incizija slepo zatvorenog distalnog kraja levog ektopičnog uretera. Endoskopsko hirurško lečenje bilo je dovoljno za prestanak tegoba. Oporavak bolesnika protekao je uredno. **Zaključak.** Pregledom literature nismo naišli na prikaz bolesnika sa renalnom displazijom i ipsilateralnim ureterom koji oponaša apsces prostate. Cistične malformacije u muškoj karlici mogu rezultirati kranijalno postavljenim ureteralnim pupoljkom, sa odloženom apsorpcijom i ektopičnom prezentacijom donjeg dela uretera.

Ključne reči:
anomalije; bubreg, bolesti; ureter, bolesti; prostatitis; apsces; dijagnoza, diferencijalna; hirurgija, urološka, procedure; lečenje, ishod.

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Introduction

Principles of ureter developement are little undestood. Ureters begin as a simple cuboidal epithelial tube with a formed lumen at 28 days of gestation. It is suggested that transient luminal obstruction occurs between the days 37 and 40 that recanalizes subsequently. The proces of recanalization starts in the mid ureter and extends cranially and caudally. Chawalla's membrane presents a two-cell thick layer over the ureteral oriffice. During ureteric developement a Chawalla's membrane separates the lumen of the ureter and the urogenital sinus. This membrane ruptures allowing urin to drain from the ureter to the urogenital sinus. In males, ectopic ureter usually drains into prostate (50%).

Case report

A subfebrile (37.3°C), 23-years-old patient, otherwise healthy, presented with persistent ascending perineal pain lasting for a week, non-responninsive to antibiotic and analgetics. His past history revealed 3 episodes of similar symptoms (although much less severe), with the first episode presented 4 years ago. In the past the patient would be typicaly treated like exacerbated chronic *prostatitis* [the diagnosis would be established based on anamnesis and laboratory tests – digitorectal examination (DRE) were not done at any time] by ciprofloxacin. The symptoms would disapeare on the standard antibiotic therapy. After anamnesis had been taken, physical examination was done. Physical examination of the abdomen and external *genitalia* as well as laboratory findings (urinalaysis, white blood cells – WBC, erythocyte sedimentation rate – SE) were unremarkable. Digitorectal examination showed the asymmetric prostate with a soft, tender, buldging left lobe mass with no discharge on massage. The diagnosis of possible prostate abscess was made and transrectal ultrasonography (TRUS) was done (Figure 1), but the defintive diagnosis was revealed by magnetic resonance imaging (MRI) (Figures 2).

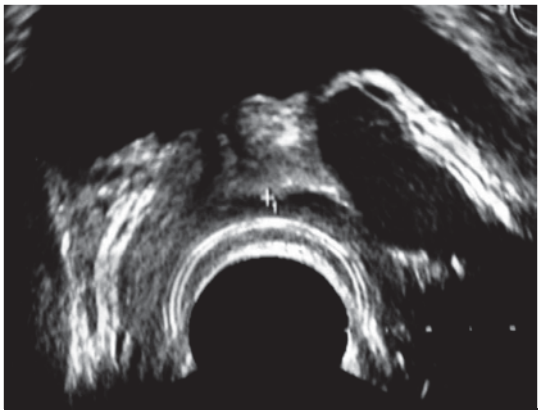


Fig. 1 – Transrectal ultrasound (TRUS) showing the anechoic tubular structure in the left lobe of the prostate with a proximal extraprostatic extension and a caudal intraprostatic blind end.

Urethrocystoscopy showed the asymmetric bladder trigone elevated on the left side (by the dilated distal part of the ectopic left ureter) with the missing left ureteral orifice. A paracolicular swelling on the left side (buldging of dilated, distal part of ectopic ureter) in the prostatic urethra (Figure 3a) was incised/minimaly resected, and was followed by turbid discharge from the ectopic ureter. A wide ectopic, dilated distal part of the left ureter was noticed (Figure 3b). Immidiately after incision/resection of the paracolicular area of the prostate, elevation of the bladder trigone disappeared.

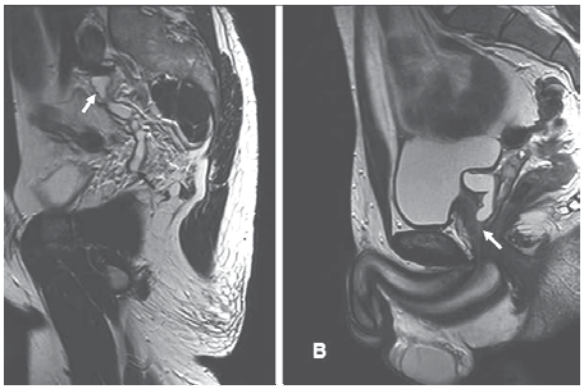
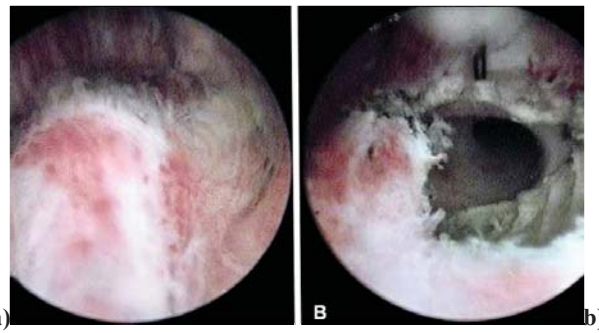


Fig. 2 – Magnetic resonance imaging (MRI) showing the small dysplastic kidney (white arrow) in the left retroperitoneum and the distended and convoluted ectopic ureter (black arrow).



a) b)

Fig. 3 – a) Endoscopic view of the prostatic urethra with the swelling on the left side above a verumontanum; b) Endoscopic view of the prostatic urethra after surgical incision of ectopic ureter.

After the procedure, upon waking up from general anesthesia, the patient was absolutely pain-free, requiring no analgesia at all. The patient was discharged from the hospital on the first postoperative day and was prescribed ciprofloxacin *per os* for 5 days. One month after the procedure, control cystoscopy was done and the same picture of intraoperative finding – a widely open ectopic, dilated distal part of the left ureter was seen. Digitorectal examination, physical examination of external *genitalia* as well as laboratory findings (urinalysis, urineculture, WBC, SE) were unremarkable.

Disscusion

To the best of our knowledge, there has been no previous report on renal dysplasia with the ipsilateral ectopic ureter mimicking prostatic abscess. Endoscopic prostate interventions can cause early and late postoperative complica-

tions such as: failure to void, urinary tract infections and transurethral resection syndrome [1, 2]. Pelvic cystic malformations in males may result from a too cranial sprouting of the ureteral bud with delayed absorption and ectopic opening of the distal end of the ureter. The symptoms are usually related to bladder or cyst distention or secondary to the obstruction of mesonephric duct derivations [3, 4]. The most probable embriological cause of blindly closed ureter is a persistent Chwalla membrane. It is physiologically seen between the weeks 37 and 39 of gestation, then it ruptures and allows normal drainage of urin [5, 6].

Conclusion

To the best of our knowledge, a case of renal dysplasia with the ipsilateral ectopic ureter mimicking prostate abscess has not been reported so far. Cystic pelvic malformations in males may result from too cranial sprouting of the ureteral bud, with delayed absorption and ectopic opening of the distal end of the ureter. A clinical algorythm consists of the history and physical exam, TRUS and MRI, and seems to be sufficient for the correct diagnosis.

R E F E R E N C E S

1. *Reich O, Gratzke C, Bachmann A, Seitz M, Schlenker B, Hermanek P*, et al. Morbidity, mortality and early outcome of transurethral resection of the prostate: a prospective multicenter evaluation of 10,654 patients. J Urol. 2008; 180(1): 246−9.
2. *Rassweiler J, Teber D, Kuntz R, Hofmann R.* Complications of transurethral resection of the prostate (TURP): incidence, management, and prevention. Eur Urol 2006; 50(5): 969−79.
3. *Mayersak JS*. Urogenital sinus-ejaculatory duct cyst: a case report with a proposed clinical classification and review of the literature. J Urol 1989; 142(5): 1330−2.
4. *MacDonald GR.* The ectopic ureter in men. J Urol 1986; 135(6): 1269−71.
5. *Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA.* Campbell-Walsh Urology. 10th ed. Philadelphia, Pa: WB Saunders; 2012.
6. *Gupta RK, Borwankar SS, Parelkar SV*. Ureteric valve: Case report with an insight into anatomy, embryology, presentation and management. Indian J Urol 2008; 24(4): 561−3.

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CASE REPORT

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Recurrent herpes zoster with segmental paresis and postherpetic neuralgia

Rekurentni herpes zoster komplikovan segmentnom parezom i postherpetičnom neuralgijom

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Abstract

Introduction. Postherpetic neuralgia and segmental paresis represent rare complications of herpes zoster infection. Recurrent herpes zoster is also rare and occurs within the first 3 years of the begining of the illness in only 1.4% of cases but it is generally higher in cases of chronic lymphatic leukemia (3.5%). **Case report.** We presented a patient with lymphatic leukemia who during the remission had 3 episodes of herpes zoster over a year. All of them took diffrent parts of the body. One of these episodes was complicated by postherpetic neuralgia and segmental paresis. A complete recovery was seen in all the three episodes. **Conclusion.** As immunosuppression is one of mechanisms of virus reactivation, it is likely associated with the described rare complications of herpes zoster.

Key words:
herpes zoster; leukemia, lymphocytic, chronic, b-cell; comorbidity; paresis; neuralgia; drug therapy.

Apstrakt

Uvod. Postherpetična neuralgija i segmentna zoster pareza neke su od ređih komplikacija herpes zostera. Rekurentni herpes zoster je, takođe, retkost a viđa se samo kod 1,4% bolesnika mada je nešto češći kod obolelih od hronične limfatične leukemije (3,5%). **Prikaz bolesnika.** Prikazali smo bolesnika sa hroničnom limfatičnom leukemijom koji je za godinu dana, u vreme remisije osnovne bolesti, imao tri epizode herpes zostera od kojih je jedna bila komplikovana segmentnom zoster parezom i postherpetičnom neuralgijom. Svako od ovih ispoljavanja zahvatalo je različite delove tela. Jedno od njih bilo je komplikovano postherpetičnom neuralgijom i segmentnom parezom. Kompletan oporavak usledio je posle svake od opisanih epizoda. **Zaključak.** Kako je imunosupresija mogući mehanizam reaktivacije virusa, moguće je da je upravo ona odgovorna i za pojavu navedenih retkih komplikacija herpes zostera.

Ključne reči:
herpes zoster; leukemija, limfocitna, hronična; komorbiditet; pareza; neuralgija; lečenje lekovima.

Introduction

Herpes zoster (HZ) is most commonly seen in older people and in cases of immunodeficiency. Despite higher incidence in immunocompromised patients, the recurrence of HZ is rare [1–5].

Postherpetic neuralgia is defined as the existence of pain at 4 to 6 weeks after vesicular skin eruptions resolve. Although postherpetic neuralgia is the most common complication of HZ, it occurs in only 5% to 10% of cases [6]. Typically, it resolves over time but some patients complain of persistent pain.

A 60 year-old patient with chronic lymphatic leukemia who in the remission developed 3 episodes of HZ within a year is presented.

Complications of HZ infection are rare. The most common is a postherpetic neuralgia and rarely segmental zoster paresis. Both complications occurred in the presented case.

Case report

A 60-year-old man with chronic lymphatic leukemia in remission developed in April 2010 an unpleasant burning pain along the inner side of the right lower leg, which progressed over time. The pain was scored 6 out of 10 on the pain scale. Herpetic rash developed within a week in the area of pain (Figure 1). Ten days after the initial pain, the patient had difficulty with moving the right foot, which prompted him to make an appointment. The neurological examination revealed a

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moderate to severe weakness of the right ankle plantar flexors and diminished ankle jerk (3/5 on MRC-scale).

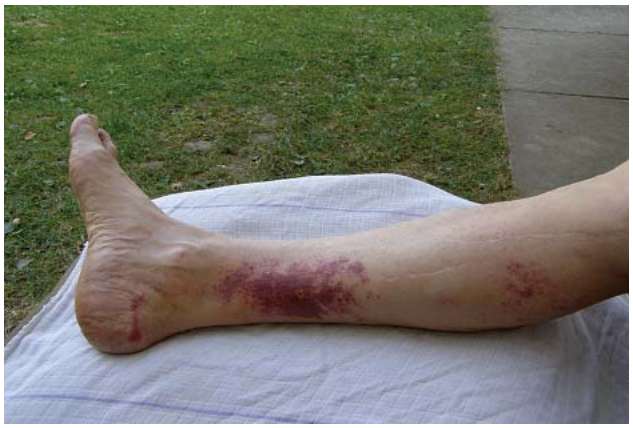

Fig. 1 – Herpetic rash along the inner lower leg.

Electrophysiological examination performed 4 weeks after the onset of symptoms did not reveal changes in the nerve motor conduction in the right leg. However, needle electromyography (EMG) at rest showed denervation potentials (fibrillations and positive sharp waves) in the right gastrocnemius muscle and paravertebral muscles innervated by S1 root. Voluntary activation during EMG examination revealed polyphasic motor unit potentials during activation of the right foot.

The magnetic resonance imaging (MRI) of the lumbosacral area showed only degenerative changes without other findings that could explain the neurologic deficits.

Testing serum (ELISA) on HZ virus was positive for IgM and IgG, which confirmed the presence of HZ infection.

The neurologic findings were ascribed to segmental herpes paresis.

The patient was referred to physical therapy and prescribed oral gabapentin (900 mg/day) for pain. The patient was on the same medication for 5 months because of severe pain, which was afterwards slowly decreased and eventually discontinued. Motor weakness completely resolved about 6 months after the onset of neurologic symptoms whereas the pain was sporadic but mild.

About 4 months after the first symptom appearance (August 2010), the patients noted new-onset burning pain in the right hand graded 8 out 10 on the pain scale. Five days later, the herpetic rash developed in the painful area. The patient did not complain of motor weakness in the right hand. The overall presentation was consistent with recurrence of HZ but without other neurological complications. Gabapentin was again prescribed for pain (1200 mg/day) but only for 6 weeks at which point the rash and pain disappeared (Figure 2).

Six months later (February 2011), the patient noted discomfort and increased skin sensation over the abdomen followed by burning pain (4/10). A few days later herpetic rash developed in the same area (Figure 3). There were no other symptoms or sign. Gabapentin was not prescribed this time considering mild pain. Pain and rash disappeared within 2 months.

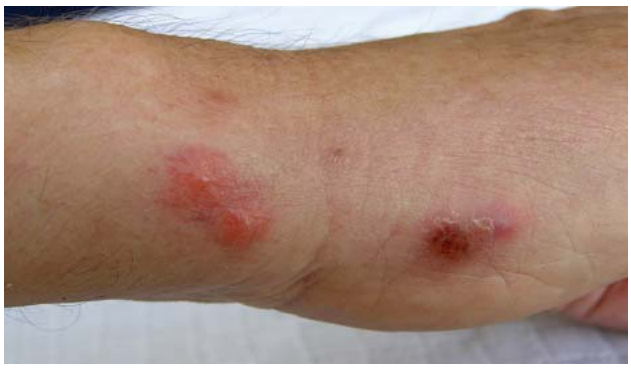

Fig. 2 – Herpetic rash in the hand area.

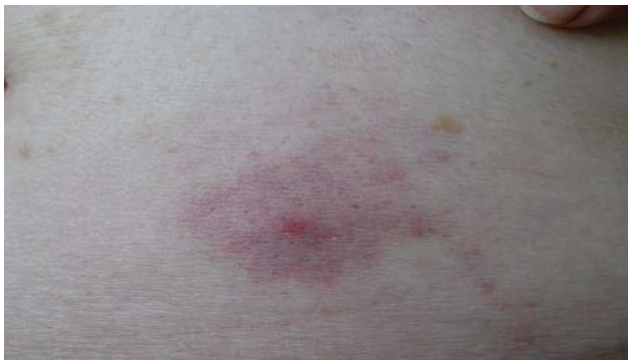

Fig. 3 – Herpetic rash over the abdomen.

Discussion

After primary infection, varicella zoster virus remains latent in the dorsal root ganglia for several years. The virus can get reactivated and migrate along the sensory nerves toward the skin innervated by the involved nerve. This results in HZ rash characterized by vesicular skin eruption accompanied by pain and dysesthesia or hyperesthesia corresponding to the affected dermatomes. Pain usually develops first followed by rash within a week of pain onset. The disease typically lasts 5–8 weeks [7]. In the presented case, pain preceded the onset of rash.

The mechanism of virus reactivation is unknown. In immunocompromised patients, it is likely associated with a decrease in the immune system, as in cases of lymphoma and AIDS [3]. The described patient had a chronic lymphatic leukemia that was in remission when the 3 HZ episodes occurred.

The annual incidence of HZ is 3.6/1,000 in general population or 300,000 new cases a year. Evidently higher incidence of HZ in 6 and 7 decades may be associated with a decrease in cellular rather than humoral immunity after 60 years of age [5]. The presented case belongs to a typical age group.

The recurrent HZ is rare and occurs within the first 3 years of the initial insult in only 1.4% of cases. The rate of recurrent infection is about 10–20% several decades later. Since the incidence of HZ is generally higher in cases of chronic lymphatic leukemia (28.6%), the recurrence rate is

also higher (3.5%) [3]. In the presented patient, 3 recurrences occurred within a year.

There are several approaches to managing post-herpetic neuralgia. Beginning treatment with antiviral agents as soon as the rash appears is considered to be the key for preventing the development of postherpetic neuralgia. Prescribing antiviral agents for a week within 3 days of rash (famcyclovir 500 mg tid or acyclovir 1 g tid), decreases pain and the incidence of postherpetic neuralgia [8–11]. Oral corticosteroids for 3 weeks (prednisone 60 mg/day in week 1,30 mg/day in week 2, and 15 mg/day in week 3), along with antiviral agents, can also reduce the overall severity and duration of pain, and thereby reduce the incidence of postherpetic neuralgia 11, 12. However, it is not clear whether the mechanism of corticosteroid action is local or systemic [10].

The second most common complication of HZ is segmental motor paresis, which occurs in 0.5% to 5% of patients [12, 13]. Although the pathophysiology of muscle weakness remains unknown, the overlap between segmental paresis and dermatomal area affected by rash suggests that HZ virus spreads from the dorsal ganglia toward anterior spinal roots [2, 4]. This is supported by histological findings typical for HZ in the anterior roots. Moreover, the absence of fasciculations in the affected muscles on EMG and slowing of motor conduction velocities suggest axonal lesions rather than the affection of the anterior horn of the spinal cord. Weakness typically develops within 2 weeks of rash, however, it may also precede pain [7, 13, 14]. The possible invasion of the anterior roots by the HZ virus was first considered by Wohlwill in 1924 [15]. In the described case, muscles paresis and the distribution of skin lesions were in the tibial nerve distribution.

In terms of neurophysiology, EMG shows denervation in the presence of motor weakness. Usual EMG findings include fibrillation potentials, PDP, and prolonged insertion activity whereas fasciculations are typically absent. Polyphasic motor unit potentials are present upon attempts of voluntary activation of weak muscles. This is typically seen in myotomes that correspond to dermatomes affected by pain and herpetic eruption. In addition, EMG may reveal a prolonged terminal latency, slowing of motor conduction velocity, occasionally absent motor response, or prolonged motor latency of the affected nerve or decrease in amplitude [16]. In the presented case, there was a denervation activity in the muscles innervated by the tibial nerve in which distribution was also present the skin lesions.

HZ more often affects proximal than distal portion of the arms. Although zoster paresis seems more common in the upper limbs, it was present in this case in the lower limbs but not in the upper limb that was later affected by the skin lesions.

The third episode of HZ affected the abdominal skin but it was not accompanied by weakness of the abdominal muscles or post-herpetic neuralgia in this region. The outcome of zoster weakness is generally good. Complete or almost complete recovery within one year occurs in 2/3 of patients [1, 5], whereas additional 9% has a partial recovery.

Recovery takes place between 1 and 2 years [17, 10]. Segmental zoster paresis treatment includes analgesics for pain control, physical therapy, and prevention of contractures [10, 18]. The presented case was treated in such a way and the degree and timing of recovery is in agreement with the literature.

Conclusion

The presented case with chronic lymphatic leukemia is unique because of 3 recurrent episodes of HZ skin eruptions in different body regions over the course of several months. Since only the first episode was accompanied by motor weakness, it appears that an earlier affliction of the motor nerve is not a risk factor for developing nerve lesions in the other area in case of HZ recurrence.

REFERENCES

1. *Akiyama N.* Herpes zoster infection complicated by motor paralysis. J Dermatol 2000; 27(4): 252−7.
2. *Fabian VA, Wood B, Crowley P, Kakulas BA.* Herpes zoster brachial plexus neuritis. Clin Neuropathol. 1997; 16(2): 61−4.
3. *Hamblin AD, Hamblin TJ.* The immunodeficiency of chronic lymphocytic leukaemia. Br Med Bull 2008; 87: 49−62.
4. *Hanakawa T, Hashimoto S, Kawamura J, Nakamura M, Suenaga T, Matsuo M.* Magnetic resonance imaging in a patient with segmental zoster paresis. Neurology 1997; 49(2): 631−2.
5. *Hoque R, Schwendimann RN, Liendo C, Chesson AL Jr.* Brachial neuritis with bilateral diaphragmatic paralysis following herpes zoster: a case report. J Clin Neuromuscul Dis 2008; 9(4): 402−4.
6. *Krause PR, Straus SE.* Zoster and its complications. Hosp Pract (Off Ed) 1990; 25(10A): 61−71, 75−6.
7. *Wendling D, Langlois S, Lohse A, Toussirot E, Michel F.* Herpes zoster sciatica with paresis preceding the skin lesions. Three case-reports. Joint Bone Spine 2004; 71(6): 588−91.
8. *Jackson JL, Gibbons R, Meyer G, Inouye L.* The effect of treating herpes zoster with oral acyclovir in preventing postherpetic neuralgia. A meta-analysis. Arch Intern Med 1997; 157(8): 909−12.
9. *Smith KJ, Roberts MS.* Antiviral therapies for herpes zoster infections. Are they economically justifiable? Pharmacoeconomics 2000; 18(2): 95−104.
10. *Schuchmann JA, McAllister RK, Armstrong CS, Puana R.* Zoster sine herpete with thoracic motor paralysis temporally associated with thoracic epidural steroid injection. Am J Phys Med Rehabil 2008; 87(10): 853−8.
11. *Whitley RJ, Weiss H, Gnann JW Jr, Tyring S, Mertz GJ, Pappas PG,* et al. Acyclovir with and without prednisone for the treatment of herpes zoster. A randomized, placebo-controlled trial. The National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Ann Intern Med 1996; 125(5): 376−83.
12. *Yawn BP, Saddier P, Wollan PC, St Sauver JL, Kurland MJ, Sy LS.* A population-based study of the incidence and complication rates of herpes zoster before zoster vaccine introduction. Mayo Clin Proc 2007; 82(11): 1341−9.

13. *Ter Meulen BC, Rath JJ*. Motor radiculopathy caused by varicella zoster virus without skin lesions ('zoster sine herpete'). Clin Neurol Neurosurg 2010; 112(10): 933.
14. *Umehara T, Sengoku R, Mitsumura H, Mochio S*. Neurological picture. Findings of segmental zoster paresis on MRI. J Neurol Neurosurg Psychiatry 2011; 82(6): 694.
15. *Wohlwill F*. Zur pathologischen Anatomie des Nervensystems beim Herpes zoster. (Auf Grund von zehn Sektionsfällen.) Z Ges Neurol Psychiat 1924; 89(1): 171−212.
16. *McAllister RK, Borum SE, Mitchell DT, Bittenbinder TM*. Thoracic motor paralysis secondary to zoster sine herpete. Anesthesiology 2002; 97(4): 1009−11.
17. *Kawajiri S, Tani M, Noda K, Fujishima K, Hattori N, Okuma Y*. Segmental zoster paresis of limbs- report of three cases and review of literature. Neurologist 2007; 3(5): 313−7.
18. *Sachs GM*. Segmental zoster paresis: an electrophysiological study. Muscle Nerve 1996; 19(6): 784−6.

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LETTER TO THE EDITOR/PISMO UREDNIKU UDC: 616.12-02-079.4(044.4)

Assessment of cardiomyopathies presenting with myocardial infarction-like clinical syndrome

(A comment on the article: *Djurić I, Obradović S, Gligić B*. Dynamics of electrocardiographic changes, brain-natriuretic peptide and cortisol levels in a patient with stress (takotsubo) cardiomyopathy: A case report. Vojnosanit Pregl 2013; 70(5): 511−5, and authors' reply)

Procena kardiomiopatija koje se javljaju sa kliničkim sindromom sličnim infarktu miokarda

(Komentar o članku: *Djurić I, Obradović S, Gligić B*. Dynamics of electrocardiographic changes, brain-natriuretic peptide and cortisol levels in a patient with stress (takotsubo) cardiomyopathy: A case report. Vojnosanit Pregl 2013; 70(5): 511−5, i odgovor autora)

Comment:

We read with great interest the article by Djurić et al. [1] entitled "Dynamics of electrocardiographic changes, brain-natriuretic peptide and cortisol levels in a patient with stress (takotsubo) cardiomyopathy – A case report", which was published in the previous issue of Vojnosanitetski Pregled.

These authors [1] presented a case of takotsubo cardiomyopathy (TCM) with the evaluation of echocardiographic and electrocardiographic changes, levels of brain natriuretic peptide (BNP) and cortisol. They concluded that TCM should be considered in emotionally stressed patients with transient-apical akinesis or dyskinesis of the LV as a differential diagnosis of acute anterior myocardial infarction (MI) and should be discriminated from MI by early coronary angiography. Although, the current study is valuable and gives detailed information about TCM and the dynamic changes of the prominent electrocardiographic findings, echocardiography parameters, BNP and cortisol levels during a follow-up period in a patient with severe stress cardiomyopathy, some comments may be of interest.

Takotsubo cardiomyopathy is a cause of reversible left ventricular systolic dysfunction and preceded by stress or exacerbation of an existing medical condition [2]. It presents with myocardial infarct-like clinical syndrome, electrocardiographic abnormalities and elevation of cardiac biomarkers. It usually tends to normalise approximately in a week and results in angiographically normal coronary arteries [2].

In patients with electrocardiographic changes and elevated cardiac biomarkers, further evaluation should be planned in detail to detect the etiology and manage spesific treatment. In determining the etiology of cardiac wall motion abnormality, coronary angiography can be used as a beneficial imaging tool. Coronary angiography has a less power to identify the etiology of cardiac wall motion abnormalities related to myocarditis, coronary vasospasm and spontaneous coronary dissection, which could be seen especially in postmenopausal women related to hormonal disturbances [3, 4]. When etiology remains unclear, cardiac magnetic resonance (CMR) appears to be a useful imaging modality for establishing the diagnosis and differentiating cardiomyopathies from each other, even in patients with angiographically normal coronary arteries [2, 5]. On CMR imaging, myocardial infarction is characteristic in subendocardial late gadolinium enhancement (LGE), which extends variably transmurally to the epicardium, whereas TCM is characteristic in no or minimal LGE [2].

Establishing the exact etiology of electrocardiographic changes and elevated cardiac biomarkers will help in managing the patients and their therapies, and increase the strength of the studies.

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REFERENCES

1. *Djurić I, Obradović S, Gligić B.* Dynamics of electrocardiographic changes, brain-natriuretic peptide and cortisol levels in a patient with stress (takotsubo) cardiomyopathy: A case report. Vojnosanit Pregl 2013; 70(5): 511−5.
2. *Quarta G, Sado DM, Moon JC.* Cardiomyopathies: focus on cardiovascular magnetic resonance. Br J Radiol 2011; 84(3): 296−305.
3. *Xu B, Jelinek VM, Hare JL, Russell PA, Prior DL.* Recurrent Myocarditis - An Important Mimic of Ischaemic Myocardial Infarction. Heart Lung Circ 2013; 22(7): 517−22.
4. *Konstantinov IE, Saxena P, Shehatha J.* Spontaneous multivessel coronary artery dissection: surgical management in a postmenopausal woman. Tex Heart Inst J 2009; 36(4): 360−1.
5. *Ivanović B, Tadić M, Maksimović R, Orbovic B.* Could it have been better? A patient with peripartum cardiomyopathy treated with conventional therapy. Vojnosanit Pregl 2012; 69(6): 526−30.

Authors' reply:

The article-letter is an interesting and valuable comment on our paper published several months ago in the *Vojnosanitetski pregled* (Vojnosanit Pregl 2013; 70(5): 511−5). MRI of the heart is an useful diagnostic tool in patients presented with chest pain, ST segment elevation in ECG, positve troponin, abnormalities in left ventricle wall motion and coronary angiography without significant atherosclerotic or thrombotic lesions. However, in the particular case, significant emotional stress preceeded the event, ECG elevation was conquave and not typical Pardee, troponin was relativlely low and had prologed plato, angio showed only a mildly slower flow through the LAD and the most important – echocardiography imaging was typical for Takotsubo. Cardiac MRI in our Institution is not avaliable, but even if we had that option, we would not do such examination for our patient, except for the scientific and curiosity reasons.

Ivica Djurić, Slobodan Obradović, Branko Gligić
Clinic for Emergancy Internal Medicine, Military Medical Academy, Belgrade, Serbia

PRIKAZ KNJIGE/
BOOK REVIEW

Naslov: Dr Emerih P. Lindenmajer – život i delo

Urednici: Prof. dr Brana Dimitrijević i dr Zoran Vacić

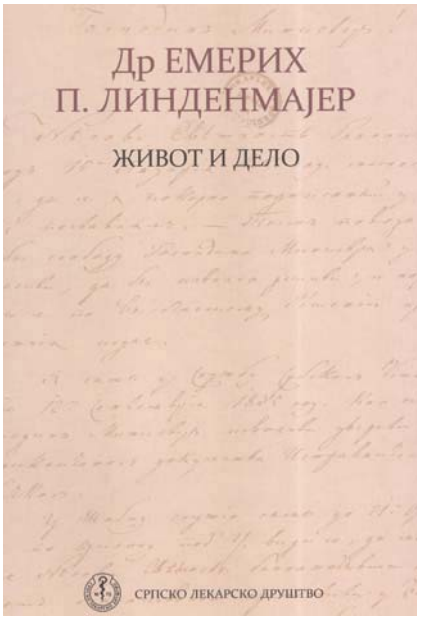
Izdavač: Srpsko lekarsko društvo

Mesto i godina izdanja: Beograd, 2013

Štampa: Poligraf, Beograd

Format 72 str; 20 cm

ISBN: 978-86-6061-040-1

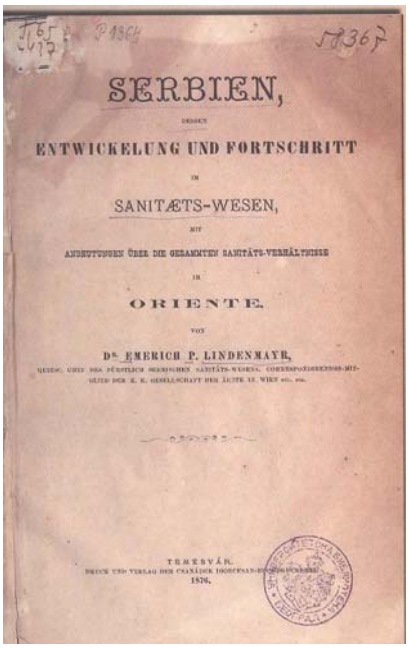


„Ne postoji ličnost u istoriji srpske medicine, koja je – u novije doba – više uradila za Srbiju, a o kojoj se manje zna. Bio je doktor gvardijskog špitalja, doktor Glavnog štaba Vojnog saniteta u Popečiteljstvu vnuterni dela, Načelnik karantinskog otdjelenija sa sanitetom, Načelnik zajedničke vojne i građanske službe u sanitetu Kneževine Srbije. Napisao je knjigu 'Srbija, njen razvitak i napredak u sanitetu, s beleškama o celokupim sanitetskim odnošajima na Istoku' (Serbien, dessen Entwicklung und Fortschritt im Sanitätswesen mit Andeuntungen über die Gesammten Sanitätsverhäaltnisse im Oriente), koju je štampao u Temišvaru 1876. na nemačkom jeziku. Ličnog života nije imao, ni lik mu se ne zna, ni grob. To je dr Emerih Lindenmajer (Emmerich Lindenmayer, 1806–1883)!" piše u knjizi, koju ćemo prikazati, prof. dr Snežana Veljković.

Odista, teško je nabrojati, čak i sada 130 godina posle smrti, sve zasluge ovog izuzetno čestitog i svom poslu sasvim odanog trudbenika, koji je punih četrnaest godina (1845–1859) bio načelnik Sanitetskog odeljenja Ministarstva unutrašnjih dela Kneževine Srbije, i u tom periodu dao niz neprocenjivih doprinosa. Njegovo, već pomenuto, memoarsko delo, koje po svojoj strukturi više liči na kakav zakonik, u kome čak i kada pominje samoga sebe, uvek govori u trećem licu, kao da mu je bezmalo neprijatno što se i to što iznosi mora reći, je, po rečima dr Vladimira Stanojevića, prva naša istorija medicine.

Zamisao o knjizi „Dr Emerih P. Lindenmajer – život i delo", i održavanju naučnog skupa povodom 130 godina od smrti pomenutog zaslužnika, potiču od člana predsedništva Sekcije za istoriju medicine Srpskog lekarskog društva (SLD) dr Zorana Vacića, uz to i jednog od njenih urednika, čija su znanja i iskustva nekadašnjeg vlasnika izdavačke kuće *Infinitas*, doprinele izgledu same knjige, a koja je po svojoj prirodi zbornik radova. Knjiga ima 69 stranica teksta, uz skroman broj ilustracija, mahom dokumenata, i fotografije naslovnih strana već pomenutih Lindenmajerovih memoara (Slika 1), i malo poznate njegove knjige „Opis mineralnih i lekovitih voda" štampane i objavljene u Beogradu 1856. godine (Slika 2). Štampana su u celini četiri rada, ne računajući „Uvodnu reč" predsednika Sekcije za istoriju medicine SLD prof. dr Brane Dimitrijevića, takođe, jednog od urednika.

U tekstu g. Ljubodraga Popovića, nekadašnjeg direktora Arhiva Srbije, „Emerih Lindemajer u Kneževini Srbiji – radni vek jednog trudbenika" izneto je u pogledu osnovnih biografskih podataka dr Emeriha Lindenmajera i njegovog kretanja u službi sve ono što se u tom pogledu od arhivskih dokumenata danas može naći. Pa, tako po prvi put imamo neku vrstu vodiča, nezaobilaznog, nadamo se, za sve buduće istra-



Sl. 1 – Naslovna strana knjige dr Emerika Lindenmajera "Srbija, njen razvitak i napredak u sanitetu, s beleškama o celokupnim sanitetskim odnošajima na Istoku", štampana 1987. god. u Temišvaru.

Sl. 2 – Naslovna strana knjige dr Emerika Lindenmajera "Opis mineralnih i lekovitih voda", objavljene u Beogradu 1856. god.

živače. Naredni tekst prof. dr Brane Dimitrijevića, pod naslovom „Majstor iz Nemačke", bavi se analizom već pomenutih Lindenmajerovih memoara „Srbija, njen razvitak i napredak u sanitetu, s beleškama o celokupim sanitetskim odnošajima na Istoku", pokušajem da se objasne razlozi i motivi njenog nastanka, a zatim, kao ilustracijom „upotrebne vrednosti" ove knjige, analizom statističkih podataka o sastavu i strukturi „lekarskih snaga" (Lindemajerov izraz) tokom prvih deset godina postojanja srpskog saniteta (1839–1849): dolazaka, ostanaka, ali i odlazaka (pa i umiranja) doktora medicine, magistara hirurgije i „upotrebljivih empiričara". Rad dr Zorana Vacića „Dr Emerih Lindenmajer i razvoj banja Kneževine Srbije (1836–1859)" otkriva nam dr Lindenmajera kao velikog istraživača mineralnih i lekovitih voda u Srbiji, i začetnika balneologije kod nas. Rad prof. dr Snežane Veljković „Srpski posinak" (naslov je uzet iz oproštajne reči dr Vladana Đorđevića nad otvorenim grobom dr Emeriha Lindenmajera) pokušava da osvetli poreklo porodice Lindenmajer. Ovaj rad skladno dopunjuje odgovore na pitanje: zašto je dr Emerih Lindenmajer „uprkos svemu ostao u Srbiji i tu proveo sav svoj život"? postavljene još u radu prof. dr Brane Dimitrijevića.

Ali to je, naglasimo, još jedna od vrednosti ove knjige: ključna pitanja povodom života i rada dr Emeriha Lindenmajera, u kojima se gdekad upliću i psihološke analize Lindemajerovog karaktera, bolje reći njegove čestitosti, i ključni odgovori povodom toga, mogu naći u sva četiri rada, pri čemu jedan autor dopunjava onog drugog ili trećeg.

„Zato sam ponosna što sam (bila) deo tima koji vam je predstavio dr Emeriha Lindenmajera po drugi put među Srbima." Završava svoj rad prof. dr Snežana Veljković. Jer i to je („Dr Emerih Lindenmajer po drugi put među Srbima") lasno mogao biti naslov ove knjige, koja je istovremeno i knjiga otrežnjenja i opomene, koja se barem u ovom slučaju ne tiče jedino Srba i njihove navodne nezahvalnosti spram velikana sopstvene prošlosti, već i – Nemaca.

Tragajući, naime, za nevelikim materijalnim sredstvima kako bi se štampao zbornik radova o dr Emerihu Lindenmajeru, predsednik Sekcije za istoriju medicine SLD obratio se i Gete institutu u Beogradu, i otud dobio munjevit odgovor „da pomaganje štampanja takvih knjižica i brošura nije predviđeno njihovim pravilnikom". Šta bi tek bilo da je tom istom institutu ponuđeno da pomogne štampanje već prevedenog dela njihovog, ipak, zemljaka, a pod naslovom „Srbija, njen razvitak i napredak u sanitetu, s beleškama o celokupim sanitetskim odnošajima na Istoku"?

Kakogod, 2014. godine navršava se 175 godina od „novog uređenja Srbije" (dr Emerih Lindenmajer), od Ustava iz 1838. godine i osnivanja Sanitetskog odeljenja Ministarstva unutrašnjih dela, naredne, 1839. Hoće li i ta godišnjica biti prepuštena hrabroj i neuništivoj Sekciji za istoriju medicine Srpskog lekarskog društva (čiji sam, s ponosom ističem, i ja dolepotpisana član) ili će se tome pridružiti i nadležne državne institucije, pod uslovom da postoje, ostaje da se vidi.

U svakom slučaju, ovaj zbornik radova „Dr Emerih P. Lindenmajer – život i delo" predstavlja snažan bljesak istraživačkih napora koji poput munje osvetljava ondašnju srpsku prošlost, dopirući svojom svetlošću i do najudaljenijeg njenog kutka. Sve je odjednom vidljivo, jasno kao na dlanu. A, naročito – putokazi...

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I N M E M O R I A M

prof. dr MIOLJUB - MIĆA KUŠIĆ pukovnik u penziji (1924–2013)

U Beogradu je 18. 03. 2013. godine preminuo pukovnik i redovni profesor Vojnomedicinske akademije (VMA) u penziji dr Mioljub - Mića Kušić. Preko 25 godina stvarao je i podizao ugled VMA po kome je ova ustanova nadaleko poznata.

Rođen je u Novom Sadu, u porodici intelektualca (otac, Milenko, inženjer hemije koju je zaršio u Tuluzu u Francuskoj, gde ga je poslala vlada Kraljevine Srbije sa Krfa).

Osnovnu školu i gimnaziju završio je u Novom Sadu i Beogradu sa odličnim uspehom i oslobađanjem od mature.

Kao dvadesetogodišnjak, dobrovoljno je stupio u redove Narodnooslobodilačke vojske (18. 11. 1944. godine) i učestvovao u proboju Sremskog fronta i oslobođenju zemlje. Kao vojni stipendista studije medicine započeo je 10. 10. 1947. godine i završio u rekordnom roku (24. 8. 1952. godine) sa izvanrednim uspehom (srednja ocena tokom studija 9,33). Lekarski staž završava u vojnim bolnicama u Sarajevu i Beogradu.

Kao mladi lekar radi u trupi, u 17. Oklopnoj diviziji u Kragujevcu gde ostaje nepune četiri godine. Iz Kragujevca je upućen na specijalizaciju iz interne medicine u Vojnu bolnicu Beograd. U toj ustanovi radi od 31. 8. 1958. do 6. 11. 1962. Na kraju je sa odličnim uspehom položio i specijalistički ispit iz interne medicine, kao i supspecijalistički ispit iz gastroenterologije, posle čega biva premešten u Opšte interno i gastroenterološko odeljenje Klinike za unutrašnje bolesti VMA u kome je radio od 20. 9. 1963. do 16. 4. 1973. godine.

Kao vrsni lekar specijalista postavljen je za ličnog lekara tadašnjeg predsednika Republike i na toj dužnosti ostaje do 5. 3. 1976. godine (sa predsednikom je obišao više zemalja Evrope, Azije i Amerike). Sa te dužnosti se vraća na rad u VMA i obavlja dužnost zamenika načelnika Klinike za unutrašnje bolesti tokom 6 godina (od 9. 8. 1978. do 20. 6. 1984. god). Nakon toga postavljen je za načelnika Klinike i na toj dužnosti ostaje do prerastanja kliničkih odeljenja u samostalne klinike. Kao načelnik Klinike za unutrašnje bolesti VMA obavljao je i dužnost glavnog terapeuta Jugoslovenske narodne armije (JNA).

Sa dužnosti načelnika Klinike za unutrašnje bolesti, raspoređen je na dužnost predsednika Glavne vojnolekarske komisije na kojoj će ostati do penzionisanja 31. 12. 1989. godine.

Za sve vreme svog stručnog rada i razvoja prof. dr Mioljub Kušić je istraživao i pisao. To je radio i za vreme specijalizacije iz gastroenterologije, kada je napisao velik broj radova iz te oblasti. Svoje stručne radove prof. Kušić je najviše objavljivao u Vojnosanitetskom pregledu, ali i u drugim poznatim domaćim i međunarodnim medicinskim časopisima.

Osim gastroenterologije, u fokusu njegovog interesovanja bila je i kardiologija i, upravo iz te oblasti, odbranio je doktorski rad pod nazivom „Kliničke i epidemiološke karakteristike hiperlipoproteinemija grupe vojnih starešina Beogradskog garnizona“. Značaj ove disertacije je u tome što je dokazano da starešine između 41. i 50. godina starosti, kod kojih su prisutni još neki faktori rizika kao što su arterijska hipertenzija, latentni ili manifestni dijabetes i

gojaznost, predstavljaju populaciju sa ozbiljnim rizikom od pojave koronarne bolesti – infarkta srca.

Visoka stručnost, uz objavljivanje većeg broja naučnih i stručnih radova razlog su što je dr Kušić biran najpre za docenta, zatim vanrednog i na kraju redovnog profesora VMA.

Sve radne i stručne ocene bile su mu najviše, što se odrazilo i na veći broj priznanja i odlikovanja, od kojih sedam ordena i sedam medalja. Među njima su najviši Orden za vojne zasluge, najviši Orden rada, Orden bratstva i jedinstva i Orden zasluga za narod kojim je odlikovan još za vreme rata.

Svojim veoma toplim, ljudskim i blagorodnim stavom i odnosom prema bolesnim i zdravim ljudima stvorio je ogroman broj prijatelja i poštovalaca, ne samo u Beogradu, već i u mnogim mestima širom nekadašnje Jugoslavije.

Za sve što je ostavio iza sebe, što je uradio u struci i nauci, kao i za odnos prema njemu najvažnijem, pacijentima, neka mu je večna slava i hvala.

dr Milić Todosijević,
pukovnik u penziji

prim. dr Branislav Popović
general-major u penziji, bivši načelnik Sanitetske uprave i predsednik Glavne vojnolekarske komisije

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Poziv za reklamiranje u 2014. godini

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

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

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

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

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MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs**.** Avoid the use of colors in graphs.

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Preparation of manuscript

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

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

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

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

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

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

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Examples of references:

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

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

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

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

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Each table should typed double-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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

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

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

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Tabele

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