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ACUTE LOWER GASTROINTESTINAL BLEEDING

AKUTNA KRVARENJA IZ DONJIH PARTIJA GASTROINTESTINALNOG TRAKTA

Tatiana JOCIĆ, Olgica LATINOVIĆ BOŠNJAK, Ljiljana HADNAĐEV, Dragomir DAMJANOV, Željka SAVIĆ and Tihomir ORLIĆ

Summary

Introduction. Acute lower gastrointestinal bleeding accounts for approximately 20% of all acute gastrointestinal hemorrhages, and they are the most common urgent cases in gastroenterology. The aim of this study was to determine the most common etiology, efficacy in diagnostics and therapy, and the outcome in patients with acute lower gastrointestinal bleeding. **Material and Methods.** Data were collected from the medical records of 86 patients who had been hospitalized for acute lower gastrointestinal bleeding in 2009 at the Ward of Gastroenterology and Hepatology, Clinical Centre of Vojvodina. **Results.** The average age of the patients was 70.4 years (ranging from 37 to 88), and the largest number of patients 41/86 (47.7%) were between the ages 71 and 80. Colon diverticulosis was the most common cause of bleeding, and it occurred in 21 patients from the study sample (24.4%), and the other causes were malignant tumors (12/86, i.e. 13.9%), polyps (10/86, i.e. 11.6%), anorectal diseases (7/86, i.e. 8.3%) and colitis (8/86, i.e. 9.3%). No diagnostic procedures were performed in 15 patients (17.4%) due to their poor medical condition and comorbidities. The total mortality rate was 6/86 (6.9%), and the largest number of deaths occurred (5/86 i.e. 5.8%) due to a multisystem organ failure and underlying diseases which were not associated with acute lower gastrointestinal bleeding. Uncontrolled bleeding was the cause of death in only 1 patient (1.2%). **Conclusions.** Acute lower gastrointestinal bleeding is most commonly found in the older population, whose age, comorbidities, and ongoing therapy have impact on bleeding lesions, diagnostic and therapeutic modalities and the outcomes of bleeding. Endoscopic procedures are still the gold standard in diagnostics.

Key words: Gastrointestinal Hemorrhage; Lower Gastrointestinal Tract; Diagnosis; Treatment Outcome; Diverticulum; Colonoscopy

Sažetak

Uvod. Akutna krvarenja iz donjih partija gastrointestinalnog trakta čine oko 20% uzroka svih akutnih gastrointestinalnih krvarenja koja predstavljaju najčešća urgentna stanja u gastroenterologiji. Cilj ove studije bio je da se utvrde najčešća etiologija, efikasnost u dijagnostici i terapiji, te ishod bolesti kod bolesnika sa akutnim krvarenjem iz donjih partija gastrointestinalnog trakta. **Materijal i metode.** Podaci su dobijeni iz istorija bolesti 86 bolesnika koji su bili hospitalizovani zbog akutnog krvarenja iz donjih partija gastrointestinalnog trakta u jednogodišnjem periodu, tokom 2009. godine, na Klinici za gastroenterologiju i hepatologiju, Kliničkog centra Vojvodine. **Rezultati.** Prosečna starost bolesnika iznosila je 70,4 godina (od 37 do 88), a najveći broj bolesnika 41/86 (47,7%) pripada starosnoj grupi od 71 do 80 godina. Divertikuloza kolona predstavljala je najčešći uzrok krvarenja 21/86 (24,4%), a ostali uzroci bili su maligniteti kolona 12/86 (13,9%), polipi 10/86 (11,6%), bolesti anorektalne regije 7/86 (8,1%) i kolitisi 8/86 (9,3%). Kod 15/86 (17,4%) bolesnika nisu sprovođene nikakve dijagnostičke procedure sa ciljem identifikacije uzroka krvarenja zbog teškog opšteg stanja i komorbiditeta. Ukupan mortalitet kod bolesnika sa akutnim krvarenjem iz donjih partija gastrointestinalnog trakta iznosio je 6/86 (6,9%), a najveći broj smrtnih ishoda 5/86 (5,8%) posledica je multisistemske organske insuficijencije i pridruženih bolesti koji nisu povezani sa krvarenjem. Samo kod 1/86 (1,2%) bolesnika uzrok letalnog ishoda bio je recidiv krvarenja. **Zaključak.** S obzirom da se akutna krvarenja iz donjih partija gastrointestinalnog trakta češće javljaju kod starije populacije, samo životno doba, prisutni komorbiditeti i terapija utiču na vrstu krvareće lezije, mogućnost dijagnostike i ishod bolesti. I dalje zlatni standard u dijagnostici predstavljaju endoskopske procedure.

Ključne reči: Gastrointestinalna krvarenja; Donji gastrointestinalni trakt; Dijagnoza; Ishod lečenja; Divertikulum; Kolonoskopija

Introduction

Acute lower gastrointestinal tract (GIT) bleeding accounts for approximately 20% of causes of all acute gastrointestinal hemorrhage. Annual incidence is 20–27 cases per 100,000 adults in western countries [1]. The source of bleeding is located distally to the ligament of Treitz, and is more com-

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Abbreviations
GIT – gastrointestinal tract
NSAIDs – non-steroid anti-inflammatory drugs
CT – computed tomography

monly found in older population (63 to 77 years of age), which affects the type of bleeding lesion. Its clinical manifestations may range from mild self-limiting bleeding to massive life-threatening hemorrhage. The mortality rate, which is up to 5% among these patients, depends on the severity of bleeding, advanced age and the presence of comorbidities [2]. After the initial evaluation of the severity of bleeding and resuscitation of the patient, the diagnostic and therapeutic approach remains the challenge for any doctor. It has to be individualized because it depends on a number of factors, such as etiology, localization and the amount of bleeding and the time of admission to the hospital. Special attention should be paid to comorbidities, coagulopathies and the use of anticoagulant and antiaggregation therapies. Identifying the cause of bleeding may be difficult as most hemorrhages stop spontaneously or the bleeding is intermittent [2–4]. In the first encounter with the patients with lower gastrointestinal bleeding it is essential to recognize those suffering from severe bleeding and should therefore be hospitalized (over the age of 60, presence of comorbidities, signs of hemodynamic instability and massive hemorrhage, use of non-steroid anti-inflammatory drugs (NSAIDs) and/or aspirin) [4].

The most common causes of lower gastrointestinal bleeding were colonic diverticulosis (17-40%), colonic vascular lesions (2–30%), colitis, which could be ischemic, post-irradiation, and infectious, inflammatory bowel diseases (9–21%), malignant colon tumors (11–14%), anorectal causes – hemorrhoidal disease, fissures, rectal varices (4-10%), other causes – lesions in the small intestine, Meckel's diverticulum, post-polypectomy bleeding and other (2–9%). In 0 to 11% of the patients the cause of bleeding was in the proximal GIT [1, 5, 6]. The diagnostic procedures performed failed to reveal the cause of hemorrhage in approximately 8% of cases [7].

Endoscopic procedures are the methods of choice in diagnostics. Taking in consideration the fact that 80% of causes of all GIT hemorrhages are located proximally to Treitz ligament, esophagogastroduodenoscopy is recommended to be performed first, especially in the patients with hemodynamic instability, history of an ulcerous disease and those taking ulcerogenic drugs. Early colonoscopy (examination performed within 24 hours after the first occurrence of bleeding) helps reveal the cause of hemorrhage in as many as 90% of patients when it is possible to perform some of the endoscopic methods of hemostasis. If the examination is conducted at a later time, the sensitivity and specificity of colonoscopy will be reduced to 45% [8]. However, no significant difference in the outcome of the disease was recorded between urgent and delayed colonoscopy although hospitalization period was shorter when the examination was performed earlier [9]. Angiography and radionuclide methods are only used in cases of massive bleeding when colonoscopy is not feasible or in cases of recurrent hematochezia when colonoscopy has failed to determine the cause of bleeding [5, 10, 11]. If the diagnostic procedures performed fail to determine the source of bleeding in the colon, various methods of small intestine exploration follow (enteroclysis, enteroscopy, videoendoscopic capsule, Meckel diverticulum scintigraphy). Surgical treatment should be considered in the cases where bleeding persists despite all measures taken, in patients who

Table 1. Patients' General Data
Tabela 1. *Osnovni podaci bolesnika*

Number of hospitalized patients/*Broj hospitalizovanih bolesnika*	86
Mean age/*Prosečna starost*	70.4 years/*godine*
Male gender/*Muški pol*	52.3%
Bleeding manifestation/*Prezentacija krvarenja:*	red/*crvena* 60 (69.7%) maroon/*bordo* 18 (21%) black/*crna* 8 (9.3%)
Abdominal pain/*Bol u trbuhu*	44.2%
Mean duration of hospitalization/*Prosečna dužina hospitalizacije*	14.2 days/*dana*
Use of medications/*Upotreba lekova:* Oral anticoagulants/*Oralni antikoagulansi* Antiaggregational therapy/*Antiagregaciona terapija* Dual therapy/*Dvojna terapija* NSAIDs/*NSAIL**	6 21 1 18
Fatal outcome/*Smrtni ishod*	6
Number of re-hospitalized patients due to bleeding relapse *Broj rehospitalizovanih zbog recidiva krvarenja*	2

*NSAIL – nesteroidni antiinflamatorni lekovi

Table 2. Endoscopic Results of Patients who Underwent Colonoscopy
Tabela 2. *Endoskopski nalazi bolesnika kojima je rađena kolonoskopija*

Colonoscopy *Kolonoskopija*	Number of patients *Broj bolesnika*	% of 54 patients who underwent colonoscopy/*% od 54 bolesnika kojima je rađena kolonoskopija*
Not performed/*Nije rađena*	32	
Diverticulosis with bleeding/*Divertikuloza sa krvarenjem*	7	12,9
Diverticulosis without bleeding stigmata *Divetikuloza bez stigmata krvarenja*	13	24,1
Malignant colon tumors/*Maligni tumori kolona*	12	22,2
Colitis/*Kolitis*	8	14,8
Polyps/*Polipi*	6	11,1
Anorectal diseases/*Anorektalna oboljenja*	6	11,1
Other/*Ostalo*	2	3,7

have received more than 6 blood units during the first 24 hours and in those who have had bleeding relapse. The purpose of preoperative diagnostics is to avoid extensive surgical intervention ("blind colectomy") and to confirm that the hemorrhage originates from the lower GIT [2].

The study was aimed at determining the most common etiology, diagnostic and therapeutic efficacy and the outcome of disease in patients with acute lower gastrointestinal bleeding.

Material and Methods

The research was conducted as a retrospective study including patients hospitalized due to acute lower gastrointestinal bleeding at the Ward of Gastroenterology and Hepatology over the period of one year, 2009. The data used for the study were obtained from the medical records.

The diagnosis of acute lower gastrointestinal bleeding at admission was based on the anamnesis data and physical examination of the patients by means of digitorectal examination, which provided the data on the clinical manifestation of bleeding. Medical history provided the data on previous diseases that could have been potential causes of bleeding, comorbidities, ongoing anticoagulant and antiaggregation therapies, usage of NSAIDs and diagnostic procedures performed. Diagnostics was not performed in a certain number of patients due to their general condition and present comorbidities, and consequently, the cause could not be established. Some of the patients had been hospitalized more than once due to hematochezia, and since their etiology in most cases had been known from before, they were discharged from the hospital after the bleeding stopped and substitution was administered without repeating the diagnostic procedures. The patients who underwent colonoscopy had the time from the moment of admission to hospital to the moment of examination calculated, and this was also taken in consideration when calculating the complete duration of hospitalization.

Results

During the period of one year (2009), 86 patients were treated at the Ward of Gastroenterology and Hepatology of the Clinical Center of Vojvodina due to acute lower gastrointestinal bleeding. The mean age of the patients was 70.4 years (ranging from 37 to 88), and the majority of patients (41/86 or 47.7%) were in the age group from 71 to 80 years.

Only 11/86 (12.8%) patients did not have comorbidities, while comorbidities increased in other patients with their increasing age. The most common underlying diseases were cardiovascular diseases, cerebrovascular insult, diabetes, chronic obstructive pulmonary disease and chronic renal insufficiency. Out of 75 patients, 55 (73.2%) with comorbidities belonged to the age group from 61 to 80 years. Anticoagulant and antiaggregation therapy was taken by 28/86 (32.6%) patients, almost half of whom were in the age group from 71 to 80. Eighteen patients had previously used NSAIDs (21.3%) **(Table 1).**

Colonoscopy was performed in 54 (62.8%) patients who were admitted due to bleeding. Total colonoscopy was performed in half of them (28/86 or 51%). On average, colonoscopy was performed on the seventh (7.8) day upon admission to the hospital **(Table 2)**.

Several potentially bleeding lesions were found in 8/54 patients.

Out of 54 colonoscopies, urgent colonoscopy was performed in two patients. Seven colonoscopies were performed on the second day upon admission, and two on the third day. Twenty examinations were performed between the fourth and seventh day of hospitalization, and the rest of them after the seventh day (in 23 patients). The success rate was 100% in colonoscopies performed on the first day (in two patients who had bleeding diverticula and hemorrhagic colitis, each). The colonoscopies performed on the second day revealed bleeding lesions or ones that had signs of recent

Table 3. Final Diagnoses at Discharge from the Hospital
Tabela 3. *Konačne dijagnoze pri otpustu bolesnika*

Diagnosis/*Dijagnoza*	Number of patient *Broj bolesnika*	%
Colonic diverticulosis/*Divertikuloza kolona*	21	24.4
Unknown bleeding lesion/*Nepoznata krvareća lezija*	15	17.4
Malignant colon tumors/*Maligni tumori kolona*	12	13.9
Anorectal disease (hemorrhoids, fissures)/*Anorektalna bolest (hemoroidi, fisure)*	7	8.1
Colitis (IBD, hemorrhagic, ischemic)/*Kolitisi (IBD, hemoragijski, ishemijski)*	8	9.3
Bleeding lesion in proximal GIT/*Krvareća lezija u proksimalnom GIT-u*	6	6.9
Colonic polyps/*Polipi kolona*	10	11.6
Colonic angiodisplasia/*Angiodisplazije kolon*a	6	6.9
Aortoenteric fistula/*Aortoenterična fistula*	1	1.2

IBD – inflammatory bowel disease/*zapaljenske bolesti creva;* GIT – gastrointestinal tract/*gastrointestinalni trakt*

bleeding in six out of seven patients. These data should be taken with reserve because total colonoscopy was performed in five patients, and in the other two only partial examination of the colon was done. There was no need for endoscopic hemostasis in these patients.

Esophagogastroduodenoscopy, which was performed in 53/86 (61.6%) patients, revealed that 6/53 (11.3%) patients who had undergone endoscopy had an actively bleeding lesion in the proximal parts of the GIT (3 patients had peptic ulcer, 2 had bleeding esophagus varicosities, and one patient had submucous tumor of the duodenum). On average, esophagogastroduodenoscopy was performed 3.6 days after admission to hospital.

Irigography, which was performed in 10 patients, was the only possible method of colon examination in three patients. The results revealed diverticulosis in five patients, negative results in two, highly suspected infiltration in two and a benign polyp in one patient. No radionuclide examination was performed in 2009. Computed tomography (CT) angiography, which was performed in three patients, revealed a lesion in two patients, whereas one patient had no signs of active bleeding during the examination. As for the other diagnostic procedures conducted in order to find the causes of gastrointestinal bleeding, the following were performed: x-ray examination of gastroduodenum (five patients) and small intestine (seven patients) with negative results, anoscopy in four patients (revealing noduli haemorrhoidales interni erosivi in three patients and an anal fissure in one patient).

The most common cause of bleeding manifested as maroon stool was diverticulosis in 7/18 patients (39%), angiodisplasia as black stool in 3/8 patients (37.5%), and benign and malignant colon tumors as red stool in 18/60 patients (30%).

In 78/86 (90.7%) of the hospitalized patients, the bleeding stopped spontaneously upon taking supportive and substitution therapeutic measures. One patient underwent urgent surgery on the first day of hospitalization due to bleeding relapse (urgent colonoscopy revealed actively bleeding diverticula, which was confirmed by the results of angiography). The outcome was fatal in one patient after bleeding relapse on the seventh day of hospitalization (arteriographically suspected aortoenteric fistula). Comorbidities and grave general condition, due to which no diagnostic procedures had been performed to determine the cause of bleeding, resulted in fatal outcome in five patients.

Bleeding relapse occurred in 14/86 patients (16.3%), one of whom suffered fatal outcome.

Only 5/86 patients (5.8%) belonged to the age group below 60. The bleeding lesions diagnosed in those patients were anorectal lesions (2 patients), malignant colon tumor (one patient), inflammatory bowel disease and colonic polyposis in one patient, each **(Table 3).**

Discussion

The one-year study analyzed the clinical manifestations and the most common causes of lower gastrointestinal bleeding, the diagnostic approaches and outcomes of the disease.

Lower gastrointestinal bleeding is more common among older population. The mean age of the patients studied was 70.4 years, the majority of them (41/86, or 47.7%) being in the age group between 71 and 80, and only 5/86 (5.8%) were below 60 years of age. Only 11/86 (12.8%) patients had no comorbidities, while the number of comorbidities increased in other patients with their increasing age, resulting in a higher rate of use of aspirin, anticoagulant therapies and NSAIDs (46/86, or 53.5%). No significant difference between the genders was found.

The exploration of the upper GIT is recommended especially in patients with history of previous ulcerous diseases, usage of potentially ulcerogenic drugs and in case of massive bleeding. Esophagogastroduodenoscopy was performed in 53/86 (61.6%) patients. Active bleeding lesion in the proximal GIT was found in 6/53 patients (11.3%). These data indicate the importance of es-

ophagogastroduodenoscopy in the diagnostics of patients who were initially hospitalized with the symptoms of lower gastrointestinal bleeding. This is also shown by the literature data, according to which the causes of bleeding proximal to Treitz's ligaments were found in 0-11% patients [1, 6, 7].

Colonoscopy was performed in 54/86 (62.8%) patients. Early colonoscopy is considered to be safe and useful because it ensures successful diagnosis and the application of some of the endoscopic hemostasis procedures decreasing the risks of complications. However, due to the advanced age of the subjects and underlying morbidities which affect the possibility and quality of preparation for examination, as well as the diagnostic procedure itself, total colonoscopy was performed only in a half of the study sample. This considerably reduced the specificity and sensitivity of the procedure itself. Early colonoscopy reduces the duration of hospitalization [12, 13].

The success of determining the bleeding lesions was 100% when colonoscopy was performed on the first day (2 patients – bleeding diverticula and hemorrhagic colitis). The colonoscopies performed on the second day upon admission revealed bleeding lesions or ones with signs of recent bleeding in 6 out of 7 patients. Colonoscopy that was performed later in eight patients revealed several potentially bleeding lesions.

In the cases where examination procedure showed the signs of recent bleeding, the diagnosis was easily determined. However, when colonoscopy performed upon the cessation of bleeding reveals diverticulosis without other potentially bleeding lesions, it can be assumed as the cause of bleeding only to a certain extent [6, 8].

Colonic diverticulosis is the most common cause of bleeding (21/86 or 24.4%), which is in line with most literature data (17–40%). According to the literature, angiodisplasia accounts for 2/30% of lower gastrointestinal bleeding. In our research it was the cause of bleeding in 6/86 (6.97%) patients [6, 14]. The other significant causes were colon malignities 12/86 (13.9%), benign colon tumors 10/86 (11.6%), anorectal diseases 7/86 (8.1%), and colitis 8/86 (9.3%). Fifteen out of /86 (17.4%) patients were discharged with the diagnosis of enterorrhagia because no diagnostic procedures had been performed to determine the cause of hemorrhage due to their general condition and comorbidities.

The most common cause of bleeding manifested as maroon stool was diverticulosis (7/18 or 39%), black stool was most frequently caused by angiodisplasia (3/8 or 37.5%) and red stool by benign and malignant colon tumors (18/60 or 30%).

In 78/86 (90.7%) patients, bleeding stopped spontaneously upon the administration of supportive and substitution therapy (infusion solutions and transfusions administered in order to maintain hemodynamic and hematological stability). One patient (1.2%) underwent urgent surgery on the first day because of active incontrollable bleeding from colonic diverticulum, and 10/86 (11.6) patients were operated on for malignancy within the elective program. Bleeding relapse was recorded in 14 patients (16.3%), while 3/86 (3.5%) patients had bleeding relapse within 3 months from the previous bleeding (angiodisplasia, diverticulosis).

Total mortality rate in patients with acute lower gastrointestinal bleeding was 6/86 (6.9%), which is higher in comparison with the mortality rates in a number of published studies (2.9%-3.6%) [6]. The highest rate of fatal outcomes (5/86 or 5.8%) was the consequence of multisystem organ insufficiency and associated diseases, which were not related to the hemorrhage. Fatal outcome resulted from disseminated malignant diseases (lungs and bladder) and the acute myocardial infarction in two patients and in one patient, respectively, whereas the cause of fatal outcome in two other patients was cardiac decompensation. Bleeding relapse was the cause of fatal outcome in 1/86 (1.16%) patient only.

Conclusion

Since acute lower gastrointestinal bleeding usually occurs in older population, the age itself, the presence of comorbidities and the ongoing therapies affect the type of bleeding lesion, diagnostic modalities and outcomes. The most common causes of hemorrhage are diverticulosis, malignancies and diseases of anorectal region, although it has to be noted that a significant number of patients remain undiagnosed. Endoscopic procedures remain to be the gold standard of diagnostic procedures (esophagogastroduodenoscopy and colonoscopy). Early colonoscopy has much higher sensitivity and specificity compared to the delayed one, although it seldom affects the outcome of the disease because the bleeding stops spontaneously in majority of cases and mortality rate is related to comorbidities.

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LOCALLY DERIVED SYSTEM FOR CARDIAC OPERATIVE RISK EVALUATION

LOKALNI SISTEM ZA EVALUACIJU OPERATIVNOG RIZIKA U KARDIOHIRURGIJI

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Summary

Introduction. During the last two decades, many authors have found that European Systems for Cardiac Operative Risk Evaluation (additive and logistic models) overestimate the risk in cardiac surgery. The new European model has recently been introduced as an update to previous versions. The aim of the study was to investigate the significance of locally derived system for cardiac operative risk evaluation and to compare its predictive power with the existing European systems. **Material and Methods.** For developing a local risk prediction model, data from 2681 patients submitted to cardiac surgery at the Institute of Cardiovascular Diseases Vojvodina have thoroughly been collected. Logistic regression analysis was used to construct a local model for prediction of outcome. The evaluation of the local model and three European systems was performed by comparing the observed and expected hospital mortality. **Results.** The difference between the predicted and observed mortality regardless of the type of surgery was statistically insignificant for the additive European system (p=0.073) and the local model (p=0.134). The logistic European system overestimated the operative risk, while the new European model underestimated mortality. In coronary surgery, all models, except the logistic European system, performed well. In valvular surgery, the new European model and the local model underestimated mortality significantly, while the additive and logistic European models performed well. In combined surgery, the new European system significantly underestimated mortality (p=0.029), while the local model performed well (p=0.252). **Conclusion.** The locally derived model shows satisfactory results, with good calibration and discriminative power. The local model specifically outperforms all other European systems in terms of discriminatory power in combined surgery subset.

Key words: Risk Assessment; Thoracic Surgery; Mortality; Treatment Outcome

Sažetak

Uvod. U poslednje dve decenije mnogi autori su ustanovili da Evropski sistemi za evaluaciju operativnog rizika (aditivni i logistički modeli) precenjuju rizik u kardiohirurgiji. Nedavno je uveden novi Evropski model za procenu rizika. Cilj rada bio je da se proceni značaj lokalno razvijenog modela za evaluaciju operativnog rizika u kardiohirurgiji i uporedi njegova moć predviđanja operativnog rizika sa postojećim Evropskim modelima. **Materijal i metode.** Prikupljeni su podaci za 2 681 bolesnika koji su operisani u Institutu za kardiovaskularne bolesti Vojvodine. Za konstrukciju modela korišćena je logistička regresiona analiza. Evaluacija lokalnog modela i tri Evropska sistema, ostvarena je upoređivanjem očekivanog i stvarnog mortaliteta. **Rezultati.** Razlika između očekivanog i stvarnog mortaliteta za čitavu grupu operisanih bolesnika nije bila statistički značajna za aditivni Evropski (p = 0.073) i lokalni model (p = 0.134).Logistički sistem je precenio, a novi Evropski model potcenio operativni rizik. U koronarnoj hirurgiji, svi modeli, osim logističkog, funkcionisali su dobro. U valvularnoj hirurgiji, novi Evropski sistem i lokalni model značajno su potcenili operativni rizik a aditivni i logistički su bili dobri. U kombinovanoj hirurgiji, novi Evropski sistem značajno je potcenio operativni rizik (p = 0.029) a lokalni model nije pokazao značajnu razliku između očekivanog i stvarnog mortaliteta (p = 0.252). **Zaključak.** Lokalni model za evaluaciju operativnog rizika u kardiohirurgiji pokazuje dobru kalibraciju i ima dobru diskriminativnu moć. U poređenju sa Evropskim sistemima, najprihvatljiviji je za oblast kombinovane hirurgije.

Ključne reči: Procena rizika; Grudna hirurgija; Mortalitet; Ishod lečenja

Introduction

An important component of modern cardiac surgery practice is the one of data recording, collection, and analysis for the purpose of assessing and improving the quality of service, surgical decision-making and preoperative patient education (informed consent) [1]. The outcome of a disease or surgery, in terms of survival, is obviously of great importance not only for the patient and his family but for his doctor as well [2]. Mortality is only one of the determinants of the success of an intervention. Numerous risk models for predicting postoperative mortality following a major cardiac surgery are commonly used, one of the more popular being the European System for Cardiac Operation Risk Evalu-

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Abbreviations

EuroSCORE	– The European System for Cardiac Operation Risk Evaluation
VojvodinaSCORE	– locally derived system for cardiac operative risk evaluation
ROC	– receiver operating characteristic
AUC	– the area under the curve

ation (EuroSCORE) [3]. The EuroSCORE, in its both additive and logistic forms, has been used extensively over the last decade for the outcome prediction and hospital performance benchmarking with a new iteration of EuroSCORE having been recently presented: the EuroSCORE II [4]. Being a model developed from a large multinational cohort, the EuroSCORE II might therefore be considered a reference group incorporating different levels of outcomes. Such risk-scoring systems provide accurate prediction when the preoperative patients' characteristics and treatment profiles are comparable with those on which the system was based.

For comprehensive assessment of the role of any risk prediction model and confirmation of its applicability in contemporary cardiac surgery practice, an external validation is mandated. Moreover, the external validation is needed to assess the service provided by the specific hospital, which should be aligned with the "gold standard," that is, the EuroSCORE II. In situations where there is no acceptable alignment between the results (prediction) produced, one has the choice to: 1) continue to use one of the standard risk prediction models being aware of all the constraints; 2) adjust (recalibrate) the model; 3) develop a local model for accurate risk prediction.

In our previous paper, we advocated the development of self-made model for a number of reasons [2]. A self-made model can usually handle input data (specific patient profile, constraints and advantages of healthcare environment) more reliably yielding better risk estimation.

Since the patient profile, as well as the quality of service provided, can significantly differ among institutions and geographical areas, we sought to develop a local outcome prediction model for cardiac surgery given all the specifics of the local population as well as customized healthcare system. Our hypothesis was that a locally derived model would provide a higher level of discrimination and would be better calibrated for the Serbian population than the models derived from other populations.

The aim of the study was to investigate the significance of locally derived system for cardiac operative risk evaluation (the VojvodinaSCORE) and to compare its predictive power with the existing European systems.

Material and Methods

For the purpose of developing a local risk prediction model, data from 2681 patients submitted to cardiac surgery at the Institute of cardiovascular diseases Vojvodina (during the period from July 2011 to December 2013) have been thoroughly collected. For each patient, 53 variables were recorded and then used in statistical analyses. The evaluation of the VojvodinaSCORE and three EuroSCORE iterations (the additive, logistic and EuroSCORE II) was performed by comparing the observed and expected hospital mortality. Data were collected prospectively and analyzed retrospectively. The study was approved by the Institutional Review Board.

Three separate models (one for each surgery subtype: coronary, valvular, combined surgery) were created using binary logistic regression. The models were developed on a cohort of 1792 consecutive patients, and then validated on a cohort of 889 patients. After the validation process, the models were compacted into a single unified model named the VojvodinaSCORE.

Descriptive statistical data were compared between the groups by using either the Pearson $\chi 2$ test or Fisher exact test. Continuous variables were compared between the groups by using the unpaired Student t-test or the Wilcoxon rank-sum test (depending on the normality of the distribution). A p-value of less than 0.05 was considered to be significant.

The calibration of models was assessed by using the Hosmer-Lemeshow test. A well-calibrated model gives a p-value greater than 0.05. The model discrimination was tested by means of receiver operating characteristic (ROC) curves calculating the area under the curve (AUC) – an index which was used to assess how well the model could discriminate between survivors and non-survivors. The accuracy and clinical performance of the VojvodinaSCORE was tested in the patient subgroups based on the type of cardiac operation, and the subgroups based on risk categorization.

The statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, Illinois, United States) and MedCalc for Windows, version 12.2.1 (MedCalc Software, Mariakerke, Belgium).

Results

The difference between the predicted and observed mortality regardless of the type of surgery was statistically insignificant for the additive EuroSCORE (p=0.073) and the VojvodinaSCORE (p=0.134) (**Table 1**). The logistic EuroSCORE overestimated the observed mortality, while the EuroSCORE II underestimated mortality. The highest AUC was observed for the EuroSCORE II model, with the value of AUC not being significantly dispersed between the models.

In coronary surgery, all models except the logistic EuroSCORE performed well. The difference between the predicted and observed mortality according to the logistic EuroSCORE was significant (p=0.045), while it was not significant concerning other three models (**Table 1).** Again, the EuroSCORE

Table 1. Cardiac operative risk evaluation using four different models
Tabela 1. *Evaluacija kardiohirurškog operativnog rizika korišćenjem četiri različita modela*

Cardiac Surgery	Model *Model*	Predicted mortality *Očekivani mortalitet*	Observed mortality *Stvarni mortalitet*	P-value *P-vrednost*	Auroc *Auroc*	Sensitivity *Senzitivnost*	Specificity *Specifičnost*
Total *Ukupno*	Additive EuroSCORE *Aditivni EuroSKOR*	4.51	3.3	0.073	0.744	76%	64.9%
	Logistic EuroSCORE *Logistički EuroSKOR*	4.78	3.3	0.034	0.738	68%	72.9%
	EuroSCORE II *EuroSKOR II*	1.86	3.3	0.002	0.769	72%	75.4%
	Vojvodina SCORE *Vojvodina SKOR*	2.48	3.3	0.134	0.759	72.4%	69.6%
Coronary surgery *Koronarna hirurgija*	Additive EuroSCORE *Aditivni EuroSKOR*	3.83	2.2	0.064	0.780	77.8%	62.6%
	Logistic EuroSCORE *Logistički EuroSKOR*	4.00	2.2	0.045	0.775	77.8%	74.3%
	EuroSCORE II *EuroSKOR II*	1.62	2.2	0.282	0.827	77.8%	74.6%
	Vojvodina SCORE *Vojvodina SKOR*	2.29	2.2	0.376	0.796	72.7%	72.3%
Valvular surgery *Valvularna hirurgija*	Additive EuroSCORE *Aditivni EuroSKOR*	5.00	4.1	0.518	0.784	75%	61.7%
	Logistic EuroSCORE *Logistički EuroSKOR*	5.23	4.1	0.427	0.793	75%	61.7%
	EuroSCORE II *EuroSKOR II*	1.87	4.1	0.010	0.792	75%	83.8%
	Vojvodina SCORE *Vojvodina SKOR*	2.19	4.1	0.042	0.706	62.5%	71.1%
Combined surgery *Kombinovana hirurgija*	Additive EuroSCORE *Aditivni EuroSKOR*	5.95	5.3	0.735	0.554	–	–
	Logistic EuroSCORE *Logistički EuroSKOR*	6.61	5.3	0.516	0.541	–	–
	EuroSCORE II *EuroSKOR II*	2.52	5.3	0.029	0.610	–	–
	Vojvodina SCORE *Vojvodina SKOR*	3.57	5.3	0.252	0.752	75%	60.8%

II yielded highest AUC (0.827) with AUC being lower than 0.8 for other three models.

In valvular surgery, the EuroSCORE II and the VojvodinaSCORE significantly underestimated mortality, while the additive and logistic EuroSCORE performed well (**Table 1**). AUC for the VojvodinaSCORE was around 0.7, which is substantially lower when compared to other three models (around 0.8)

In combined surgery, the difference between the predicted and observed mortality according to the additive EuroSCORE, logistic EuroSCORE and VojvodinaSCORE was not significant, while the EuroSCORE II underestimated mortality significantly (p=0.029). Discriminative power was satisfactory only for the VojvodinaSCORE (AUC – 0.752).

Discussion

Preoperative risk prediction models have a critical role in current cardiac surgical practice and the use of risk models to risk-stratify patients appropriately is well established [5, 6]. Choosing the most reliable model among many other models raises a question about how good the model really is in terms of effectiveness in relation to other models. In our previous studies [7–10], we analyzed the predictive value of the EuroSCORE model in coronary surgery, as well as the trends of risk factors included in the EuroSCORE model. It was observed that the profile of coronary patients undergoing surgery in one of the cardiac surgery centers is drastically changing primarily due to the significantly advanced percutaneous techniques for myocardial revascularization. In spite of the differences in the patients' characteristics between our databases and that of the EuroSCORE, the latter performed quite well in our databases.

The EuroSCORE, in its both additive and logistic form, has been extensively used over the last decade for the outcome prediction and hospital performance benchmarking. It is generally believed

that the model shows a good level of accuracy, with a C-statistic of around 0.75 to 0.80, but could use an improvement or recalibration especially for high-risk patients [11]. The internal validation of the EuroSCORE II shows an improved C-statistic compared with the previous logistic EuroSCORE model ($C\text{-}Index_{EuroSCOREII} = 0.81$ vs. $C\text{-}Index_{EuroSCORE} = 0.78$) and good calibration (Hosmer– Lemeshow $\chi 2 = 15.48$; $p = 0.0505$) [3]. Risk models are most valid in patient populations where the preoperative patients' characteristics and treatment protocols are comparable with those of the original environments. That is why a model should not be used elsewhere as such before its validity has been tested in the local patient material [12].

The majority of the cardiac surgery centers perform less than 1500 cardiac surgical procedures annually. Surgeons in these institutions have three possibilities: 1) to apply and use some of the existing models ("ready-made" model); 2) to recalibrate the existing model by defining new coefficients for specific factors (recalibrate); 3) to develop a completely new local model based on their experience calibrated in relation to their patient population (remodel) [13]. The later solution offers the best possibility to achieve adequate accuracy and good distinctive features of the model. Certain clinical factors, which have not been taken into account within the EuroSCORE, might have significant impact on the postoperative outcome and therefore potentially important morbidity and prognostic information may be missing. Thus, a well-calibrated model specifically designed to predict the risk for patients undergoing different cardiac surgery procedures would be applicable to improve patient selection and plan the care for high-risk patients more efficiently [14].

Due to the known constraints of ready-made models and somewhat limited results in our population published in the previous papers [2, 7, 8, 10], we have decided to create a local model. This endeavor has been implemented through support of the Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (Serbia) (Grant number 114–451–2131/2011). The main goal that we wanted to achieve was to provide our patients with the most accurate information on what their operative risk was and to provide the hospital with the most accurate tool for clinical benchmarking. Our intention was also to promote the VojvodinaSCORE as a national model for outcome prediction in cardiac surgery. Unfortunately, the idea was not accepted by others [15]. In an era of transcatheter aortic heart valve replacement when indications of valve replacement are heavily based on risk scores, the importance of having a score that accurately predicts the outcome is of paramount importance. We believe that we have developed the model capable to serve this purpose (again in conjunction with the EuroSCORE II). Clinical benchmarking and comparison of the results with other hospitals around the world is extremely important and only possible through standardized models such as the EuroSCORE. However, certain risk factors, not included in the EuroSCORE, have significant impact on the postoperative outcome.

The main limitation of our model is the relatively low sample size on which it was developed and tested. Therefore, other (external) validation would be warranted. The model reflects the experience of a single healthcare institution and may not represent national and international practice and outcomes, which may lead to a potential bias and results requiring further examination with a large number of patients across the multicenter database.

Conclusion

The latest iteration of the European System for Cardiac Operation Risk Evaluation outperforms earlier iterations in terms of discriminatory power, although it universally underestimates the risk. The Vojvodina System for Cardiac Operation Risk Evaluation –a locally derived model – shows satisfactory results, with good calibration and discriminative power. The locally derived model specifically outperforms all other European Systems for Cardiac Operation Risk Evaluation in terms of discriminatory power in combined surgery subset, where the European Systemse believe that the locally derived model would be of great use in everyday clinical practice since it faithfully illustrates the actual state of patient population of the region where it was developed.

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TERMINAL REMISSION IS POSSIBLE IN SOME PATIENTS WITH JUVENILE MYOCLONIC EPILEPSY WITHOUT THERAPY

TERMINALNA REMISIJA BEZ TERAPIJE MOGUĆA JE KOD BOLESNIKA SA JUVENILNOM MIOKLONIČNOM EPILEPSIJOM

Nebojša J. JOVIĆ, Ana KOSAĆ and Miloš D. BABIĆ

Summary

Introduction. Juvenile myoclonic epilepsy is considered to be a chronic disease requiring lifelong antiepileptic treatment. The aim of this study was both to identify factors predicting the kind of seizure control and to investigate the outcome in patients after therapy withdrawal. **Material and Methods.** The study included 87 patients (49 female, 38 male), aged from 17.5 to 43.5 years, referred to our Department between 1987 and 2008, with the seizure onset at the age of 14.3±2.9, and followed up for 13.3±5.8 years on average (from 5 to 23 years). **Results.** Sixty seven (77.0%) patients were fully controlled; whereas 13.8% had persistent seizures and 9.2% showed pseudoresistance. The combination of three seizure types and focal electroencephalogram features were independent factors of poor seizure control. Therapy was discontinued in 34 patients either by the treating physician (in 21 patients) or by the patients themselves (in 13 cases). In 18 subjects, all seizure types relapsed after 1.1 year on average (from 7 days to 4 years) and therapy was resumed in them. All patients but three (10/13), who stopped the treatment themselves, experienced recurrences. Seizure freedom off drugs was recorded in 10.3% patients. Nonintrusive myoclonic seizures recurred in 0.5-3 years as their only seizure type in four patients, but without reintroducing medication in three patients. **Conclusion** Combination of seizure types and focal electroencephalogram features are significant factors of pharmacoresistancy. Continuous pharmacotherapy is required in majority of patients, although about 10% of them appear to have permanent remission without therapy in adolescence.

Key words: Myoclonic Epilepsy, Juvenile; Treatment Outcome; Anticonvulsants; Seizures; Drug Therapy; Recurrence; Drug Resistance; Risk Factors; Electroencephalography

Sažetak

Uvod. Juvenilna miokloničnа epilepsija smatra se hroničnim stanjem koje zahteva doživotnu primenu antiepileptičkih lekova. Cilj ovog istraživanja bio je da utvrdi činioce predikcije vrste kontrole napada i da istraži ishod posle obustave terapije kod ovih bolesnika. **Materijal i metode.** Sačinjena je grupa od 87 bolesnika (49 ženskog, 38 muškog pola), upućenih na našu Kliniku u periodu 1987–2008. godine, starosti 17,5–43,5 godina, sa prosečnim početkom napada u životnom dobu od 14,3±2,9 godina i praćenih u proseku 13,3±5,8 godina (u rasponu 5–23 godine). **Rezultati.** Potpuna kontrola napada postignuta je kod 77% bolesnika, dok su se kod 13,8% napadi i dalje javljali a kod 9,2% postojala je pseudorezistencija. Pojava tri tipa napada i žarišne elektroencefalografske promene su nezavisni prediktivni faktori loše kontrole napada. Terapija je obustavljena kod 34 bolesnika: na predlog terapeuta kod 21 i po sopstvenoj odluci 13 bolesnika je samo obustavilo terapiju. Kod 18 ispitanika došlo je do recidiva svih tipova napada posle prosečnog perioda od 1,1 godine (raspon 7 dana do 4 godine) pa je terapija ponovo uvedena. Većina (10/13) je imala recidiv napada posle samoinicijativne obustave terapije. Potpuna remisija napada bez terapije zabeležena je kod 10,3% bolesnika. Blagi miokloničnі napadi javili su posle 0,5–3 godina kao jedini tip napada kod 4 osobe, bez ponovnog uvođenja leka. **Zaključak.** Više tipova napada i žarišne elektroencefalografske promene značajni su činioci farmakorezistencije. Stalna primena lekova neophodna je kod većine bolesnika sa juvenilnom miokloničnom epilepsijom, iako oko 10% postiže u adolescenciji dugotrajnu remisiju bez hronične terapije.

Ključne reči: Juvenilna miokloničnа epilepsija; Ishod lečenja; Antiepileptici; Epileptični napadi; Terapija; Rekurenca; Rezistencija lekova; Faktori rizika; Elektroencefalografija

Introduction

Juvenile myoclonic epilepsy (JME) is a common idiopathic generalized epilepsy syndrome with the prevalence of 6 to 12% among all patients with epilepsy based on hospital and clinical records [1, 2]. As a specific electroclinical syndrome, it is characterized by a genetic predisposition, no evidence of neurological or intellectual deficit and by mandatory or typical myoclonic seizures alone (irregular jerks of the shoulders and arms) or combined with generalized tonic–clonic seizures (GTCS) in 80% or the absence seizures in 15–30%. Bilateral myoclonic seizures and GTCS are provoked by sleep deprivation and predominantly occur after awakening [1, 3, 4]. The seizures may also be precipitated by fatigue, alcohol intake, and stress [4]. Series of myoclonic seizures often precede GTCS

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Abbreviations

JME – juvenile myoclonic epilepsy
GTCS – generalized tonic–clonic seizures
EEG – electroencephalography
CAE – childhood absence epilepsy
AEDs – antiepileptic drugs
MRI – magnetic resonance imaging
VPA – valproate
TPM – topiramate
LTG – lamotrigine
LEV – levetiracetam
PPR – photoparoxysmal response

[5]. JME with isolated jerks usually developed in patients between 12 and 18 years of age with the female predominance. Interictal electroencephalography (EEG) in JME shows interictal epileptiform discharges such as generalized spikes, polyspikes, bilateral-synchronous 4–6/s spike-wave complexes, or combinations of these [6, 7]. Myoclonic jerks appear to be time-locked to generalized 3–5 Hz spike-and wave complexes [8]. Photoparoxysmal response was seen in 40% of patients. Some authors consider cases of childhood absence epilepsy (CAE) developing to JME to be subtypes of JME with a different outcome [2]. Despite the well-defined clinical and EEG features, JME is very often underdiagnosed and errors and considerable delays in diagnosis have been attributed to many factors. Focal features may contribute to misdiagnosis of JME as focal epilepsy [9].

Many large case series suggest the efficacy of valproate for the therapy of JME but the relative value of other, newer antiepileptic drugs (AEDs) have been suggested [10]. Valproate is very effective and leads to total control of seizures in about 80% of patients.

Although JME is recognized as a common form of epilepsy, its long-term follow-up has rarely been performed. Earlier studies reported JME to be a chronic disease that required lifelong AED treatment with virtual certainty of relapse if medication was discontinued [2, 3]. Only a limited decrease (or with no decrease at all) in seizure propensity was stressed [3]. Withdrawal of medication even in well controlled patients may precipitate seizures [11, 12]. In many case series, a small percent of patients with clear-cut JME can discontinue AEDs and enter a very long (if not permanent) remission. It is usually assumed that continuous pharmacotherapy is required, although about 10% of them appear to have permanent remission in adolescence [13]. Discontinuing AED treatment in JME is very important for the long-term social outcome and quality of life of the patients and requires an individual risk assessment [9, 14].

The aim of this study was both to identify factors that are predictive for seizure remission/drug resistance and to investigate the long-term seizure outcome in patients with JME after the AED withdrawal. Patients were followed up for at least 5 years since the onset of seizure. While the majority of people with JME have a chronic disorder, there are some adolescents with a long-term remission without AEDs according to our experience.

Material and Methods

This retrospective study included the records of JME patients who had been referred to our Department for the first time between 1987 and 2008. An epilepsy syndrome diagnosis was made for each patient, based on the clinical and EEG features according to the International Classification of Epilepsies [6] and on classic treatment [1, 4]. The study sample consisted of 87 patients (49 female, 38 male) aged from 17.5 to 43.5 years (the mean age being 27.6), who had had their first seizure at 14.3±2.9 years of age (their age ranging from 8.3 to 20.5) and were followed up for 13.3±5.8 years on average (from 5 to 23 years). Our Epilepsy Ward is a part of a University Department and a well-established tertiary referral center for children and adolescents with epilepsy in Serbia. Having given their consent, these patients were followed up periodically.

All patients were independently evaluated by at least two of the experienced epileptologists during the long-term follow-up.

The analysis of the seizure type distribtion revealed myoclonic seizures in all patients, GTCS in 82.8% of them, and absences in 21.8%. Myoclonic jerks only were present in 10 patients. All types of seizures (myoclonic, GTC and absence) were observed in 14 patients, whereas the combination of myoclonic and GTC seizures in 58 and the combination of myoclonic seizure and the absence of seizures were found in five patients. Epilepsy remission at follow-up was defined as a terminal seizure-free period lasting for at least 5 years. Relapse was defined as a recurrence of seizures after being seizure-free for at least 5 years. The patients were divided into three groups: I- fully controlled patients, II- truly resistant patients defined as persisting seizures despite adequate lifestyle and treatment that included adequate doses of AED, III pseudo-resistant patients due to inadequate lifestyle, low compliance, or inadequate choice of drugs.

EEG studies at the time of diagnosis and during the course were performed in all patients using the international 10–20 system of electrode placement. Focal EEG features were defined as regional or lateralized EEG epileptiform patterns. Photoparoxysmal response (PPR) is defined as the occurrence of spikes, spike waves, poly-spike waves, or repetitive spikes in response to intermittent photic stimulation, which was observed in 30 patients (34.5%), while focal EEG abnormalities were recorded in 25 of our patients (28.7%). EEG when asleep, after the whole-night sleep deprivation, was recorded in 47/87 patients with bilateral par-

oxysms of spike-waves activated in 36 of 47 (76.5%) sleep deprived subjects.

Brain imaging was not usually required. Magnetic resonance imaging (MRI) of the brain was obtained in 57/87 patients. Neuroimaging was normal in 46 and abnormal in 11 patients.

It was found that AED treatment was discontinued in 34/87 patients (39.1%) which was not proposed to all seizure-free patients. Discontinuation of AED treatment in 21 seizure-free patients was attempted on physician's proposal and only if desired by the patient (group A). In addition, 13 patients who discontinued therapy by their own choice and without agreement of the treating physician (group B) were separately considered. In a non-standardized interview during the regular visit after >3 years since the seizure onset, the patients commented on lifelong therapy irrespective of seizure remission. In addition, they were asked regularly about AEDs side effects (e.g. weight gain and/or teratogenicity).

Data were stored and analyzed in Statistical Package for the Social Sciences (SPSS)17.0 (SPSS Inc., Chicago, U.S.A.), which was used for statistical processing of the data. The statistical methods were descriptive statistics with frequency analysis and cross-tab analysis, as well as mean and standard deviation calculation in parametric data. Statistical significance was assessed using Fisher's exact test to compare categorical variables and χ^2 test, with the significance defined as a p-value of <0.05. Fisher exact tests were selected in order to be comparable with similar investigations [15, 16].

Results

At the end of the follow-up, 67 patients (77.0%) were found to be fully controlled (I), whereas 12 patients (13.8%) had persistent seizures (II). Long-term remission was achieved by valproate (VPA) in 55 of 67 our JME patients with complete seizure control (82.1%), while in 12 patients (17.9%) other AEDs were used, such as topiramate (TPM), lamotrigine (LTG), levetiracetam (LEV) in 2, 6 and 4 patients, respectively. The most common reasons for breakthrough seizures reported by the patients were sleep deprivation, stress, alcohol, non-compliance and menses. Other reported triggers for seizures were fatigue, hyperventilation, photosensitivity and physical exertion. A pseudoresistance was noted in 8 patients (9.2%) (III). Ten patients were initially misdiagnosed as having focal epilepsy. After a change of treatment, all patients who had shown aggravation on carbamazepine (8/10) got obviously better either on VPA monotherapy or on bitherapy combining VPA with LTG, phenobarbital, or a benzodiazepine.

Clinical features associated with poor seizure control despite adequate treatment (group II) were assessed in comparison with the patients with good control (group I). Family history of epilepsy among the first and second degree relatives (23.8% in group I *vs.* 33.3% in group II), age at onset of seizures (mean 14.7±2.9 in subjects with seizure freedom vs. 13.8±2.3 years in those with poor seizure control) and sex (females 60% of patients in group I *vs.* 41.6% of patients in group II) were not significantly associated with drug resistance. In addition to the clinical parameters, the presence of photoparoxysmal response (38.8% in group I vs.-16.7% in group II), and delay of diagnosis since seizure onset (mean 8.0 years, SD 7.5 in group I *vs.* 8.5 years, SD 6.7 in group II) had no significant influence. However, focal EEG abnormalities were significantly more often recorded in the patients with incomplete seizure control than in those with seizure freedom (66.7% of patients vs. 27.4%, Fisher's exact test, p=0.016). Psychiatric disorders were found in 41.6% of AED-resistant patients and in 22.2% of well-controlled patients (Fisher's exact test, p=0.156, p>0.05), which was nearly significant for resistant course. Non-specific abnormal neuroimaging findings were found in eleven of 57 patients (11.7% of patients in group I v*s.* 25% of patients in group II), including a common arachnoid cyst (two), a mild ventricular enlargement (two), a mild diffuse cerebral atrophy and ventricular enlargement (five), intracranial calcifications (one), and pituitary hyperplasia (one). All of these patients but three were fully controlled and the course of the disease was benign. Thus, the abnormal neuroimaging findings seemed to be non-relevant for the clinical expression and evolution of JME.

The influence of the combination of seizure types to the therapeutic response was not statistically significant (χ^2 test, p=0.067). More precisely, the coexistence of all three types of seizures was associated with drug resistance, and was found in 41.7% of resistant cases *vs.* 11.8% in non-resistant cases (Fisher's exact test, p=0.022). Only one of the resistant patients had myoclonic jerks as the only seizure type vs. 10.4% in group I, and none had a combination of absence seizures and myoclonic jerks vs. 7.3% patients with seizure freedom.

Presence of the combination of three seizure types and focal EEG features are independent factors of poor seizure control despite adequate treatment, compared with patients with seizure freedom, achieved with AEDs.

Termination of AED treatment was not proposed to all seizure-free patients. Analysis of medical records from the follow-up visits about 5 years after the seizure onset disclosed that more than half of well-controlled patients (37; 55.2%) stated that assertion on lifelong therapy was hardly acceptable after years of remission. In addition, a number of them (21; 31.4%) at that time reported their concern about VPA effects (weight gain and / or teratogenicity).

Therapy was discontinued in 34 patients. In 21 patients (group A), the discontinuation of therapy

Table 1. Clinical outcome in 34 patients with discontinued therapy
Tabela 1. *Klinički tok kod 34 bolesnika posle obustave terapije*

Characteristic/*Karakteristika*	Number/*Broj*	% and range/*% i raspon*
Therapy discontinued/*Obustavljena terapija*	34/87	39.1%
Group/*Grupa* A – Decided by the physician/*po odluci lekara*	21	24.1% (61.8%)
Group/*Grupa* B – Patients themselves decided/*samoinicijativno*	13	14.9% (38.2%)
Seizure relapses/*Recidivi napada*	22/34	64.7%
Relapses in group A/*Recidivi u grupi A*	12/21	(57.1%)
Relapses in group B/*Recidivi u grupi B*	10/13	(76.9%)
Relapses of GTCS/A/MS/*recidivi GTKN/AN/MN*	18	52.9%
Relapses of myoclonic seizures only/*Recidivi miokloničkih napada samo*	4	11.8%
No relapses/*Bez recidiva napada*	12/34	35.3%
AED restarted/*Ponovo uveden AEL*	20/34	58.8%
after seizure relapses/*posle recidiva napada*	18	52.9%
after EEG aggravation/*posle EEG pogoršanja*	2	5.9%
AED (and Seizure) freedom/*Bez AEL (i bez napada)*	12/87	13.8%
Seizure and AED freedom/*Bez napada i bez AEL*	9	10.3%
AED freedom (MS only)/*Bez AEL (MN samo)*	3	3.4%

Legend: GTCS – generalized tonic-clonic seizures, A-absences, MS-myoclonic seizures, *GTKN – generalizovani toničko-klonički napadi, AN − apsansni, MN − miokloničk napadi*

followed the protocol of AED withdrawal. Majority were treated by VPA-16, while four patients used LTG and one patient received TPM. The drug withdrawal process was managed by the treating physician after 8.5±5.2 years of seizure freedom. However, 13 patients (group B) themselves attempted the drug discontinuation after 5.3±2.4 years of complete seizure remission (**Table 1.**). Majority in group B were also treated by VPA-9, while two patients used LTG or LEV each. In 18 subjects, all seizure types relapsed after 1.1 year on average (from 7 days to 4 years) and AED was restarted in them. In two patients, AED (VPA and LTG) was reintroduced because of EEG aggravation without overt seizure relapse. No seizure occurred in them after such therapeutic decision during the period of 2.5 and 1.5 years, respectively. Nonintrusive myoclonic seizures recurred in 0.5-3 years as their only seizure type in 4 patients, but without restarted medication in 3 patients. No GTCS occurred during the follow-up in the period from 2 to 4.5 years in them. One patient was lost for the follow-up. No relapses were observed in 12 of 34 patients. Seizure freedom off drugs lasting for 4.1 years on average (from 2.8 to 9 years) was observed in 10.3% patients.

No specific AED was associated with the type of therapy termination ($p>0.05$). Neither the type of therapy discontinuation (group A and group B) nor the prescribed AED was significantly associated with the recurrence rate. Statistical significance was not reached despite the fact that all patients but three (10/13), who themselves stopped the treatment, experienced recurrences ($p=0.21$). The reason for AED withdrawal (either decided and advised by the physician or by the patients themselves) was not significantly associated with a) the duration of remission with AED (8.5±5.2 years in group A, 5.3±2.4 in group B) (M W=90.0, $p=0.256$) nor b) the duration of remission after AED withdrawal till the recurrence (A-1.8±1.2 years, B-0.5±0.4 years; T=1.840, DF=32, $p=0.579$). Age at chronic AED withdrawal was not significantly different in patients with various types of therapy discontinuation (21.4 years in group A, 19.4 years in group B; MW-85.0, $p=0.180$).

Discussion

When Delgado-Escueta and Enrile-Bacsal [3] described their experience with 43 patients in 1984, they said that 86% of them had seizure remission with valproic acid but 12 of 13 who discontinued medication had their seizures return. It has been generally accepted that JME is lifelong and that it is unwise to discontinue treatment once seizure control has been established [1, 4, 13].

Generally speaking, JME is not considered a severe condition. The diagnosis of JME is often delayed. Complete, long-term seizure control was achieved in 77% of our patients, whereas 13.8% of them had persistent seizures. Additional 9.2% had pseudoresistant seizures. These findings are in accordance with the experience reported by others, who found that 66-88.3% of patients were controlled on valproate monotherapy [3, 11, 13, 16, 17]. True resistance to adequate drugs is not uncommon, and it was found in 11.7-16.7% of patients [8, 15–17). Pseudoresistance was found in 9.7-16.7% of patients [15, 16]. The course of disease may vary and some patients with a benign form can have a transient period of aggravation, whereas others who are more difficult to treat may experience spontaneous improvement later.

Despite the favorable seizure outcome in most of the cases, 3/4 of patients with JME have at least one major unfavorable social outcome [18]. Clinical features associated with drug resistance in our study

were the presence of the combination of seizure types and focal EEG features. The presence of three types of seizures was found in 41.7% of resistant cases *vs.* 11.8% of well-controlled cases. Only one of the resistant patients had myoclonic jerks as the only seizure type vs. 10.4% of those in group I, and none had a combination of absence seizures and myoclonic jerks vs. 7.3% of those seizure-free. Similar results were found in a number of studies [15–17]. Family history of epilepsy, age at onset of seizures, sex, presence of PPR, results of conventional magnetic resonance imaging (cMRI) and delayed diagnosis were not significantly associated with drug resistance as reported in some previous studies [15, 16]. Contrary to our results, the true-resistant course was significantly associated with psychiatric disorders [14–16] and the presence of thyroid diseases [16]. Myoclonic jerks alone, without GTCS or absence seizures have been reported to occur in 7-17% of JME probands [1]. Patients with myoclonic jerks alone may represent a benign subgroup of JME that may be genetically distinct from classic JME and the jerks may even spontaneously remit in a few cases [5]. The possible sub-syndromes of JME, its genetic background, and its pathophysiological and neuroimaging correlations should be further investigated [18].

A normal EEG while the patient is receiving daily AED treatment is a good sign for the prognosis [10]. Focal clinical and/or EEG features in patients with JME are common. Focal EEG abnormalities, consisting of transients of localized spikes, slow and sharp waves have been found in 16.5–36.7% of JME patients depending on the methodology applied [8, 12, 13]. They were recorded in our patients with poor seizure control significantly more often than in seizure-free patients (66.7% vs. 27.4%). According to recent results, focal findings indicate poor response to treatment of JME [19]. A few EEG and neuroimaging studies indicate that the cortex precedes the thalamus at the onset of generalized spike-wave discharges [9]. PPR is obtained in 27-33% of JME patients [12, 13, 20]. Unexpectedly, the PPR in our patients (38.8% in group I vs 16.7% in group II) was nearly significant for favorable course, contrary to the study conducted by Baykan at al.[16, 18]. The PPR could be a final expression of pathogenic phenomena occurring in the striato-thalamocortical system, possibly a core feature of system epilepsy JME. Abnormal response to photic stimulation in JME in an EEG-functional magnetic resonance imaging (fMRI) study disclosed prominent involvement of basal ganglia circuitry [21].

Janz stated that JME could be controlled very well by valproate and/or primidone. A complete cure, nevertheless, does not seem to be possible [1]. Long-term remission was achieved by VPA in 82.1% of our JME patients with complete seizure control, while other AEDs were used in 17.9% of them. No randomized trials specifically address the treatment of JME [10]. It has been suggested that VPA is more effective than other medications; however, consideration of weight gain, concern of teratogenicity in unplanned pregnancy, and the disorder of polycystic ovaries may lead to an attempt of treatment with LTG, TPM, LEV or clonazepam [13, 20]. However, management of JME should also include interventions in lifestyle, with strict avoidance of sleep deprivation and management of co-pathologies, including the cognitive and psychiatric problems that are often encountered [22]. Detailed results on both initial and maintenance AED therapy in our patients will be soon separately reported.

International workshops on JME were conducted in Avignon, France (2011) and in the Hague, the Netherlands (2012) and included a group of experts on JME, together with one of the founding fathers of the syndrome of JME (*Janz syndrome*), Prof. Dr. Dieter Janz from Berlin. Most clinicians are still reluctant to withdraw medication. Only if the patient is seizure-free for at least 5 years, has a normal EEG, and has a low risk of recurrence of GTCSs the AED(s), the withdrawal could be considered. For women with JME, who are planning to get pregnant, most clinicians attempt to withdraw or reduce VPA dosage as much as possible before the planned conception [23].

After the AED discontinuation in 34/87 patients, 22 patients relapsed. In 18 subjects, all seizure types relapsed and continuous AED was restarted in them. Seizure freedom of drugs was observed in 10.3% patients. Termination of AED treatment was not proposed to all seizure-free patients, so there was selection bias from the physician's side.

Wolf et al. hypothesized that in many patients with epilepsy who are seizure-free under treatment, a gradual decrease in seizure propensity occurs that allows AED dose reduction and, in some patients, discontinuation of AED. Relapses were not increased in their patients with JME (10/21; 47.6%) [24]. A relatively low relapse rate of these patients with JME could not be explained by a slower in-study reduction rate. The more rapid reduction used in some other studies seemed to cause more relapses. Oller-Daurella et al., who also used a very slow rate of reduction after 5 seizure-free years, observed no relapses in 16 cases of JME [25]. In a Norwegian clinic study, 7% of 43 patients with definite or probable JME had a terminal remission without AEDs and an additional 10 patients were seizure-free for >5 years with AEDs [26]. As in the case of absence epilepsy, the enduring propensity of the system to generate seizures needs to be demonstrated. Brain maturation seems to influence cerebral hyperexcitability, which could explain why JME and related conditions appear/abate at a certain age [27].

Pannaiyotopoulos et al. reported that VPA dosage was successfully reduced in 15 patients who were seizure-free for >2 years and had infrequent

seizures before treatment, but 9 of 11 patients relapsed after VPA discontinuation [13]. Long-term follow-up of different clinical JME subtypes (classic JME; childhood absence epilepsy evolving to JME, JME with adolescent absence and JME with astatic seizures) conducted by Martinez-Juarey et al. indicates that all sub-syndromes are chronic and perhaps lifelong [2]. Only 8 of 161 (5%) patients with classic JME were in remission from all seizures without treatment. Epilepsy was clearly chronic as shown by recurrence of seizures upon discontinuation of medications. In addition, only 3 of 35 patients with CAE evolving into JME achieved complete freedom from seizures [2]. In 5/12 our patients with poor seizure control, absences occurred, comparing with 14/75 with complete seizure freedom. In a study conducted by Pavlovic et al., all 17 studied patients with JME, who had discontinued AED, relapsed after the AED withdrawal [12]. In their study, Canevini et al. reported relapses in 8 out of 60 JME patients who had attempted to discontinue AED. They relapsed during the follow-up period lasting from 6 months and 1 year [15]. In a study of Nicolson et al. [20], the relapse rate following AED withdrawal was 93.6%.

Camfield and Camfield [28] conducted a population-based study of 24 JME patients after 25-year long follow-up. All seizure types in JME resolved in 4 (17%) and for 3 (13%) only myoclonus persisted after stopping AEDs. Therefore, troublesome seizures vanish in one-third of people with JME and AED treatment is no longer needed. A Turkish clinical study showed that although a great majority of the patients with JME had continuing seizures after a follow-up of 20 years, almost all had either 5-year remission or a substantial alleviation of the myoclonic seizures in the fourth decade. In 54.2% of the patients, myoclonia were in remission for a mean duration of 8.4 ±7.7 years [16]. The majority of patients with JME have continuing seizures after a follow-up of two decades. However, 17% are able to discontinue medication and remain seizure-free thereafter [18]. Nonintrusive myoclonic seizures recurred in 2.4-4 years as the only seizure type in 4 of our subjects, and without restarted medication. No GTCS occurred during the follow-up in them. It is possible that relatively optimistic rate of remission is related to the age of patients at the end of follow-up: seizure-provoking factors such as binge drinking and sleep deprivation are likely to decrease with age [28]. The tendency of myoclonic seizures in JME toward remitting with age could be unrelated to the AED treatment or to the lifestyle changes. We could not comment on this issue, because our series included a small number of patients having myoclonus only.

Pavlovic et al. stated that EEG worsening during and/or after the AED withdrawal was an independent prognostic variable associated with the higher risk of seizure relapse in generalized epilepsy [9, 12, 21]. In two of our patients, the AED was reintroduced because of EEG aggravation without overt seizure relapse. Although no seizure occurred in them during the period of 2.5 and 1.5 years, there is no strong evidence to justify such therapeutic decision.

Conclusions

The presence of the combination of seizure types and focal electroencephalogram features are independent factors of poor seizure control despite adequate treatment. With 64.7% of patients having seizure recurrences upon antiepileptic drug discontinuation after >5-year stable seizure freedom, and the necessity to restart therapy in them, there is little doubt that the majority of people with juvenile myoclonic epilepsy have a lifelong disorder. Our experience indicates that there are some (10.3%) patients with a relatively short active phase followed by a long-term remission without antiepileptic drugs. Recurrence of myoclonic seizures only may not lead to the reintroduction of chronic therapy. Identifying juvenile myoclonic epilepsy patients prospectively who will have remission of their epilepsy and be able to discontinue antiepileptic drug treatment should be an issue of further research.

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C-FOS PROTEIN EXPRESSION IN THE ANTERIOR AMYGDALOID AREA AND *NC. ACCUMBENS* IN THE HYPOXIC RAT BRAIN

EKSPRESIJA C-FOS PROTEINA U ANTERIORNOJ AMIGDALOIDNOJ REGIJI I NC. ACCUMBENS U HIPOKSIČNOM MOZGU PACOVA

Siniša S. BABOVIĆ, Sonja ŽIGIĆ and Branislav ŠAKIĆ

Summary

Introduction. By examining the production of c-Fos protein, we analyzed the response to the ischemic attack in different brain tissue, two of which are regions of the limbic system: the anterior amygdaloid area and nc. accumbens. **Material and Methods.** We used the model of rat brain ischemia - four-vessel occlusion, and Pulsinelli's method. The rats were treated in two ways, according to which they were divided into two groups: a total ischemia (ligation of four blood vessels, i.e. electrocauterization of the vertebral artery with bilateral ligation of the carotid artery - the so-called R-group rats), and transient ischemic attack (ligation of four blood vessels, i.e. electrocauterization of the vertebral artery, with mutual re-ligation of the carotid arteries in the form of transient ischemia - the so-called T-group rats, which can also be called "pre-conditioned group"). Both groups had their own control group. **Conclusion**. We have concluded that parts of the brain with an important role for the survival have a strong expression of c-fos gene.

Key words: Hypoxia; Proto-Oncogene Proteins c-fos; Amygdala; Brain Ischemia; Rats; Nucleus Accumbens; Limbic System; Ischemic Attack, Transient

Sažetak

Uvod. Ispitivali smo reakciju na ishemijski atak u vidu stvaranja c-Fos proteina u različitim moždanim tkivima, među kojima i u dva regiona limbičkog sistema (*area amygdaloidea anterior* i *nc. accumbens).* **Materijal i metode.** Korišćen je model ishemije mozga pacova − okludiranjem četiri krvna suda (*four-vessel occlusion*) i Pulsinelijeva metoda. Pacovi su tretirani na dva načina, prema kojima su podeljeni u dve grupe: kao totalna ishemija (ligaturom četiri krvna suda, tj. elektrokauterizacijom vertebralne arterije uz obostranu ligaturu karotidnih arterija – tzv. R-grupa pacova) i kao ishemijski tolerantni atak (ligaturom četiri krvna suda, tj. elektrokauterizacijom vertebralne arterije, uz obostranu ponovljenu ligaturu karotidnih arterija uz uspostavljanje recirkulacije između dve ligature što je dovodilo do tranzitorne ishemije – tzv. T-grupa pacova koja se može nazvati i „prekondicionirana grupa"). Obe posmatrane grupe imale su svoju kontrolnu grupu. **Zaključak**. Došli smo do rezultata da delovi mozga sa važnom ulogom za preživljavanje jedinke imaju izraženu ekspresiju c-Fos gena.

Ključne reči: Proto-Onkogen c-fos protein; Amigdale; Moždana ishemija; Pacovi; Nucleus Accumbens; Limbički sistem; Tranzitorni ishemički napad

Introduction

Brain ischemia, as a local interruption of blood flow in the brain, is becoming the most common death cause in developed countries; while the transient global ischemia, caused usually by cardiopulmonary arrest, is a more significant cause of cerebral stroke today. Various external and internal factors can cause ischemia, and despite advances in the research field of brain ischemia in experimental rat models, no effective therapy has been established [1]. In order to find an appropriate neuroprotective treatment, various experimental studies have been conducted in the rat brain by causing ischemic changes induced by occlusion of blood vessels in the brain, hypotension and hypovolemia [2]. The most common way was the occlusion of four blood vessels (four-vessel occlusion) [3]. Occlusion of the four major blood vessels of the brain (two carotid and two vertebral arteries) creates the conditions for the occurrence of ischemic changes.

So far, various studies have been conducted in an attempt to reduce the consequences of ischemic attacks on the functioning of neural pathways. Ferreira et al. pointed out the significant potential of fish oil in reducing amnesia after a transient ischemic attack, but not in chronic cerebral hypoperfusion [4]. On the other hand, in their research, the River-Auty et al. failed to confirm the hypothesis according to which the cannabinoid CB2 receptor agonists should improve the condition of neurons in rats after brain ischemia [5]. In this area, experimental studies are still performed in order to improve the condition and function of neurons after ischemic attack.

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Abbreviations

ATP – adenosine triphosphate
NAMD – N-acetyl-O-methyldopamine
R – groups of rats total ischemia
T – a group of rats with transient attacks
CA – *Cornu Ammonis*

At the onset of ischemia, there is an expression of the so-called group of early genes or proto-oncogenes (*immediate early gene expression*) [6].

The role of Proto-Oncogenes and their Importance

Proto-oncogenes are genes which are rapidly and temporarily expressed as an early cellular response to various stimuli, and they are also called a "gate of genomic response". Ischemia as a stimulus leads to a post-ischemic expression regulated by transcription factors, the important mediators between the extracellular and cellular response signal in the form of long-term changes in the cell phenotype.

So far, about forty proto-oncogenes have been found, most notably the human homologs of retroviral oncogenes – the Fos family (c-fos, fos-B, fra-1, fra-2) and the Jun family (c-jun, jun-B, jun-D). The regional and occasional models of proto-oncogene induction are related to gene expression regulated by neurotransmitter, which implies the transcription in physiological and pathological conditions. The physiological stimuli lead to a discrete production of expression of these genes. However, pathological causes (tumor proliferation, cardiac disorder, occlusion of blood vessels of the brain, etc.) lead to the induction of expression of these genes and their proteins in the wide regions of the brain [7], even beyond the initial damaged areas, which serves as a marker of early neuronal activity [8].

Different models of global ischemia are used in experiments, such as Pulsinelli's method [9], which we also applied, but it is very difficult to set clear barrier between a lethal and reversible injury caused by various models of global cerebral ischemia.

In animal models, we can perceive an ischemic tolerance, transient cerebral ischemia, which protects the brain from subsequent ischemic attacks. It functions by inducing endogenous neuroprotectants, based on the program of post ischemic genetic expression, which is regulated by the transcription factors of early genes [10]. This functional-protectoral role of the proto-oncogenes depends on the degree of created ischemic tolerance. A prolonged activation of c-fos gene leads to the programmed cell death [11]. In their research, Jee et al. have shown that the antidepressant fluoxetine can inhibit apoptosis of hippocampus neurons caused by transient global ischemia, protecting the integrity of the blood-brain barrier [12]. Hua-Juan et al. suggest the need to examine the therapeutic potential of chlorpromazine as a neuroprotective agent in the treatment of ischemic stroke [13].

The increase in stress factors, neurotrophic and early genes in response to stress as a condition of mild stroke may indirectly prevent lethal ischemic insult by opening the adenosine triphosphate (ATP)-sensitive potassium channels [14]. The level of ATP, as the most important parameter for cell survival, directly affects the intensity of the stroke, and therefore the type of cell death caused by experimentally induced ischemia. This biochemical parameter cannot be below 20% [15].

Global ischemia activates an early gene as a trigger of transcription factor, which plays the role in signal transduction from extracellular signal to long-term changes in cellular phenotype. All early genes, including c-fos gene, can be activated by different harmful stimuli, such as cocaine, heroin, and other psychoactive drugs [16], "heat stress", adrenalectomy, intracortical injection of nerve growth factor (NGF), some medicines, and reduced diet. Jungenitz et al. showed that the induction of c-Fos gene can be encouraged by high frequency stimulation in their research on hippocampal granule cells [17]. Expression of c-Fos proteins can be inhibited by conditioned fear [18] and noise [19].

Since the role of c-Fos protein in neurodegeneration and neuroprotection is controversial, it is the subject of many studies. There are different *in vivo* and *in vitro* studies showing that prolonged induction of c-fos proto-oncogene leads to neuronal death after ischemia, and that this proto-oncogene and its products cause diverse neuronal damages in addition to the neurodegenerative nature of c-Fos induction [20]. On the other hand, the expression of c-Fos protein is considered essential to the recovery of the neurons after ischemia in certain cases [21].

The role of c-Fos proto-oncogene was examined by several types of experimental models in the rat brain, each securing global transient ischemia, followed by unilateral or bilateral cerebral ischemia. Initial studies of behavior of hippocampus cells [22] and the vulnerable neurons of Cornu Ammonis (CA1)as well as neurons slightly more resistant to hypoxia-dentate gyrus and CA2-4 have shown that the level of c-Fos protein drops all parts of the hippocampus up to 6 hours after the reperfusion. Its level increases in the presence of N-acetyl-O-methyldopamine (NAMD), which can be used for pharmacological purposes [9]. Sanz et al. published studies with similar findings [23].

After the blood pressure had decreased by 30% during the period of 60 minutes, immunohistochemical reaction was recorded in the following structures: *nc. tractus solitarii, area postrema, nc. parabrachialis lateralis, nc. paraventricularis et supraopticus, nc. amygdalae centralis* [24].

The striatal neurons were studied in immature rat brains, and it was found that the induction of this protein started 0 to 12 hours after ischemia. Caudoputamen of adult brain was examined on the type of unilateral, focal ischemia and the c-Fos protein level was found to decrease 4 hours after reperfusion [25].

On the hypoxia model of immature rat brain during unilateral ligature it has been proved that the fastest messenger ribonucleic acid (mRNA) expression was in the ipsilateral cortex, and then in all other vulnerable regions, i.e. hippocampus and striatum [26]. The increase in the level of c-Fos protein appeared in the contralateral hemisphere in hypoxia, and it was shown that different parts of the brain c-Fos are immunopositively activated [8].

The most comprehensive study of transient brain ischemia was done by Wassel and Volpe [27].

The increase in c-Fos expression in global ischemia can be preventive, causing the so-called preconditioning. Tolerance to ischemia achieved in this way protects the vulnerable regions of the brain [28].

Seo et al. obtained similar results in their study of using proton magnetic resonance spectroscopy, with which they confirmed the reduction of neuronal damage by the method of ischemic and hypoxic preconditioning [29].

Ischemia and Anterior Amygdaloid Area and *Nc. Accumbens*

Anterior amygdaloid area and *nc. accumbens* were the two brain structures of the examined brain regions which were among the most prominent ones in the expression of c-Fos protein according to semiquantitative method. These two regions belong to the limbic part of the brain, which is the backbone and morphological basis of higher nervous activity, responsible for behavior, affects, and emotional behavior. In the limbic system, the impulses, which are sent from the neocortex, have been modified and thus processed are sent to the hypothalamus and the hypothalamus affects the function of vegetative functions and character of behaving. The results of our study should show whether these brain structures are somehow protected from the consequences of ischemic attack by specific molecular and biochemical way.

The aim of the study was to determine the expression of c-Fos protein in the anterior amygdaloid area and *nc. accumbens* in various models of global ischemia.

Material and Methods

Distribution of c-Fos protein was examined in 10 adult male Wistar rats, weighing 200 to 635 g. Ischemia was created by the method of Pulsinelli:

– total ischemia ligation of four blood vessels (electrocauterization of the vertebral artery, with bilateral ligation of carotid artery by paraffined thread for 10 min, followed by perfusion lasting 60 min), marked as R-group, 4 animals

– tolerant ischemic attack (electrocauterization of vertebral artery with bilateral ligation of carotid arteries by paraffined thread for 4 minutes, and then repeated after 72 hours for 10 min), marked as T-group, 4 animals,

– the first control group in which there was no intervention, one animal;

– the second control group for tolerant ischemia (vertebral electrocauterization with ligation of both carotid arteries for 4 minutes without 10-minute religature, one animal.

The experimental R-group included a subgroup of R1 and R2, in which we observed anterior amygdaloid area, and *nc. accumbens,* respectively. In the T1 and T2 subgroup, from the experimental T-group, we observed anterior amygdaloid area, and *nc. accumbens*, respectively.

The rats were anesthetized with ketamine on the first day (100 mg/kg), when electrocauterization of the vertebral artery was done. The rat was anesthetized with halothane (1.5–2.0%) the following day, and both of its common carotid arteries were ligated with paraffined thread to avoid the damage to the vessel wall when tightening the thread.

In the total ischemic group, the occlusion of the carotid arteries lasted 10 minutes, after which the blood recirculation was achieved by cutting the thread and after 60 min the animals were sacrificed. In the tolerant group, 72 hours after the first occlusion, the common carotid artery was occluded for 10 min and 60 min after the occlusion, the animals were executed in under anesthesia with ketamine (100 mg/kg), and intracranial brain became reperfused with 4% paraformaldehyde.

After the overnight fixation, the tissue was in cryoprotective, 20% sucrose, and then cut by free-floating technique, the incision being 50 μm thick. In this study, we used the immunohistochemical technique of avidin-biotin-peroxidase, as well as semiquantitative technique as a method of detection of the examined protein. The sections were washed with 0.1 M phosphate buffer, and incubated in Triton X-100 and 10% normal goat solution. This was followed by 48 h incubation with the specific c-Fos antibody (Santa Cruz, 1:100 000). The next step was the incubation in the rabbit secondary antibody for 60 min, and then avidin-biotin-peroxidase was added for the same time. Elution was carried out with 0.1 M phosphate buffer (pH 7.26). Antigen-antibody complex was visualized by 3.3'- diamino benzidine and 0.03% of H_2O_2. The preparations were observed and analyzed by the Leica microscope. Photos were taken by Analisis program and analyzed by Scion J program, converting images into binary representation.

Results

The observed c-Fos protein was found in both experimental groups, with no difference in coloration. The photos of stained preparations of examined structures were converted into binary representations in order to obtain numerical values of some

Figure 1. Binary representation of the coloring of preparation of anterior amygdaloid area by c-Fos protein in rat brain ischemia. 20x magnification.
Slika 1*. Binarni prikaz prebojenosti preparata area amygdaloidea anterior c-Fos proteinom pri ishemiji mozga pacova. Uvećanje 20 x.*

Figure 2. Binary representation of the coloring of preparation of *nc. accumbens* by c-Fos protein in rat brain ischemia. 20x magnification.
Slika 2. *Binarni prikaz prebojenosti preparata nc. accumbens c-Fos proteinom pri ishemiji mozga pacova. Uvećanje 20 x.*

Table1. The level of staining of neurons in the examined subgroups according to the semiquantitative method
Tabela 1. *Stepen obojenosti neurona u ispitivanim podgrupama po semikvantitativnoj metodi*

Subgroup/*Podgrupa*	Minimal values/*Minimalne vrednosti*	Maximal values/*Maksimalne vrednosti*
R1	20.00	46.00
T1	23.00	127.00
R2	10.00	413.00
T2	21.00	73.00

discoloration of the observed image, and were subjected to the statistical processing (**figures 1 and 2**).

Minimum and maximum values were obtained by semiquantitative method of determining the degree of staining neurons in the examined areas of the brain (**Table 1**).

The R and T values of samples were grouped according to the analyzed parameters based on the measured values and shown in graphs. The order of column values is presented in such a way that every two columns (one pair of columns) are the left and the right side of the same location. We noticed an uneven expression of observed protein on the left and right in the same rats (**graphs 1 and 2**).

A clear dominance of T numeric values was noticed by grouping the values of coloration degree of c-Fos immunoreactive neurons at the location R1/T1 (anterior amygdaloid area). Grouping the values of staining degree of c-Fos immunoreactive neurons at the location R2/T2 (*nc.*) shows that the numerical values of T brains are grouped around the middle and the end points of a series take R values.

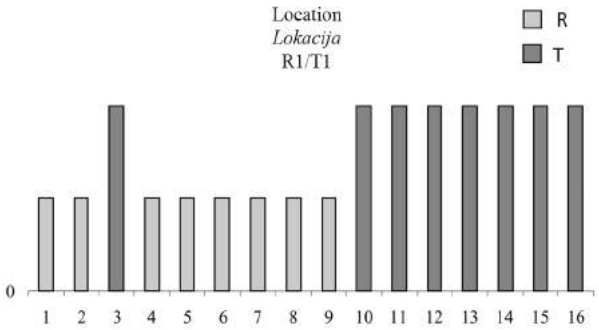
Discussion

All the medical conditions leading to a reduced blood flow not only through the brain but also through the heart, kidneys and other organs are common epidemiological problems that should be researched in this way.

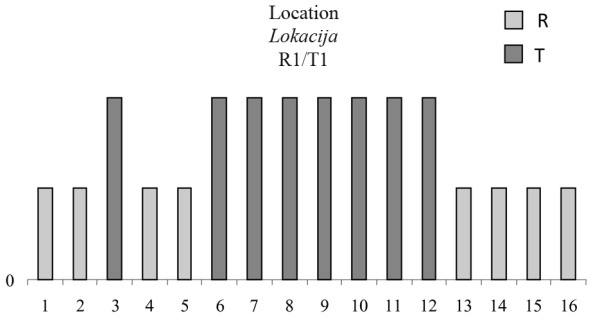
Recent studies in this area deal with the expression of c-Fos protein after ischemic attack mainly in the hippocampus of rats, without taking some other parts of the limbic system as subject of their interest, such as the area amygdaloidea anterior and *nc. accumbens.* Our experiment was a part of a larger study of all structures of the adult brain in hypoxia, which relied on previous research conducted by scientists such as Nytrai et al. [6], Cho et al. [10] Soriano et al. [25], Xie et al. [30] and Johansen et al. [14], whose studies marginally presented results on cells of other (non-hippocampal) regions.

Semiquantitative method showed a high degree of neuron discoloration in the anterior amygdaloid area, which was determined by measuring the degree of discoloration and shown in the photos. The simiral results were obteined examing the parietal cortex and the olfactive tubercle by Puškaš et al. [31].

Nc. accumbens showed a distinct expression of c-Fos protein in the T-group of rats, which is clearly seen as a graphic shift of T-values to the right side, to a higher value. However, the absolute values indicative of greater expression of the R-group of rats were



Graph 1. The order of the value of c-Fos protein by size in the anterior amygdaloid area
Grafikon 1. *Redosled po veličini vrednosti c-Fos proteina u area amygdaloidea anterior*



Graph 2. The order of the value of c-Fos protein by size in *nc.accumbens*
Grafikon 2. *Redosled po veličini vrednosti c-Fos proteina u nc. accumbens*

obtained by semiquantitative method for determining the degree of staining of neurons. This contradiction of results can be explained by technically poor adhesion of antibodies or by other technical artifacts.

The graphs clearly show the grouping of R and T treated brains against the intensity of the reaction, where the T group showed a more prominent reaction and more apparent grouping towards higher values.

The results of our study are correlated with the research of Seo et al. who, using proton magnetic resonance spectroscopy, came to the conclusion that ischemic and hypoxic preconditioning could reduce the extent of neuronal damage in ischemia of the affected part of the brain [29].

Our research should continue into several directions - to observe the brain in different stages of gestational development and adult age, to determine the degree of reactivity of c-Fos protein in the unilateral ligation and in the direction of pharmacological quantification of c-Fos protein in relation to the NAMD, as a potential therapeutic agent for brain ischemia in humans.

Conclusion

Based on our research, we concluded that there is a statistically significant difference of the observed c-Fos protein in the group of rats belonging to the group with total ischemia of compared to the group with tolerant ischemic attack, and in both examined structures of the brain. In this way, the expression of the investigated protein where the organ is "accustomed" to the reduced flow of oxygen and nutrients is prominent, which confirms its importance for the survival of neurons.

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INFLUENCE OF HEMODIALYSIS DURATION PER WEEK ON PARAMETERS OF DIALYSIS ADEQUACY AND CARDIOVASCULAR MORBIDITY

UTICAJ NEDELJNOG TRAJANJA HEMODIJALIZE NA PARAMETRE NJENE ADEKVATNOSTI I KARDIOVASKULARNI MORBIDITET

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Summary

Introduction. The optimal length of dialysis is still under debate and current regimen of 12 hours a week is medically acceptable. The aim of this observational study was to confirm the relationship between different length of dialysis per week and the parameters of dialysis adequacy and cardiovascular morbidity. **Material and Methods.** The study included 206 patients (128 man and 78 females) who were on maintenance hemodialysis for more than 6 months. They were classified into three groups according to the length of dialysis per week: group I (12 hours), group II (15 hours) and group III (≥17.5 hours). **Results.** Index of dyalysis adequacy values did not differ among the groups (group I, II, III = 1.32 vs. 1.51 vs. 1.42; p>0.05); however, the patients from group III had the best bicarbonate level (group I, II and III= 22.7; 21.4; 17.6 mmol/L; p<0.001). In comparison with group I (12 hours), longer dialysis duration was associated with significantly higher hemoglobin values (12.2 vs. 11.4 vs. 10.5 g/dL), less frequent use of erythropoietin stimulating agents (26.9% vs. 65% vs. 86.3%), lower stimulating agents weekly dose (median in group I, II, III=2000 vs. 5000 vs. 4000 I.J.), lower stimulating agents resistance index (4.9 vs. 7.8 vs. 8.8), significantly higher level of serum albumin (42.3 vs. 40.7 vs. 38.2 g/dL), total cholesterol (5.1 vs. 4.7 vs. 4.5 mmol/L) and serum calcium level (2.38 vs. 2.42 vs. 2.28 mmol/L), less frequent use of phosphate binders (53.8% vs. 85% vs. 84.4%) and calcitriol (19.2% vs. 65% vs. 50.6%) and lower intact parathyroid hormone level (336 vs. 363 vs. 446 pg/ml). In addition, longer dialysis duration was associated with lower cardiovascular morbidity score (0.52 vs. 1.05 vs. 1.26). **Conclusion.** Duration of dialysis per week above the current standard positively correlates with parameters of hemodialysis adequacy.

Key words: Renal Dialysis; Morbidity; Cardiovascular Diseases; Anemia; Minerals + metabolism

Sažetak

Uvod. Optimalna dužina lečenja hemodijalizom još je neutvrđena, a trenutni režim od 12 sati nedeljno je medicinski prihvatljiv standard. Cilj ove opservacione studije bio je da utvrdi povezanost različite nedeljne dužine lečenja hemodijalizom sa parametrima adekvatnosti hemodijalize i kardiovaskularnim morbiditetom. **Materijal i metode.** Ovom opservacionom studijom analizirano je 206 bolesnika (128 muškaraca i 78 žena) lečenih duže od 6 meseci koji su bili podeljeni u grupe shodno nedeljnom trajanju dijalize: grupa I (12 h), grupa II (15 h) i grupa III (≥ 17,5 h). **Rezultati.** Vrednosti indeksa adekvatnosti dijalize nisu se razlikovale između grupa (grupe I, II, III = 1,32 vs 1,51 vs. 1,42 : p > 0,05), ali su bolesnici grupe III imali najbolje korigovanu acidozu (u grupi I, II i III = 22,7; 21,4; 17,6; p < 0,001). U odnosu na standardne dijalize od 12 h, duže nedeljno trajanje dijalize bilo je praćeno značajno višim vrednostima hemoglobina (12,2 vs 11,4 vs 10,5 g/dl), redom upotrebom agenasa stimulacije eritropoeze (26,9% vs 65% vs 86,3%), manjom nedeljnom dozom agenasa stimulacije eritropoeze (medijana u grupi I, II, III = 2000 vs 5 000 vs 4 000 i.j.), njihovim nižim indeksom rezistencije (4,9 vs 7,8 vs 8,8), značajno višim vrednostima serumskih albumina (42,3 vs 40,7 vs 38,2 g/dl), ukupnog holesterola (5,1 vs 4,7 vs 4,5 mmol/L) i serumskog kalcijuma (2,38 vs 2,42 vs 2,28 mmol/L), redom upotrebom vezivača fosfata (53,8% vs 85% vs 84,4%) i kalcitriola (19,2% vs 65% vs 50,6%) i nižim vrednostima intaktnog paratiroidnog hormona (336 vs 363 vs 446 pg/ml). Duža dijaliza bila je praćena i nižim skorom kardiovaskularnog morbiditeta (0,52 vs 1,05 vs 1,26). **Zaključak.** Nedeljna dužina lečenja hemodijalizom preko trenutnog standarda pozitivno korelira sa parametrima adekvatnosti hemodijalize.

Ključne reči: Dijaliza; Morbiditet; Kardiovaskularna oboljenja; Anemija; Minerali + metabolizam

Introduction

During the 1960s, chronic hemodialysis (HD) usually included three sessions per week in duration of 8 to 12 hours [1, 2]. Advanced dialysis membranes and techniques lead to reduction in the length of dialysis to 4 hours three times a week since the clinical outcome of such prescription was considered acceptable [3].

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Abbreviations

HD – hemodialysis
ESA – erythropoietin stimulating agents
BMI – body mass index
ERI – erythropoietin resistance index
CRP – C-reactive protein
HDL – high-density lipoprotein
LDL – low-density lipoprotein
iPTH – intact parathyroid hormone
KtV – index of dialysis adequacy
NCDS – National Cooperative Dialysis Study
HEMO – hemodialysis study

Generally speaking, the adequacy of dialysis implies not only the clearance of uremic toxins and partial control of the condition of patients having terminal renal failure but optimal rehabilitation as well [4]. The following depend directly on the level of adequacy of dialysis: mortality rate, anemia severity, damage to immune competence, renal osteodystrophy, hypertension, cardiomyopathy, nutrition disorders and general quality of life [4].

In the first randomized clinical study which involved patients treated with HD, "National Cooperative Dialysis Study" (NCDS), no statistical correlation between the duration of HD treatment and treatment outcomes was found [5]. Data from the NCDS gave the ground to introduce the concept of 'dialysis dose' in the form of the Kt/V urea formula, based on urea as a marker of uremia, and for more than two decades, the clearance of low-molecular weight uremic toxins remained the measure of dialysis adequacy [6]. Several guidelines recommend minimum target values of Kt/V formula aimed at delivering an adequate dialysis dose to all patients [7,8]. As a result of the NCDS, dialysis time was not been considered the only key factor in the dialysis prescription. The randomized Hemodialysis study (HEMO) did not find advantages in survival of patients treated with higher dialysis dose (expressed by Kt/V) or using high-flux dialysis membrane [9]. However, the HEMO did not take into account the duration of HD treatment because this parameter is not believed to be the key determinant of treatment outcomes, regardless of its contribution to Kt/V urea value. In the last few years, several studies have shown that increased dialysis length can lead to better correction of anemia parameters, along with the reduction in the frequency of administration and dose of erythropoietin stimulating agents (ESA) preparations. At the same time, the increased dialysis length was associated with better control of hyperphosphatemia and prevention of secondary hyperparathyreoidism, along with reduced frequency of administration of phosphate binders and metabolite of vitamin D [10–13]. Furthermore, there are several reports about the positive effects of increased HD session duration on nutritional parameters of patients [10, 14, 15] and higher survival rate [6, 16–20].

The aim of this one-year observational study was to compare parameters of dialysis adequacy and duration of HD treatment per week.

Material and Methods

This retrospective study included a total of 206 patients (128 man and 78 women/, their mean age being 61.0±11.7 years) treated with chronic HD for more than 6 months at the Department of Renal Diseases ''Prof. Dr Vasilije Jovanovic'', Zvezdara University Medical Center, Belgrade. The patients were divided into 3 groups according to the total duration of dialysis treatment per week: group I - 12 hours, group II - 15 hours, and group III – 17.5 or more hours per week. The patients from group I and II were treated by hospital HD, while the patients from group III were treated by home HD.

Data on patients, which had been taken from their medical records, included age, sex, duration of dialysis (expressed in months), presence of diabetes and hypertension, cardiovascular diseases until the beginning of the study; intake of vitamin D metabolites and phosphate binders, cumulative dose of calcium carbonate and vitamin D metabolites during the previous year, the use of statins and weekly dose of ESA. Body mass index (BMI) was calculated according to the patients' weight and height [21]. Erythropoietin resistance index (ERI) was expressed as the quotient of average weekly ESA dose and body mass of patient divided by average hemoglobin value. Adequacy of dialysis is expressed using Kt/V for urea in accordance with Daugirdas formula [22].

Samples for laboratory analyses were taken at the beginning of dialysis procedure after a weekend pause once in three months and the following laboratory parameters were analyzed: total proteins, serum albumins, serum bicarbonates, C-reactive protein (CRP), hemoglobin (Hb), ferritin, calcium (Ca), phosphorus (P), total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, which were measured by standard laboratory techniques. The average of analysis was calculated for the period of one year except the values of parathormon, which was controlled at least twice a year using chemiluminescent assay (DPC, Diagnostic Product Corporation, USA).

Cardiovascular morbidity score was calculated for each patient and on the basis of previous medical dialysis data file, one point was given for each of the following diagnoses: cardiomyopathy, ischemic heart disease, peripheral vascular disease and stroke.

Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS) (version 16.0) program. Data were expressed as percentage for discrete factors, and mean values for continuous variables. Statistical analyses included exploratory analysis method (descriptive and analytic statistics). Analysis of variance (ANOVA) was used to compare variables with normal dis-

Table 1. Characteristics of patients regarding duration of hemodialysis treatment per week (mean ± S.D.)
Tabela 1. *Karakteristike bolesnika u odnosu na ukupnu nedeljnu dužinu lečenja hemodijalizom (srednja vrednost ± S.D.)*

	Group I/*Grupa I* (12 h) N=160	Group I/*Grupa II* (15 h) N=20	Group III/*Grupa III* (≥17.5 h) N=26	p
Gender male/*Pol muški* %	58,8	65,0	80,0	0,096
Age, years/*Godine starosti*	62,6±11,7	57,1±9,8	54,2±9,7	<0,001*
Dialysis vintage, months/*Dužina HD, meseci*	76,9±59	152,8±75	207,0±105	<0,001**
DM/*DM* %	17,5	5,0	3,8	0,026
HTN/*HTA*%	89,4	100,0	88,5	0,511
Statin use/*Upotreba statina* %	15,0	40,0	7,7	0,478
Low-flux/*Niskopropusna* %	43,8	5,0	0,0	
High-flux/*Visokopropusna* %	40,0	95,0	6,2	<0,001
HDF/*HDF* %	16,2	0,0	53,8	
HCO_3^-/ HCO_3^- (mmol/L)	17,6±2,1	21,4±2,2	22,7±2,1	<0,001†
Kt/V/ *Kt/V*	1,32±0,25	1,51±0,39	1,42±0,32	0,176

DM - diabetes mellitus/*dijabetes melitus*; HTN/HTA - arterial hypertension/*arterijska hipertenzija*; HDF - haemodiafiltration/ *hemodijafiltracija*; Kt/V - index of dialysis adequacy/*indeks adekvatnosti dijalize/*; HCO_3 - bicarbonate/*bikarbonat*, *group I vs. group III/*grupa I vs. grupa III/*, p=0,002; ** group I vs. group II/*grupa I vs. grupa II*, p<0,001, group I vs. group III/*grupa I vs. grupa III/*, p<0,001; † group I vs. group II/*grupa I vs. grupa II/*, p<0,001, group I vs. group III/*grupa I vs. grupa III*, p<0,001

tribution in different groups while Bonferroni test was used for setting the difference between groups (*post hoc* analyzing). In case where variables did not have normal distribution, Kruskal Walis test was used for comparing groups, and the differences between groups were analysed by Mann-Withney test.

The results are shown in tables and graphics. In all comparisons, a p value <0.05 was considered statistically significant.

Results

Table 1 shows the characteristics of patients in relation to the HD treatment duration per week. The patients from group I were significantly older compared to the patients from group III. HD vintage was significantly different between the groups because the patients from groups III and II were treated longer with HD compared to the patients from group I. Group I included significantly more patients with diabetes while the groups did not differ regarding the presence of hypertension and use of statin therapy. The patients from group I were treated with low-flux membranes while the patients from group III were treated by hemodiafiltration (HDF) more frequently than patients from the two other groups. The patients from group I had the worst acid-base status, which was significantly different from the patients from two other groups. Adequacy of dialysis expressed through Kt/V was not statistically different among groups even though the patients in group II (treated with high-flux membrane) had the highest value of Kt/V.

The parameters of anemic syndrome are presented in **Table 2**. The patients in group III had the highest values of hemoglobin which was statistically different from the patients in group I and it was near to the statistical significance compared to the patients from group II. The patients in group I had the highest values of ferritin while the

Table 2. Parameters of anemia in relation to duration of hemodialysis treatment per week (mean ± S.D.)
Tabela 2. *Parametri anemije u odnosu na ukupnu nedeljnu dužinu lečenja hemodijalizom (srednja vrednost ± S.D.)*

	Group I/*Grupa I* (12 h) N=160	Group I/*Grupa II* (15 h) N=20	Group III/*Grupa III* (≥17.5 h) N=26	p
Hemoglobin/*Hemoglobin*, g/dL	10,5±1,0	11,4±1,1	12,2±1,7	<0,001*
Ferritin/*Feritin,* umol/L	366,6±179	356,0±438	150,5±234	<0,001**
ESA use/*ASE upotreba*, %	86,3	65,0	26,9	<0,001†
ESA, IU/week/*ASE, IJ/nedeljno*	5636±4348 (med 4000)	6061±4107 (med 5000)	3785±3314 (med 2000)	0,321
ERI/*ERI*	8,8	7,8	4,86	0,029‡

ESA - erythropoietin stimulating agents/*ASE - Agensi stimulacije eritropoeze;* ERI - erythropoietin resistance index/*ERI - indeks rezistencije na ASE*; *group I vs. group II/*grupa I vs. grupa II*, p<0,001, group I vs. group III/*grupa I vs. grupa III/*, p<0,001, group II vs. group III/*grupa II vs. grupa III*, *p=0,056;* ** group I vs. group II/*grupa I vs. grupa II*, p=0,018, group I vs. group III/*grupa I vs. grupa III*, p<0,001, group II vs. group III/*grupa II vs. grupa III/*, p<0,001; † Chi-square (Cohrane – Armitage test for trend)/*Hi kvadrat test/*: p<0,001; ‡ group I vs. group III/*grupa I vs. grupa III*, p=0,029

Table 3. Parameters of nutrition and inflammation in relation to weekduration of hemodialysis treatment per week (mean ± S.D.)
Tabela 3. *Parametri nutricije i inflamacije u odnosu na ukupnu nedeljnu dužinu lečenja hemodijalizom (srednja vrednost ± S.D.)*

	Group I/*Grupa I* (12 h) N=160	Group I/*Grupa II* (15 h) N=20	Group III/*Grupa III* (≥17.5 h) N=26	p
BMI/*BMI*, kg/m²	24,1±4,3	24,7±4,4	25,7±4,9	0,223
CRP/*CRP*, mg/L	9,6±10,1	6,8±8,0	8,0±6,2	0,181
Serum albumin/*Serumski albumin*, g/L	38,2±2,8	40,7±2,3	42,3±2,5	<0,001*
Total cholesterol/*Ukupni holesterol*, mmol/L	4,5±1,0	4,7±1,4	5,1±1,0	0,017**
LDL cholesterol/*LDL holesterol*, mmol/L	2,6±0,7	2,5±1,0	2,9±0,9	0,163
HDL cholesterol/*HDL holesterol*, mmol/L	1,1±0,3	1,1±0,3	1,1±0,4	0,599
Triglycerides/*Trigliceridi*, mmol/L	1,8±0,8	2,1±1,3	2,4±1,6	0,110

* group I vs. group II/*grupa I vs. grupa II*, p<0,001, group I vs. group III/*grupa I vs. grupa III*, p<0,001; ** group I vs. group III/*grupa I vs. grupa III*, p=0,013, LDL - lipoprotein male gustine, HDL - lipoprotein veike gustine

lowest value was observed in the patients from group III and this difference reached the statistical significance. A significant difference was observed in ESAs dosing: ESAs agents were given more frequently in the patients from group I and less frequently in the patients from group III. There was no difference between average weekly doses of ASE among the groups including those patients who had it in therapy. There was a difference regarding ERI between groups I and III – the ERI was lower in group III.

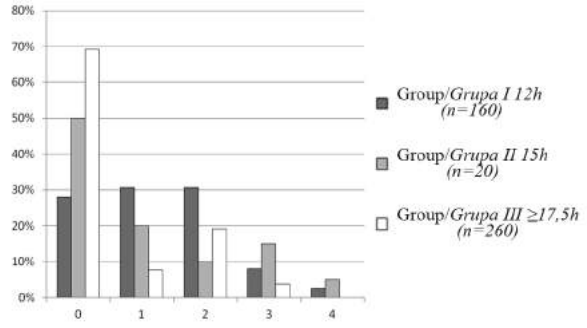
The parameters of nutrition and inflammation are shown in **Table 3**. BMI did not differ significantly among the groups. The highest value of serum albumin was found in the patients from group III and the difference was statistically significant compared to other two groups. There was no difference among the groups regarding CRP value. The patients from group III had significantly higher value of total cholesterol than the patients from group I. There were no differences among the groups in serum levels of low-density lipoprotein (LDL) and HDL cholesterol.

Parameters of mineral metabolism are shown in **Table 4**. The lowest values of intact parathyroid hormone (iPTH) were observed in the patients from group III while the highest values were found in the patients from group I and the difference was statistically significant. The patients in group I had the lowest value of serum calcium and it was statistically different compared to other two groups. There was no difference among the groups in values of serum phosphorus but the patients from group III used phosphate binder less frequently than the patients from two other groups. At the same time, the patients from group III had the highest cumulative yearly dose of calcium-carbonate which was significantly higher compared to the patients from group I. The patients from group III used the metabolite of vita-

Table 4. Parameters of mineral metabolism in relation to duration of hemodialysis treatment per week (mean ± S.D.)
Tabela 4. *Parametri metabolizma minerala u odnosu na ukupnu nedeljnu dužinu lečenja hemodijalizom (srednja vrednost ± S.D.)*

	Group I/*Grupa I* (12 h) N=160	Group I/*Grupa II* (15 h) N=20	Group III/*Grupa III* (≥17.5 h) N=26	p
iPTH/*iPTH*, pg/ml	446±500 (med 280)	363±382 (med 245)	336±481 (med 87)	0,041*
Calcium/*Kalcijum*, mmol/L	2,28±0,17	2,42±0,19	2,38±0,18	<0,001**
Phosphorus/*Fosfor*, mmol/L	1,60±0,41	1,45±0,30	1,62±0,49	0,137
Phosphate binders/*Vezivači fosfata*, %	84,4	85,0	53,8	<0,001†
Yearly cumulative $CaCO_3$ dose *Kumulativna godišnja doza* $CaCO_3$	1139,3±545	1371,2±678	1546,4±836	0,047‡
Vit D metabolites/*Vit D metaboliti*, %	50,6	65,0	19,2	0,020§
Yearly cumulative dose of vit D metabolites *Kumulativna godišnja doza metabolita vit D*	367,0±284	480,4±311	462,0±404	0,374

*group I vs. group III/*grupa I vs. grupa III*, p=0,011; **group I vs. group II/*grupa I vs. grupa II*, p=0,003, group I vs. group III/*grupa I vs. grupa III*, p=0,012; †Chi-square (Cohrane–Armitage test for trend)/*Hi kvadrat test*, p<0,001; ‡ group I vs. group III/*grupa I vs. grupa III*, p=0,036; § Chi-square (Cohrane–Armitage test for trend)/*Hi kvadrat test,* p=0,02; $CaCO_3$ - calcium-carbonate/*kalcijum-karbonat*; iPTH - intact parathyroid hromone/*intaktni paratiroidni hormon*



Graph 1. Cardiovascular morbidity score in relation to duration of hemodialysis treatment per week
Grafikon 1. *Skor kardiovaskularnog morbiditeta u zavisnosti od ukupne dužine trajanja nedeljnog lečenja hemodijalizom*

min D least frequently, but there was no significant difference among the groups in total dose of vitamin D metabolite per year.

Scores of cardiovascular morbidity are shown in **Graph 1**. The patients in groups I and II had higher cardiovascular morbidity score compared to the patients in group III. Compared to the average value of cardiovascular morbidity score there was a statistically significant difference. The patients in group III had the lowest value of cardiovascular morbidity score (0.58) compared to the patients in group II (1.05) and group I (1.26).

Discussion

During the early years of HD treatment, a session lasted for as long as 20 hours and it was conducted once or twice per week in hospital setting depending on residual kidney function and patient's symptoms [23]. This way of treatment depended on the equipment availability and the quality of dialysis material. Home HD, which was conducted three times per week, was introduced into clinical practice in 1964 and soon became far more efficient (excellent blood pressure control, rare intradialytic collapse, satisfactory nutrition, no neuropathy and almost full rehabilitation) [24, 25]. Simultaneously with the development of dialysis technology, the regime of hospital treatment changed and dialysis sessions became shorter. As the consequence of shorter dialysis session during the 1980s, patients and nephrologists faced numerous problems including frequent intradialytic symptoms, nausea after HD and lower quality of patient's life.

In our study, we classified 206 patients treated with chronic HD into 3 groups depending on overall dialysis duration per week – group I (12 hours), group II (15 hours) and group III (≥ 17,5 hours). The patients in group III were the youngest but they were treated by HD for the longest period of time – 207 months on average. Although the patients in group I were older, had diabetes mellitus more frequently and were dialyzed with low-flux membranes more often, their Kt/V was not significantly different compared to the patients in groups II and III. These data differ from other studies data, which could be the consequence of unequal groups examined regarding the number and characteristics of patients (residual kidney function). However, the patients with longer duration of dialysis procedure per week had better corrected acidosis, which was observed in other studies as well [10, 23].

Well-corrected anemia is one of crucial indicators of dialysis adequacy. This paper shows favorable effect of longer duration of HD on anemia correction considering the fact that the values of hemoglobin were higher in the groups with longer duration of HD treatment despite significantly less frequent use of ESAs (only 27% of patients in group III vs. 86% in group I) and lower values of ferritin. The patients with the longest duration of HD had significantly lower resistance to ESAs applied, which could be a possible explanation for their lowest average dose of ESAs. Ok et al. reported similar results. After a year of treatment, the patients who had 8 hours treatment three times per week experienced the increase of hemogloblin value and at the same time, there was a decrease in frequency of ESAs use – from 55% to 24.7% with simultaneous reduction of weekly ESAs dose [10]. The reason of favorable effect of longer dialysis on anemia in the setting of similar Kt/V is probably multifactorial. It is possible that longer HD leads to an increase in the clearance of middle molecules including the inhibitors of erythropoiesis [26].

Numerous studies, including this one, have indicated the importance of nutritional status for survival of dialysis patients. BMI values were not significantly different among the groups. However, the patients with longer duration of HD procedure were found to have significantly higher values of serum albumin and total cholesterol. These findings suggest better nutritional status of these patients, which is in accordance with data reported by other authors [10, 14, 15, 17, 27, 28]. Since CRP levels did not differ among the groups, better correction of anemia and nutritional status could not be the result of apparent or silent inflammation in our groups of patients. Better correction of acidosis in patients with longer duration of HD treatment may have contributed to better appetite and at the same time to better nutrition parameters. This study did not monitor leptine values but there is a possibility that prolonging the time of dialysis increases the elimination of leptin and consequently increases the appetite and parameters of nutrition.

This study confirmed the relationship between the control of mineral metabolism and duration of dialysis treatment. Lower values of iPTH were recorded in the patients from group III compared to the patients from group I (iPTH: 336 vs. 447, $p<0.05$) although the patients in group III used vitamin D metabolites least frequently (19 % of pa-

tients). The patients from group I had the lowest calcium value, which is in accordance with iPTH values. There was no significant difference among the groups in phosphorus values even though the patients from group III used phosphate binders least frequently (group I- 84,4% vs. group II-85% vs. group III-54%). There was no difference among the groups in cumulative yearly dose of vitamin D metabolites, but a yearly dose of calcium carbonate was significantly higher in group III compared to other two groups, which could be explained by better appetite of these patients and more liberal diet. Reduced frequency of phosphate binder prescription following the prolonged duration of dialysis procedure as well as an increase in serum calcium were observed by other authors [10]. Lower frequency of secondary hyperparathyroidism was recorded in Tassin group of patients as compared to other parts of France and Europe with less frequent use of phosphate binders [6].

The patients with the shortest duration of HD procedure had the highest cardiovascular morbidity score on average, which was statistically different in comparison to other two groups. In other words, the patients with longer duration of HD treatment were less likely to have any of cardiovascular diseases. As the result of less frequent morbidity, the lower mortality may be expected in patients treated with longer HD procedure. Numerous studies suggest lower mortality of patients whose HD procedures are longer than traditional four-hour dialysis session [10, 17, 19, 20, 29, 30].

This study has its weak points. Although the aim was to examine the impact of HD duration per week on adequacy parameters, the groups of patients were not homogenous by the type of dialysis membrane and dialysis techniques. Yet, Kt/V values did not differ among groups, which indicates a similar level of small molecules clearance. Elimination of molecules with higher molecular mass requires longer dialysis time, which justifies our criteria for creating groups according to dialysis duration. Namely, experience indicates that the application of high-flux membranes is not the only precondition for more adequate dialysis and centers which enable longer and slower dialysis have the best experience.

Conclusion

Patients treated with hemodialysis in duration longer than 12 hours had better dialysis adequacy expressed by better correction of acidosis and anemia, less frequent use of erythropoietin stimulating agents and lower resistance. At the same time, these patients had better correction of nutrition and mineral metabolism parameters with less frequent use/lower dose of phosphate binders and vitamin D metabolites. All these may be a possible reason for less frequent cardiovascular morbidity recorded in this group of patients.

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PROGNOSTIC FACTORS OF PRIMARY CUTANEUS MELANOMA

PROGNOSTIČKI FAKTORI ZA PRIMARNI KUTANI MELANOM

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Summary

Introduction. The purpose of this study was to identify tumor characteristics of primary malignant melanoma predictive of sentinel lymph node positive status, and then to determine whether sentinel lymph node status has an impact on recurrence and survival. **Material and Methods.** A total of 100 patients with primary malignant skin melanoma were analyzed. The prospective melanoma database identified patients with histologically confirmed cutaneus melanoma, clinically negative and clinically positive regional lymph nodes with no evidence of distant disease, who had undergone surgery between April 2001 and May 2012. Univariate and multivariate analyses were performed to assess factors that predict sentinel lymph node positive status, recurrence and survival. **Results.** We identified Breslow's thickness and lymphocytic response as independent predictors of sentinel lymph node status in cutaneous melanoma patients. Sentinel lymph node status was a significant predictor of disease free survival. **Conclusion.** Despite the limitation, this study confirms Breslow's thickness and tumor lymphocytic infiltration as two factors predictive of sentinel lymph node metastasis in cutaneous melanoma patients. We also found sentinel lymph node status to be the most significant independent predictor of disease free survival and identified sentinel lymph node status as an important variable to consider when estimating the risk of regional recurrence.

Key words: Prognosis; Recurrence; Melanoma; Skin Neoplasms; Sentinel Lymph Node Biopsy; Disease-Free Survival

Sažetak

Uvod. Cilj ovog rada bio je da se identifikuju tumorske karakteristike primarnog malignog melanoma koje predviđaju pozitivan status sentinelnih limfnih čvorova i da se zatim odredi da li njihov status ima uticaja na recidiv i preživljavanje. **Materijal i metode.** Studija je obuhvatila ukupno 100 pacijenata sa primarnim malignim kutanim melanomom. Na osnovu prospektivne baze podataka identifikovani su pacijenti sa histološki potvrđenim kutanim melanomom, klinički negativnim i klinički pozitivnim regionalnim limfnim čvorovima bez dokaza o udaljenoj bolesti, koji su operisani u periodu od aprila 2001. do maja 2012. godine. Izvedene su univarijantne i multivarijantne analize da bi se procenili faktori koji predviđaju pozitivan status sentinelnih limfnih čvorova, recidiv i preživljavanje. **Rezultati.** Identifikovali smo Breslovljevu debljinu i limfocitnu reakciju kao nezavisne prediktore statusa sentinelnih limfnih čvorova kod pacijenata sa kutanim melanomom. Status limfnih čvorova bio je značajan prediktor preživljavanja bez bolesti. **Zaključak.** Uprkos ograničenju, ova studija potvrđuje Breslovljevu debljinu i tumorsku limfocitnu infiltraciju kao dva faktora koji predviđaju metastazu na sentinelnim limfnim čvorovima kod pacijenata sa kutanim melanomom. Takođe smo utvrdili da je status sentinelnih limfnih čvorova najznačajniji nezavisni prediktor preživljavanja bez bolesti i identifikovali status sentinelnih limfnih čvorova kao značajnu varijabilu prilikom procenjivanja rizika od regionalnog recidiva.

Ključne reči: Prognoza; Rekurenca; Melanom; Karcinom kože; Biopsija sentinel limfnog čvora; Preživljavanje bez bolesti

Introduction

Most authors seem to agree that several clinical and pathological characteristics such as age, sex, tumor localization, Breslow's thickness, ulceration, mitotic count, vessel invasion and the presence of tumor infiltrating lymphocytes are independent prognostic factors for relapse and overall survival of the patients with skin melanoma [1–7]. The status of the regional lymph nodes draining the primary melanoma (sentinel lymph nodes – SLN) has also been referred to as a very important factor for patients' prognosis and overall survival. Thus, since its first introduction in 1992 by Morton, SLN biopsy has become widely regarded as a standard procedure in the staging of melanoma and it has proven to be the most important prognostic factor [8–14].

The purpose of this study was to identify tumor characteristics of primary malignant melanoma predictive of SLN positive status, and then to determine whether SLN status had an impact on recurrence and survival.

Material and Methods

A total of 100 patients with primary malignant skin melanoma were analyzed. The prospective melanoma database identified patients with histologically confirmed cutaneus melanoma, clinically negative and clinically positive regional lymph nodes with no evidence of distant disease, who

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Abbreviations

SLN – sentinel lymph node
DFS – disease free survival
OS – overall survival
ELND – elective lymph node dissection

had undergone surgery at the Clinic of Plastic and Reconstructive Surgery, Clinical Center, Skopje in the period from April 2001 until May 2012. An approval to conduct the study was obtained from the Institutional review board of the University Clinical Center in Skopje.

All patients included in the study were informed about the planned surgical procedure and gave their written informed consent.

The prospective melanoma database included patients who met the inclusion criteria for the study or for the control group, each consisting of 50 patients.

The patients in the study group met the following inclusion criteria: primary malignant histologically confirmed skin melanoma, without clinically present signs for metastases in the regional lymph nodes or distant metastasis. Exclusion criteria were the presence of any type of metastasis except of SLN, patients after any surgical procedure that could disrupt lymphatic drainage patterns from the primary site and pregnancy.

The patients in the control group met the following inclusion criteria: primary malignant histologically confirmed skin melanoma, with clinically present metastases in the regional lymph nodes, without signs for distant metastases. Exclusion criteria were the presence of distant metastasis.

All patients underwent routine preoperative examinations.

Thirty patients from the study group were intradermally injected with 1% solution of methylene blue at four places around the primary lesion 40 minutes before the surgery. During the surgery, the radical excision of the malignant melanoma lesion through uninvolved healthy tissue was made depending on the clinical finding (1–3 cm from the margins of the lesion). The skin defect was managed by three surgical techniques depending on the situation (direct suture, local surgical flap and free skin transplant).

A small skin incision superficial to the lymph group in which the region was drained was used to identify the blue colored afferent lymph vessels as well as the first blue colored lymph nodes. After the identification, the nodes were carefully removed and histologically examined.

The wound was closed with direct suture and passive drainage.

Due to technical limitations, only twenty of the patients from the study group underwent an intradermal injection of Technetium [Tc]-99m sulfur colloid at the site of the primary melanoma. After the injection, the patients were imaged using a large field of view γ camera until enough radioactivity accumulated in the SLN to allow localization, which generally takes between 30 and 60 minutes. Dynamic as well as static images were obtained to differentiate the first from the second echelon nodes. Anterior, posterior, and oblique images serve to localize the node in three dimensions. Once the SLN was visualized, the patient was taken to the operating room. The skin over the SLN was localized by a hand-held γ probe, and general or local anesthetic was administered. In order to facilitate identification of SLN, blue dye was used in conjunction with radiocolloid, using the same method as in the first 30 patients. Approximately 1 ml of 1% solution of methylene blue was injected into the skin at the site of primary melanoma. A small incision was made over the radioactive SLN, and all blue-stained and radioactive lymph nodes were removed. After removal of the last SLN, the γ probe was used to determine that bed counts were less than 10% of the *ex vivo* counts of the least radioactive SLN. This ensures that all radioactive SLNs have been removed.

The radical lymphadenectomy was performed as a separate procedure in the patients from the study group with SLN positive for metastasis (SLN+). The patients with SLN negative for metastasis (SLN-) did not undergo lymphadenectomy.

All patients from the control group underwent radical skin excision of the primary malignant lesion and radical lymphadenectomy of the regional lymph nodes with clinically present metastasis.

Pathohistological analysis was made on primary melanoma lesion and on lymph nodes sectioned at 2 mm intervals. Sections were evaluated after hematoxylin-eosin staining. The features routinely examined included Breslow's thickness (measured in millimeters), Clark level, the presence or absence of ulceration and histological type. Tumors were routinely assessed for lymphocytic infiltration and classified as brisk, non-brisk and absent according to criteria formulated by Clark et al. [15].

The removed lymph nodes were assessed for the presence of metastasis.

Demographic data about the age and sex of the patients, and location of the skin melanoma, number of harvested regional lymph nodes and number of positive regional lymph nodes were prospectively collected from all patients.

The patients were followed up for evidence of recurrence (local and regional) using physical examination, laboratory analysis and appropriate radiologic investigations.

The statistical analysis of the collected data was made using statistical program Statistical Package for the Social Sciences (SPSS) for Windows 17.0. In univariate analysis, the statistical comparison between groups was performed using the t test for continuous variables or the χ^2 test for categorical variables. Multivariate logistic regression was performed to identify clinical and pathologic features predictive of SLN metastases and to control potential confounding factors. Because of the limited data available, multivariate regression analysis was performed using the factors that were significant in the univariate analysis. Disease free survival (DFS) and overall survival (OS) were calculated from the date of SLN biopsy to the date of first recurrence, death, or last follow-up. The

survival distributions were estimated using the Kaplan-Meier method. Univariate comparisons or survival distributions were made using the long-rank test and multivariate comparisons using the Cox proportional hazard model. $P \leq 0.05$ was considered statistically significant in all analyses.

Results

A total of 50 patients in the study group underwent SLN mapping with 1% solution of methylene blue and SLNs were identified, removed and histologically examined. Of these, 14 (28%) had shown positive for metastases sentinel lymph nodes (SLN+) and 36 (72%) had shown negative for metastases sentinel lymph nodes (SLN-). The median age of patients was 52.5 years. Nineteen (38%) of them were males and 31 (62%) were females. There were no significant differences in age or sex between the SLN+ and SLN- patients.

Table 1 shows comparison of the analyzed demographic and tumor characteristics between the SLN + and SLN- patients within the study group. Using the t test for continuous variables or χ^2 test for categorical variables, we found that the median Breslow's depth was 2.9 mm in the SNL- patients, and 4.3 in positive SLN+. Positive SNL status was significantly correlated with tumor thickness (p=0.03). Breslow's level 4 was evaluated in 6 (42.9%) SLN+ patients, and in 10 (27.8%) SNL- patients. The variable of lymphocyte response collapsed into positive versus negative, and there was a significant difference between SLN+ and SLN- patients. Those with absent lymphocytic response were significantly more likely to have positive SLN status, compared with patients with positive lymphocytic response (p=0.05). Severe lymphocytic response was present in 17 (47.2%) SLN- patients, and in 3 (21.4%) SLN+ patients. There were no significant differences in other tumor characteristics evaluated (ulceration, histological type, location, the number of SLN harvested), although the upper extremity and the

Table 1. Demographic and tumor characteristics of patients in study group
Tabela 1. *Demografske i tumorske karakteristike pacijenata iz eksperimentalne grupe*

		SLN status, no/*Status SLČ*, br (%)*		
Characteristic *Karakteristika*	No of patients *Broj pacijenata*	Negative *Negativni* N= 36	Positive *Pozitivni* N= 14	P value *P vrednost*
Age, years, median/*Starost, godine, srednja*	50	52.5	51.5	0.88
Sex/*Pol*				
Male/*Muški*	19	12 (33.3)	7 (50)	0.44
Female/Ženski	31	24 (66.7)	7 (50)	
Tumor location/*Lokacija tumora*	50			0.23
Upper extremity/*Gornji ekstremitet*		6 (16.7)	5 (35.7)	
Lower extremity/*Donji ekstremitet*		27 (75)	7 (50)	
Trunk/*Trup*		3 (8.3)	2(14.3)	
Head and neck/*Glava i vrat*		/	/	
Histological type/*Histološki tip*	50			
Nodular/*Nodularni*	39	27 (75)	12 (85.7)	0.65
Amelanotic/*Amelanotični*	2	1 (2.8)	1 (7.1)	
Lentigo/*Lentigo*	3	2 (5.6)	1 (7.1)	
Superficial spreading/*Širi se po površini*	2	2 (5.6)		
Acral/*Akralni*	4	4 (11.1)		
Desmoplastic/*Dezmoplastični*				
Breslow's thickness, mm, median *Breslova debljina, mm, srednja*	50	2.9	4.3	0.03
Clark's level, median/*Klarkov nivo, srednji*	50	3	4	0.19
Ulceration (positive)/*Ulceracija (pozitivna)*	26	18 (50)	8 (57.1)	0.89
Lymphocytic response/*Limfocitna reakcija*				0.052
Positive/*Pozitivna*	39	31 (86.1)	8 (57.1)	
Negative/*Negativna*	11	5 (13.9)	6 (42.9)	
Mean no of harvested SLNs/*Srednji broj uzetih SLČ**	50	2.7	1.7	
Mean no of positive SLNs/*Srednji broj pozitivnih SLČ**	50	0	1.3	0.000
Recurrences (positive)/*Recidivi (pozitivni)*	13	5 (13.9)	8 (57.1)	0.004

SLČ –sentinel limfni čvorovi*

Table 2. Demographic and tumor characteristics of patients of the study group in comparison with demographic and tumor characteristics of patients in the control group
Tabela 2. *Demografske i tumorske karakteristike pacijenata iz eksperimentalne grupe u poređenju sa demografskim i tumorskih karakteristikama pacijenata iz kontrole grupe*

		SLN status/*Status sentinel limfnih čvorova*		
Characteristic *Karakteristika*	No of patients *Broj pacijenata*	Negative *Negativni* N= 50	Positive *Pozitivni* N= 50	P value *P vrednost*
Age, years, median/*Starost, godine, srednja*	100	52.5	56.5	0.68
Sex/*Pol*				0.23
Male/*Muški*	45	19 (38)	26 (62)	
Female/Ženski	55	31 (52)	24 (48)	
Tumor location/*Lokacija tumora*	100			0.08
Upper extremity/*Gornji ekstremitet*		34 (68)	22(44)	
Lower extremity/*Donji ekstremitet*		11 (22)	17 (34)	
Head and neck/*Glava i vrat*			1 (2)	
Trunk/*Trup*		5(10)	10 (20)	
Histological type/*Histološki tip*	100			
Nodular/*Nodularni*		39 (78)	44 (88)	0.215
Amelanotic/*Amelanotični*		2 (4)	2 (4)	
Lentigo/*Lentigo*		3 (6)	0 (0)	
Superficial spreading/*Širi se po površini*		2 (4)	0 (0)	
Acral/*Akralni*		4 (4)	3 (0)	
Desmoplastic/*Dezmoplastični*		0 (0)	1 (2)	
Breslow's thickness, mm, median *Breslovljeva debljina, mm, srednja*	100	3.0	4.25	0.02
Clark's level, median/*Klarkov nivo, srednji*	100	3	4	0.00
Ulceration (positive)/*Ulceracija (pozitivna)*	60	26 (52)	34 (68)	0.15
Lymphocytic response/*Limfocitna reakcija*				0.05
Positive/*Pozitivna*	68	39 (78)	29 (58)	
Negative/*Negativna*	32	11 (22)	21 (42)	
Mean no of harvested lymph nodes *Srednji broj uzetih sentinel limfih čvorova*	100	2.4	10.2	0.00
Mean no of positive lymph nodes *Srednji broj pozitivnih limfnih čvorova*	100	0.36	4. 29	0.00

trunk localization of the primary malignant melanoma was more frequent in SLN+ patients.

Table 2 shows the comparison of the demographic and tumor characteristics between the patients of the study and the control group. This comparison confirmed the tumor characteristics, Breslow's thickness and lymphocyte response to be significantly different in patients with metastases in the regional lymph nodes, (Breslow's thickness p=0.002, lymphocytic response p=0.005). The location of primary malignant melanoma on the trunk was more frequent than on the extremities in the patients with metastases in the regional lymph nodes.

We examined all variables in the study group by univariate logistic regression to assess the correlation with positive SLN status. Breslow's thickness significantly correlated with positive SLN status (p=0.04), and lymphocytic infiltration significantly correlated with negative SLN status (p=0.03). For each unit increase in Breslow's value, the odds of positive SLN biopsy results increased by about 1.4 times. Positive lymphocytic response decreased the

Table 3. Univariate regression analysis of factors predictive of SLN+ status
Tabela 3. *Univarijantna regresiona analiza faktora koji predviđaju status pozitivnih sentinel limfnih čvorova*

Variable *Varijabila*	B *B*	SE *SE*	Wald *Vald*	df	p	Odds ratio *Verovatnoća*	95% CI
Breslow's thickness/*Breslovljeva debljina*	0.33	0.16	4.28	1	0.039	1.40	1.02- 1.92
Lymphocytic response/*Limfocitna reakcija*	-0.096	0.44	4.72	1	0.03	0.38	0.16-0.91

Table 4. Multivariate regression analysis of factors predictive of SLN+ status
Tabela 4. *Multivarijantna regresiona analiza faktora koji predviđaju status pozitivnih sentinel limfnih čvorova*

Variable/*Varijabila*	B	SE	Wald	df	p	Odds ratio	95% CI
Breslow's thickness/*Breslov debljina*	0.38	0.18	4.13	1	0.042	1.46	1.013-2.093
Lymphocytic response/*Limfocitna reakcija*	- 0.81	0.48	2.80	1	0.094	0.44	1.172-1.148
Upper extr. Localization *Lokalizacija na gornjem ekstremitetu*	1.47	0.86	2.97	1	0.085	4.42	0.82-23.92
Trunk localization/*Lokalizacija na trupu*	0.90	1.14	0.62	1	0.43	2.46	0.26-23.02

Table 5. Cox regression analysis of factor predictive of disease free survival
Tabela 5. *Koks regresiona analiza faktora koji predviđaju preživljavanje bez recidiva*

Variable/*Varijabila*	B/*B*	SE/*SE*	Wald/*Vald*	df	p	Hazard ratio/*Rizik*	95% CI
SLN status/*Status SLČ**	-1.62	0.805	4.04	1	0.04	0.198	0.041-0.96

SLČ - sentinel limfnih čvorova*

odds of having positive SLN status to about 38% of that of the negative lymphocyte response (**Table 3**).

The other variables, Clark's level, histological type, tumor location, ulceration and number of SLN harvested, were not significantly correlated with positive SLN status.

We next used multivariate regression model that included the two significant factors from univariate analysis (Breslow's thickness, lymphocytic infiltration) and tumor location as well. Breslow's thickness remained the predictive factor of positive SNL status. Our model was statistically significant, χ^2 (4, N 50) =11.87 p=0.018. The model as a whole explained between 21% (Cox & Snell R Square) and 30% (Nagelkerke R squared) of the variance of SLN status and correctly classified 82% of cases. The strongest predictor of positive SLN status was Breslow's thickness. The odds ratio of 1.46 for Breslow's thickness indicate that for each unit increase in Breslow's thickness the odds of having SNL positive biopsy results increase by about 1.5 times (**Table 4**).

Recurrence and Survival

SNL status was the most significant predictor of DFS. SLN- patients had a 5-year DFS of 60% compared with 12.5% of SLN+ patients (p=0.027) (**Figure 1**). Negative SLN status had a significant effect on the increase of DFS and the reduction of the hazard rate of recurrence compared with positive SLN status. Negative SLN status reduced the hazard ratio of recurrence to about 19.8% of that of positive SLN status (**Table 5**).

There was no significant difference in 5-year overall survival (OS) between SLN+ and SLN- patients, although SLN- patients demonstrated survival advantage in 5-year overall survival. SLN- patients had a 5-year OS of 95.5% compared with 76.9% of SLN- patients.

When SLN status was not taken into consideration, the 5-year DFS was better for patients with lymphocytic response (33%) compared with those in whom lymphocytic response was absent (25%), but this was not statistically significant. Also the 5-year DFS was better for patients with Breslow's thickness ≤ 4mm (37.5%) compared with those with Breslow's thickness ≥ 4 mm (20%).

The 5-year overall survival was statistically different between the study and the control group, being 88.6% in the study and 58.2% in the control group (p = 0.000), as it was the 5-year disease free survival, being 30.8% in the study and 10% in the control group (p = 0.025).

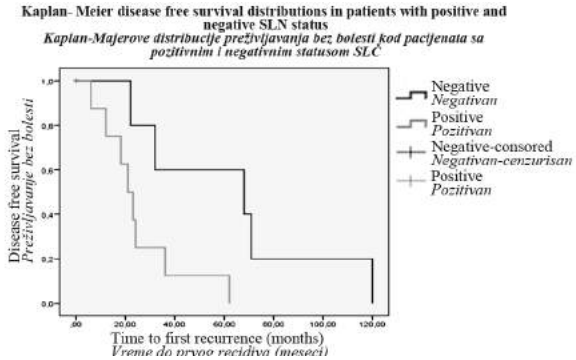

Figure 1. Kaplan-Meier disease free survival distributions in patients with positive and negative SLN status
Slika 1. *Kaplan-Majerove distribucije preživljavanja bez bolesti kod pacijenata sa pozitivnim i negativnim statusom sentinel lifmnih čvorova (SLČ)*

Discussion

The current study has identified Breslow's thickness and lymphocytic response as independent predictors of SLN status for cutaneous melanoma patients. Furthermore, we found an independent relationship between SLN status and local and regional recurrences.

According to the literature, Breslow's thickness, as the most important independent predictor for SLN status, is regularly used to identify patients for sentinel node biopsy [8]. For other tumor characteristics (localization, ulceration, mitotic count, vessel invasion and the presence of tumor infiltrating lymphocytes), there is a variability in the results and according to some studies, it might be a consequence of institution or re-

gion specific differences in measuring and reporting histological variables [1]. After a review of 910 patients with cutaneous melanoma who had undergone SLN biopsy, Peak et al. found that the Breslow's depth, younger age, angiolymphatic invasion, mitotic rate and trunk or lower extremity location were predictive of a positive SLN biopsy result [1, 16]. In a review of 682 melanoma patient who had undergone SLN biopsy, Kuper et al. found that only Breslow's thickness, tumor infiltrating lymphocytes and mitotic rate were predictive of positive SLN status [1, 17]. The significance and predictive value of tumor infiltrating lymphocytes in primary cutaneous melanoma has been examined in numerous previous studies. Lymphocytic response has long been considered a manifestation of host immune response to tumor, but its prognostic value remains controversy. Several studies have reported that the presence of tumor infiltrating limphocytes is associated with better prognosis [18, 19].

In this study, the results of comparing the data of SLN+ and SLN- patients within the study group showed that median Breslow's depth was 4.3 in SLN+ patients, and 2.9 mm in the SNL- patients. Breslow's thickness significantly correlated with positive SLN status (p=0.03). Breslow's level 4 was evaluated in 6 (42.9%) SLN+ patients and in 10 (27.8%) SNL- patients. Our study demonstrated an independent relationship between the lymphocyte response and the histological status of the SLN. Lymphocytic infiltration significantly correlated with negative SLN status (p=0.05). Severe lymphocytic response was present in 17 (47.2%) SLN- patients, and in only 3 (21.4%) SLN+ patients. The other variables, Clark's level, histological type, tumor location, ulceration and number of SLN harvested, were not significantly correlated with SLN status. Breslow's thickness was evaluated in multivariate analysis as well and found to be a predictor of SLN status even when other significant parameters were taken in consideration.

In addition, we compared the data on tumor and demographic characteristics of 50 patients with clinically present metastasis in regional lymph nodes (control group) with the data on patients having clinically negative regional lymph nodes from the study group (14 of them were SLN+). This comparison demonstrated that Breslow's thickness and lymphocyte response were significantly different between the two groups and confirmed their prognostic value. The trunk localization of the primary melanoma was more frequent than other localizations in the control group compared to the study group.

Our Clinic offers SLN biopsy to all patients as a routine preoperative procedure. For the mapping of SLN, 1% solution of methylene blue is used because our experience confirmed this technique as effective and easy to master, and we have not encountered allergic reactions to the dye. Complete lymphadenectomy is performed only in patients found to have positive SLNs. In this study, we found SLN status as a significant independent predictor of disease free survival (p=0.027). Five-year DFS was 60% in SLN- patients, whereas it was 12.5% in SLN + patients.

Our study also demonstrated a survival advantage, although not statistically significant, in 5-year overall survival (OS) in SLN-patients (95.5% in SLN- versus 76.9% in SLN+ patients). We found 28% of positive SLN rate, but all patients (not only patients with thick melanomas) underwent lymphatic mapping. We have to emphasize that 42.9% of the SLN + patients in our study were with thick melanomas (≥ 4 mm), while Breslow's thickness was <4 mm in 57.1% of them. These results confirmed our decision to include all patients, except those with Breslow's thickness ≤ 1mm in a routine preoperative lymphatic mapping and SLN biopsy. Numerous studies have reported survival for patients with thick melanomas and positive SLN status [9, 20]. In a review of 120 patients with thick melanoma, Kim et al. also found that SNL status was an independent factor predicting survival [9, 21]. SNL histology appeared to be the strongest predictor of survival in a retrospective analysis by Gershenwald et al. of 126 patients with melanomas thicker than 4 mm having undergone SLN biopsy. They reported 39% of positive SLN rate, that being in agreement with other recent studies, thus demonstrating occult regional lymph node metastases in 30% to 40% of patients with thick melanomas [9, 22]. Similar results were also found in an Australian review of patients with melanomas ≥4 mm thick performed by Thompson and Show who reported SLN positi-vity of 30% and 5-year overall survival of 74% in patients with negative SLN status and 41% in patients with positive SNLs [23].

According to the literature, the regional nodal basin is the first site of recurrence in 60% to 70% of melanoma patients with the clinically localized disease who develop metastases [24, 25]. Despite the intuitive appeal of removing the draining lymph nodes in patients with clinically localized disease, several prospective, randomized trials failed to demonstrate a survival advantage [26, 27]. Retrospective studies, however, suggest a survival benefit for patients undergoing elective lymph node dissection (ELND) compared with those undergoing only wide excision [28, 29]. Once regional nodal metastases are palpable, a patient's opportunity for long-term survival is reduced by 20% to 50% over those found to have microscopically positive lymph nodes at ELND. Our study demonstrated a reduction in 5-year overall survival as well as in the disease free survival in the patients with clinically palpable regional lymph nodes compared with patients with clinically negative regional lymph nodes (58.2% versus 88.6% in OS, 10% versus 30.8% in DFS). Patients positive to metastasis in regional lymph nodes had OS of 58.2% in our study, similar to the results presented by Weide et al. (60.1%) [30] and Balch et al. (69%) [31].

The limitation of this study was the small number of patients, which corresponds to the population in our country.

Conclusion

Despite the limitation of this study, we have identified two factors predictive of sentinel lymph node metastasis in cutaneous melanoma patients in

our country, which are Breslow's thickness and tumor lymphocytic infiltration. We also found that sentinel lymph node status was the most significant independent predictor of disease free survival and identified sentinel lymph node status as an important variable to take into consideration when estimating the risk of regional recurrence.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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MESENCHYMAL HAMARTOMA OF THE LIVER IN ADULTS – CASE REPORT

MEZENHIMALNI HAMARTOM JETRE U ADULTNOM DOBU – PRIKAZ SLUČAJA

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Summary

Introduction. Mesenchymal hamartoma of the liver is a benign lesion presenting as an enlarging abdominal mass in children less than 2 years of age. Fewer than 5% cases are present in individuals over 5 years of age, and this lesion is extremely rare in adults. It may affect the left or the right lobe of liver as a cystic or solid mass or both components may be present. The pathogenesis remains incompletely understood, but these lesions have generally been considered to represent a development abnormality in the bile duct plate formation. **Case Report.** In this report, we present a case of a 44-year-old man who was surgically treated at the Department of Abdominal, Endocrine and Transplantation Surgery of the Clinical Center of Vojvodina due to cystic lesion in the liver segment IV that had been verified by computed tomography imaging diagnostics. The patient was sent from a smaller health center with the diagnosis of echinococcosis. After the adequate preparation of the patient, surgical excision of the liver cystic lesion was done. Once a thorough histological examination had been performed, the diagnosis of mesenchymal hamartoma was made. **Conclusion.** Mesenchymal hamartoma of the liver is a benign tumor resulting from abnormal, intra-uterine development of bile ducts and has a delayed clinical manifestation, thus this lesion appears to be related to the processes of maturation. It is potentially premalignant lesion presenting as a solid and/or cystic neoplasm. Symptoms, laboratory results and radiographic imaging are nonspecific and inconclusive, so surgical excision of the whole lesion is the imperative for the definitive diagnosis.

Key words: Liver Neoplasms; Hamartoma; Adult; Diagnosis; Tomography, X-Ray Computed; Hepatectomy

Sažetak

Uvod. Mezenhimalni hamartom jetre je benigni tumor koji se manifestuje kao rastuća masa u abdomenu obično kod dece uzrasta do 2 godine. U manje od 5% slučajeva je dijagnostikovan kod uzrasta starijeg od 5 godina, dok je u odraslom dobu izuzetno redak. Može zahvatati levi ili desni režanj jetre, a javlja se kao solidni ili cistični oblik, ili kombinacija ove dve forme. **Prikaz slučaja.** U radu je prikazan slučaj pacijenta starosti 44 godine koji je hirurški lečen na Klinici za abdominalnu, endokrinu i transplatacionu hirurgiju Kliničkog centra Vojvodine zbog cistične promene u IV segmentu jetre koja je verifikovana kompjuterizovanom tomografijom. Pacijent je poslat iz zdravstvenog centra iz unutrašnjosti sa dijagnozom ehinokokne ciste. Nakon adekvatne pripreme pacijenta urađena je hirurška ekscizija ciste. Posle detaljnog patohistološkog pregleda postavljena je dijagnoza mezenhimalnog hamartoma jetre. **Zaključak.** Mezenhimalni hamartom jetre je benigni tumor porekla poremećaja razvoja žučnih vodova koji su nastali intrauterino, ali imaju odložene kliničke manifestacije, te se smatra da postoji povezanost ovih lezija sa procesima maturacije. To je potencijalno premaligna lezija koja može da bude solidnog i/ili cističnog izgleda. Kako je klinička slika nespecifična kao i laboratorijski i radiološki nalazi, radi dobijanja definitivne dijagnoze ovakvih promena jetre, hirurška ekscizija cele promene se nameće kao imperativ.

Ključne reči: Karcinomi jetre; Hamartom; Odrasli; Dijagnoza; CT; Hepatektomija

Introduction

Mesenchymal hamartoma of the liver is a benign lesion presenting as an enlarging abdominal mass in children less than 2 years of age, which is more frequent in boys [1]. This is the third most common liver tumor in this age group after hepatoblastoma and infantile hemangioendothelioma [2]. More than 50% of described cases are diagnosed before 1 year of age [3]. In less than 5% of cases, tumor was diagnosed after 5 years of age and though it is extremely rare in adults, if it appears, it is more frequent in female population. This tumor was first described by Maresch in 1903, but Ed-

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Abbreviations
CT – computed tomography
HCG – human chorionic gonadotropin

mondson was the first to use this name of the tumor in 1956 [4]. Mesenchymal hamartoma of the liver can be found in the left or right lobe either as a cystic or solid mass, although both components may be present. Concerning localization and structure, pediatric and adult populations have different characteristics. Mesenchymal hamartoma of the liver is more common in the left liver lobe in children, while in adults it is equally common in the left (about 40%) and the right lobe of liver (40%), and in about 20% of cases, tumor is in both lobes [5]. It has been noticed that mesenchymal hamartoma of the liver in male patients is more common in the right lobe, while in women it is more common in the left lobe of liver, and cases when the tumor is in both lobes is more frequent in women. From the histological point of view, mesenchymal hamartoma of the liver consists of epithelial and stromal components. The epithelial component consists of hepatocytes looking relatively normally and bile ducts, both being surrounded by myxoid and fibrous stroma. Hepatocytes are arranged in groups of different sizes maintaining architectonics of cell plates as in a normal liver. Bile ducts have typical branching layout and they are frequently surrounded by acute inflammatory infiltrate in the wall of bile duct. Cystic spaces, if there are any, can be coated by thinned to cuboidal epithelium or they can be nonepithelialized and surrounded by loose or dense fibrous stroma. Stroma contains a large number of vascular spaces, spindle-shaped and inflammatory cells. Normal ports are not present. In adults, stroma is more fibrous and more densely hyalinized by only focal myxoid areas. In some cases, mesenchymal component can be dominant in a lesion with infrequent ductal elements [6]. The pathogenesis of mesenchymal hamartoma of the liver remains incompletely understood, but one can say that these lesions have generally been considered to represent a development abnormality in the bile duct plate formation. Several cytogenetic studies have suggested that this tumor can appear with the creation of embryonic sarcoma of the liver [7].

Case Report

A 44-year old male patient was admitted to the Department of Abdominal, Endocrine and Transplantation Surgery of the Clinical Center of Vojvodina for the surgical treatment of a change in liver that had been verified by computed tomography (CT) imaging diagnostic in another institution. The CT image attached hereby showed that there was a relatively sharply encapsulated focal hypodense lesion in the liver segment IV, 29 x 31 x 35 mm in size with internally localized hyperdense ring change 5 mm thick. Right next to the described change, subcapsularly localized, there was a hypodense area 13 x 19 mm in size, hence the patient was diagnosed with echinococcus cyst

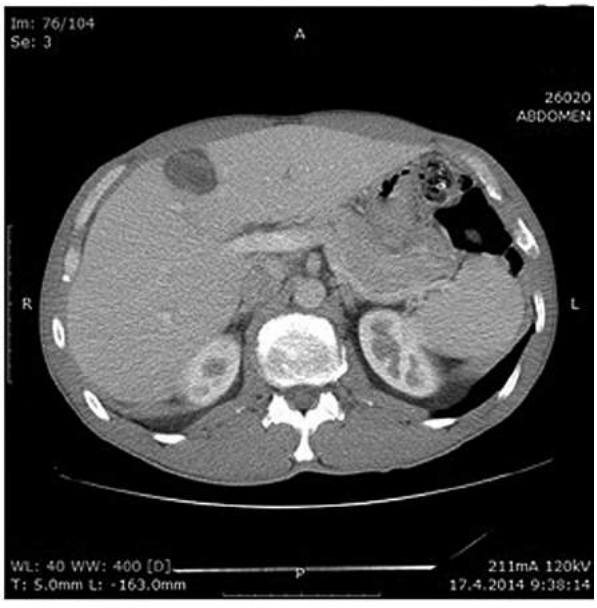

Figure 1. A change on the IV segment of the liver - hypodense lesion 29x31x35 mm in size with ring hyperdense zone and subcapsular hipodense zone
Slika 1. *Promena IV segmenta jetre - hipodenzna lezija dimenzija 29x31x35 mm sa prstenastom hiperdenznom i subkapsularnom hipodenznom zonom*

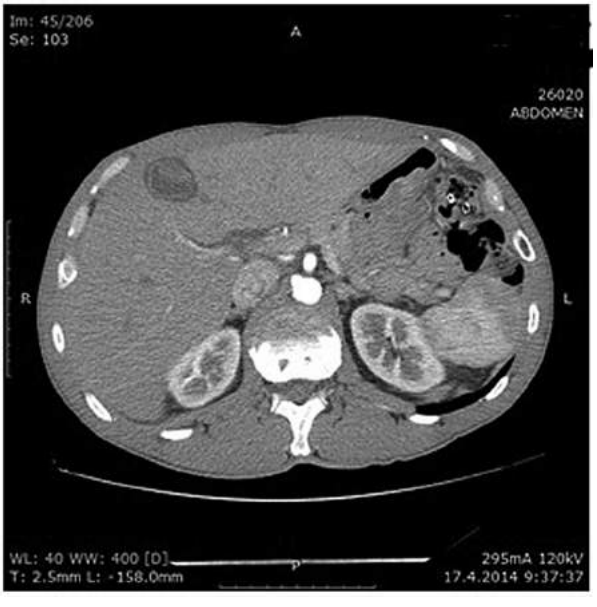

Figure 2. A change on the IV segment of the liver – hypodense lesion 29x31x35 mm in size with ring hyperdense zone and subcapsular hipodense zone
Slika 2. *Promena IV segmenta jetre - hipodenzna lezija dimenzija 29x31x35 mm sa prstenastom hiperdenznom i subkapsularnom hipodenznom zonom*

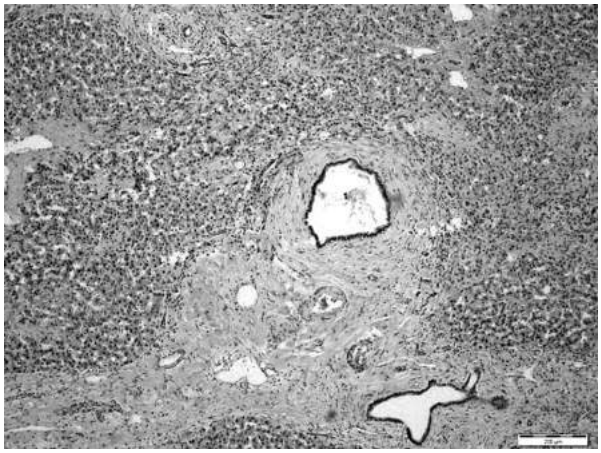

Figure 3. Slightly proliferated and dilated bile ducts (HE, magnification 40 x)
Slika 3. *Blago proliferisali i dilatirani žučni vodovi (HE, uvećanje 40x)*

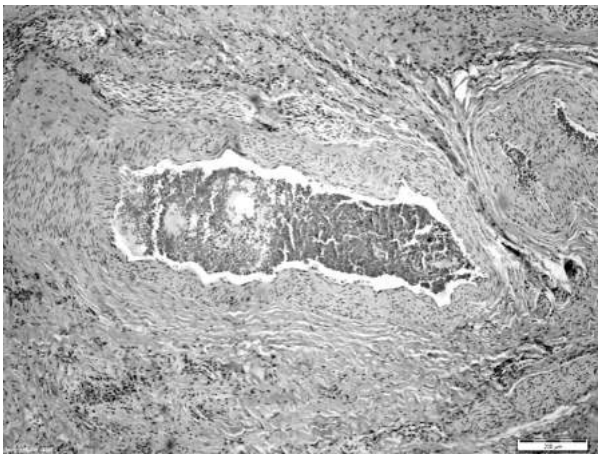

Figure 4. Dilated blood vessels with thickened wall (HE, magnification 40 x)
Slika 4. *Dilatirani krvni sudovi zadebljalog zida (HE, uvećanje 40x)*

(figures 1 and 2). The patient claimed to have had a numb pain in the stomach region under the right costral arch for the previous six months, but denied any other discomforts as well as any other hereditary diseases. After the clinical examination, laboratory tests were conducted which indicated serological negativity, therefore the referral diagnosis was rejected and the working diagnosis of tumor in the liver segment IV with indicated surgical treatment was made.

The laboratory affiliated to the Institute of Pathology and Histology of the Clinical Center of Vojvodina received operative material, a fragment of liver parenchyma, triangular in shape, 6.5 x 3.5 x 3 cm in size with dark pink, blurred capsule. A cystic formation 3.5 cm in diameter with yellowish, smooth inner surface surrounded by the liver parenchyma about 0.2 cm wide was seen on the intersection subcapsularly.

After classical material processing, histological preparations of cyst formations were obtained. Detailed pathohistological examination verified the existence of cystically dilated bile duct which was coated by focally multiplied cylindrical epithelial whose lumen contained a large amount of bile. Around the cystically dilated bile duct, there was a prominent connective tissue containing multiplied and partly dilated bile ducts **(Figure 3).** In addition to the described bile ducts, there were intersections of blood vessels with thickened wall and peripheral nerves (**figures 4, 5, and 6**). The proliferated connective tissue was partly and unclearly separated from the surrounding liver parenchyma which had appropriate histomorphological characteristics. The diagnosis of mesenchymal hamartoma of the liver was based on the typical morphological appearance of the change. In addition to the operative material,

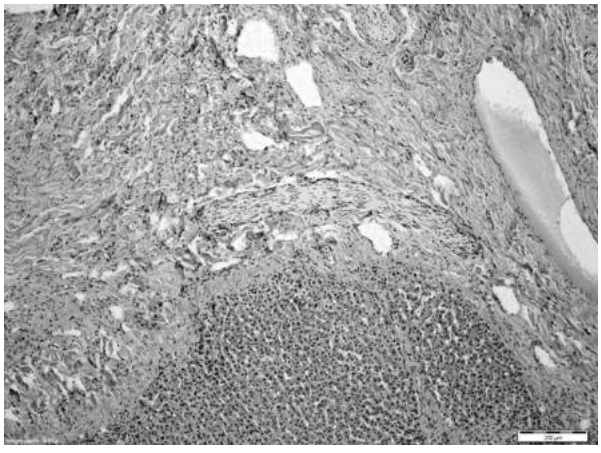

Figure 5. Connective, vascular and nervous tissue surrounding the liver parenchyma (HE, magnification 40 x)
Slika 5. *Vezivno, vaskularno i nervno tkivo koje okružuje jetrin parenhim (HE, uvećanje 40x)*

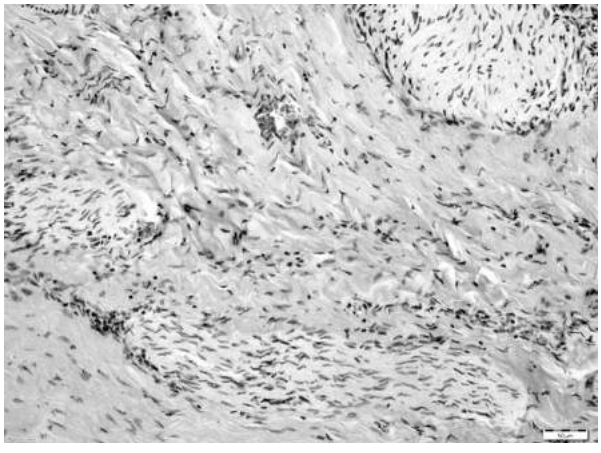

Figure 6. Peripheral nerves with proliferation of connective tissue (HE, magnification 100 x)
Slika 6. *Periferni nervi sa proliferacijom vezivnog tkiva (HE, uvećanje 100x)*

the gallbladder, whose pathohistological finding indicated chronic cholecystitis, was received.

The postoperative period and hospital treatment were uneventful so the patient was discharged after five days for home care and recovery with advice to have examinations and monitoring regularly.

Discussion

Mesenchymal hamartoma of the liver accounts for about 8% of all tumor changes in pediatric age group. The majority (about 80%) of cases are diagnosed before 2 years of age. Mesenchymal hamartoma of the liver can rarely appear in an adult liver, but there are only a few case reports in adults, more frequently in women [7]. The clinical picture is different and it depends on the age of patients. The most common clinical picture of pediatric patients is painless enlargement of the abdomen usually noticed by parents. If the enlargement of the abdomen is significant or liver function is compromised, there could be ascites and/or jaundice. In some cases, there could be compression of the diaphragm or lungs with consequential respiratory disorders. Adult patients are usually asymptomatic, although some authors have given case reports of patients who complained about discomforts such as diffuse abdominal pain [7], and some have presented patients who, in addition to diffuse abdominal pain, had clinical signs and symptoms of hepatomegaly, pain in the right hypochondrium and in the left upper quadrant as well [8]. Since the appearance of tumor can vary from cystic to completely solid mass as well as a combination of both components, there is no consensus on which form of tumor is more common. Some authors have reported results which show higher frequency of cystic form in children [2], whereas others claim quite the opposite, that is they have found a higher frequency of solid mass tumor in pediatric age group [5]. The situation with adult patients is similar. Chau et al. [5] have reported higher frequency of cystic tumors, and Hernandez et al. [9] have recorded higher frequency of cystic tumors in both adult and pediatric patients. It is believed that women are more prone to developing cystic tumors, and for men there is no tendency to any kind of tumor. However, it has been noticed that if there is a case of tumor which has both cystic and solid component, then it is more frequent in women.

Because of its non-specificity, mesenchymal hamartoma is extremely hard to be diagnosed by laboratory or other methods. In laboratory tests, alkaline phosphatase, β-human chorionic gonadotropin (HCG), serum transaminases as well as the α-fetoprotein are mostly within normal range in these patients. Radiograph of mesenchymal hamartoma can vary from predominantly cystic to completely solid change. If it is a cystic mass, differential diagnosis suggests echinococcus cyst and hepatic abscess in the first place [10]. Ultrasonography is usually the first in line of the radiological evaluation of the change to show the suspected liver mass to be either cystic or solid as well as its dimensions. CT imaging is the next step to show anechogenic cystic change with thin partitions. If a contrast is applied, a solid change will show; however, a cystic component does not show on contrast CT [11]. Thus, all imaging methods provide a nonspecific finding of mesenchymal hamartoma of the liver. A differential diagnosis allows various pathological processes in the liver such as a simple liver cyst, hydatid cyst, biliary cystadenocarcinoma or cystic metastases. If it is a solid mass, what follows is differential diagnosis of focal nodular hyperplasia, hepatic adenoma, cavernous hemangioma, angiomyolipoma as well as hepatocellular carcinoma. One should also take into consideration Von Meyenburgov complex (biliary hamartoma) which is histologically manifested by multiplying bile ducts surrounded by a more or less connective tissue which can be found as tiny nods 1-2 mm in diameter scattered on the liver parenchyma [12]. In most cases, the definitive diagnosis of mesenchymal hamartoma of the liver is made after surgical excision of the lesion, and extremely rarely by needle biopsy.

Since the tumor has the potential for massive growth as well as the potential for malignant alteration into embryonal sarcoma or angiosarcoma, the treatment of mesenchymal hamartoma of the liver is surgical excision of the tumor in operable patients. Although we live in an era of highly developed laparoscopic surgery, the gold standard in the treatment of mesenchymal hamartoma of the liver is liver resection with negative margins [1].

Conclusion

Mesenchymal hamartoma of the liver is a benign tumor resulting from abnormal, intra-uterine development of bile ducts and has a delayed clinical manifestation, thus this lesion seems to be related to the processes of maturation. It is potentially premalignant lesion presenting as a solid and/or cystic neoplasm. It predominantly appears in children before 2 years of age and it is extremely rare in adult population, which happened to be in our case. The clinical picture as well as laboratory and radiological findings are nonspecific. Needle biopsy is not a diagnostic method, therefore, surgical excision of the lesion and detailed pathohistological analysis are the imperative for the therapy and the definitive diagnosis.

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TICK ON THE TYMPANIC MEMBRANE

KRPELJ NA BUBNOJ OPNI

Dragoslava ĐERIĆ, Bojan PAVLOVIĆ, Miljan FOLIĆ, Srbislav BLAŽIĆ and Ljiljana ČVOROVIĆ

Summary
Introduction. Different foreign bodies can reach the lumen of the external auditory canal. Clinical presence of the foreign bodies depends on the nature of the foreign body, localization, morphological features, and the presence of pathological process. **Case Report.** This study gives a report on a rare foreign body – a tick on the eardrum, which is a very rare localization in European countries. **Conclusion.** Identification, determination of the nature of the foreign body and the way of extracting it depend on the application of adequate diagnostic and therapeutic approaches.
Key words: Ticks; Tympanic Membrane; Diagnosis; Ear Canal; Bites and Stings; Surgical Instruments; Drug Therapy

Sažetak
Uvod. Različita strana tela mogu dospeti u lumen spoljašnjeg slušnog hodnika. Klinička slika zavisi od prirode stranog tela, lokalizacije, morfologije spoljašnjeg slušnog hodnika i prisustva patoloških procesa u njemu. **Prikaz slučaja.** Rad prikazuje retko starno telo u uvu − krpelj u bubnoj opni. Ovo je redak slučaj krpelja sa ovakvom lokalizacijom na području Evrope. **Zaključak**. Identifikacija, određivanje prirode stranog tela i plana načina ekstrakcije zavisi od primene adekvatnih dijagnostičkih i terapijskih pristupa.
Ključne reči: Krpelji; Bubna opna; Dijagnoza; Ušni kanal; Ujedi i ubodi; Hirurški instrumenti; Terapija

Introduction

Different foreign bodies can reach the lumen of the external auditory canal. Clinical presence of foreign bodies depends on a few factors (the nature of foreign body, localization, morphological features, and the presence of pathological process). Identification, determination of the nature of the foreign body and the way of extracting it depend on the application of adequate diagnostic and therapeutic approaches.

The aim of this case report is to present a rare foreign body – a tick on the eardrum in a seven-year old girl, who was admitted to hospital after one-month outpatient treatment due to repeated bleeding from the ear. This is a very rare case of tick on the eardrum in European countries.

Case Report

A seven-year old girl was an outpatient in the regional medical centre for a month due to repeated bleeding from the right ear.

The child was treated with local therapy and unsuccessful removal of an unidentified foreign body. During the intervention, massive bleeding occurred and then she was sent to the Ear, Nose and Throat (ENT) Department with the glomus tympanicus diagnosis.

The patient did not complain of discharge, tinnitus or hearing loss.

Otoscopic and otomicrosopic check-up showed a foreign body, situated in the upper part of the right external auditory canal, next to eardrum. Since a leg had been identified, it was concluded that the foreign body was an insect. The external auditory canal and tympanic membrane showed normal morphology, without any sign of bleeding and hematoma. A blood sample was taken for serology.

Both otomicroscopic examination and extraction of the foreign body by microforceps were carried out under short general anesthesia. A dead tick was identified in the upper posterior region of the eardrum near to annulus, without perforation. Parasitological analysis confirmed a hard tick - Rhipicephalus sanguineus (female) (**Figure 1**).

Systemic antibiotic therapy (ceftriaxone) and local treatment were administered postoperatively. The postoperative course had no complications, and audiometry confirmed normal hearing. During the six-month follow-up period, the child's condition was good.

Serological analysis on Borrelia burgdorferi was negative and further serological analyses were not suggested by infectologist.

Informed consent had been obtained from the child's parents prior to the procedures performed.

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Discussion

Ticks are blood-sucking ectoparasites of the class Arachnida. They are classified as hard ticks and soft ticks, depending on their covering cuticles. They feed with human and animal blood, and can transmit pathogens such as rickettsiae and spirochetes, which induce spotted fever, Lyme disease, and spirochetal infectious disease. There is not much data about this kind of foreign bodies in the external ear canal and eardrum, and the most common cases have not been reported in European countries [1–8].

Careful otoscopy and otomicroscopy are necessary to identify foreign bodies. The shape and diameter of the outer ear canal can present an obstacle.

One of the most distressing experiences in case of a foreign body is having a live insect in the ear canal, especially in children. The insect's movement can cause a buzzing in the ear and may be quite uncomfortable. In some cases, a foreign body in the ear canal will go undetected. Sleeping on the floor or outdoors would increase the chance of this unpleasant experience.

Successful removal of foreign bodies depends on a number of factors such as the nature of foreign body, the cooperation of the patient during removal, the ability to visualize the foreign body, the equipment and tool available for the removal of foreign body, the experience and skill of otorhinolaryngologist [9].

Literature recommends different therapeutic procedures, such as mere inaction until a tick falls off spontaneously [4], extraction by microforceps [1, 2, 5], extraction with the skin around it, and extracting the abdomen of tick along with suction of the body fluid and the extraction of the whole body of the tick after three days [4].

Some authors recommend killing the tick by pouring warm water, mineral oil [2], 4% solution of lidocain [2] or glicerin [5] into the outer ear canal before the extraction of the tick .

The tick can be extracted from the canal under local anesthesia, while general short-term anestehesia is recommended for its removal from an eardrum in the pediatric population. Antibiotic therapy is recommended after extracting the tick [5, 6], or to treat complications [4].

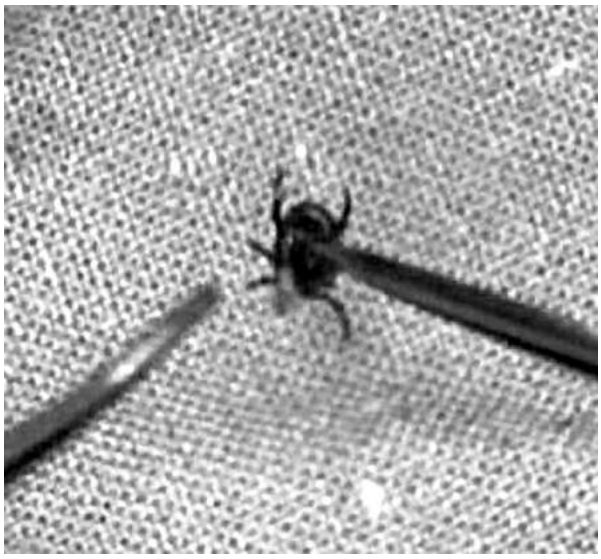

Figure 1. Removed tick - *Rhipicephalus sanguineus*
Slika 1. *Izvađeni krpelj - Rhipicephalus sanguineus*

Ticks and other foreign bodies in the outer ear can damage the eardrum and lead to inflammation of the middle ear [2].

There are reports of luxation of the incudomalleal or incudostapedial joints and the dislocation of the stapes from the oval window [7], but they are mostly the consequence of an attempt of extraction by inexperienced clinician.

Less common is the paralysis of a facial nerve as an effect of neurotoxin [2, 8]. Inadequate extraction, due to the pressure on the abdomen of a tick, can cause regurgitation of the content and cause the infection of the host [4].

Conclusion

In conclusion, in case of ear bleeding a differential diagnosis should include the existence of a tick on the tympanic membrane, and the accurate diagnosis is given with careful direct otoscopy and otomicroscopy. The right diagnosis and urgent treatment in referral centers prevent possible complications.

Our recommendation is extraction of a tick under general anesthesia by an experienced clinician.

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Case report
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A CASE REPORT OF LISTERIA MONOCYTOGENES MENINGOENCEPHALITIS IN GENERAL HOSPITAL "DR RADIVOJ SIMONOVIĆ" SOMBOR

PRIKAZ SLUČAJA MENINGOENCEFALITISA IZAZVANOG LISTERIJOM MONOCITOGENES U OPŠTOJ BOLNICI "DR RADIVOJ SIMONOVIĆ" SOMBOR

Snežana DELIĆ[1], Snežana BRKIĆ[2], Aleksandar DELIĆ[3] and Ivana B. ĆIRKOVIĆ[4]

Summary

Introduction. *Listeria monocytogenes* is one of the most common causes of bacterial central nervous system infections in adults. It often affects immunicompromised and elderly patients. Even with appropriate antimicrobial treatment, mortality due to *Listeria monocytogenes* meningoencephalitis is among the highest of all causes of bacterial central nervous system infections. **Case report.** We presented a previously healthy, 79-year-old farmer with typical clinical features of meningoencephalitis. The initial treatment with vancomycin and meropenem did not produce any clinical effect. On day six, *Listeria monocytogenes* was isolated from the cerebrospinal fluid and blood culture and identified by using conventional and automated microbiology methods. Antimicrobial susceptibility testing was performed by E test method. After bacterial isolation and identification, the administration of ampicillin and gentamicin was followed by the complete recovery of our patient. **Conclusion.** This case is presented to emphasize the negative outcome of empirical treatment when *Listeria monocytogenes* is not taken into consideration. Furthermore, the administration of ampicillin and gentamicin combination for treatment should be considered as the best therapeutic option in *Listeria monocytogenes* meningoencephalitis.

Key words: Meningoencephalitis*;* Listeria monocytogenes; Meningitis, Listeria; Central Nervous System Bacterial Infections*;* Aged; Male; Diagnosis; Anti-Bacterial Agents

Sažetak

Uvod. *Listeria monocytogenes* jedna je od najčešćih uzročnika bakterijskih infekcija centralnog nervnog sistema kod odraslih. Obično pogađa imunokompromitovane osobe ili pacijente u odmaklom životnom dobu. Čak i sa odgovarajućom antibiotskom terapijom, smrtnost od meningoencefalitisa koji izaziva *Listeria monocytogenes* je jedna od najviših među akutnim bakterijskim infekcijama centralnog nervnog sistema. **Prikaz slučaja.** U radu je prikazan poljoprivrednik star 79 godina, prethodno zdrav, sa tipičnom kliničkom slikom meningoencefalitisa. Terapija započeta vankomicinom i meropenemom nije dala kliničko poboljšanje. Šestog dana od prijema, *Listeria monocytogenes* je izolovana iz likvora i hemokulture i identifikovana korišćenjem konvencionalih i automatizovanih mikrobioloških metoda. Osetljivost na antimikrobne lekove ispitana je E-testom. Primena ampicilina i gentamicina, mada odložena, dovela je do potpunog oporavka bolesnika. **Zaključak.** Ovaj slučaj je predstavljen da bi se naglasio negativan ishod empirijskog lečenja bakterijskog meningoencefalitisa kada se ne sumnja na *Listeria monocytogenes*. Pored toga, kombinaciju ampicilina i gentamicina treba smatrati najboljim terapijskim izborom kod meningoencefalitisa izazvanih *Listeria monocytogenes*.

Ključne reči: Meningoencefalitis; Listeria monocytogenes; Meningitis, Listerija; Bakterijske infekcije centralnog nervnog sistema; Stari ljudi; Muško; Dijagnoza; Antibiotici

Introduction

Listeria monocytogenes is a Gram-positive, rod-shaped and facultative anaerobic bacterium. It is motile via flagella at room temperature and usually not at 37°C, but can move within eukaryotic cells by polymerization of actin filaments. *Listeria monocytogenes* is a facultative intracellular bacterium that can grow and reproduce inside the host's cells and is one of the most virulent food-borne pathogens [1]. Listeriosis is the leading cause of death among food-borne bacterial pathogens, with mortality rates surpassing even *Salmonella spp.* and *Clostridium botulinum* [2].

Listeria monocytogenes is the common cause of the central nervous system (CNS) infections and bacteremia in immunicompromised and elderly patients, as well as severe infections in pregnant women and their newborns. It can also cause gastroenteritis in healthy people who have ingested a large inoculum of the organism [3].

Few reports of *Listeria monocytogenes* infections are available from Serbia [4, 5] and hereby, a

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Abbreviations

CNS – central nervous system
CSF – cerebrospinal fluid
WBC – white blood cell
CT – computed tomography
PBPs – penicillin-binding proteins
MIC – minimum inhibitory concentration

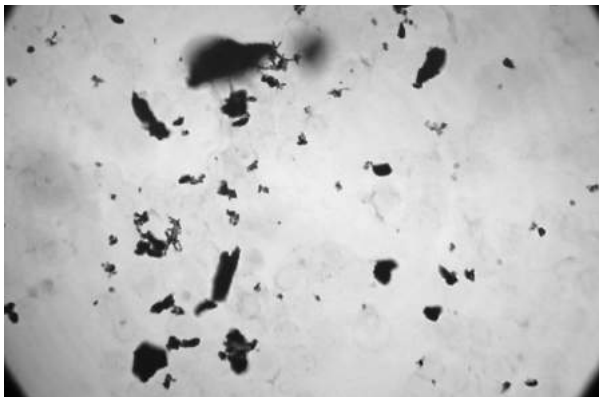

Figure 1. Direct Gram stained smear (blood culture) (1000x)
Slika 1. *Direktni preparat obojen po Gramu (hemokultura) (1 000 x)*

case of listerial meningoencephalitis from general hospital in Sombor is presented.

Case Report

A 79-year-old male farmer, previously healthy with a 3-day history of severe headache, nausea, vomiting and fever was admitted to the Department. On examination, he was febrile (38.9ºC), adynamic, dehydrated, with arterial blood pressure of 25/13 kPa, heart rate 100/minute, ventilation 25/minute. There were neck stiffness and positive Kernig's and Brudzinski's signs. Other results of physical check-up were normal. Leukocyte count was 16.100/mm^3 (89% neutrophils), platelet count, 141.000/mm^3; erythrocyte sedimentation rate 28 mm/hour; and C reactive protein 15 mg/dL. The values of aspartate aminotransferase (80 U/L), alanine aminotransferase (53 U/L), lactate dehydrogenase (866 U/L), urea (56 mg/dL), creatine (1.1 mg/dL) were within normal ranges. On admission, the cerebrospinal fluid (CSF) was obtained from the patient and the analysis of slightly turbid CSF showed pleocytosis (white blood cell (WBC) 134/mm^3, 58% neutrophils and 42 lymphocytes), elevated concentrations of proteins (1.70 g/L) and decreased glucose concentration (2.3 mmol/L; simultaneous serum glucose 5.3 mmol/L). The patient was first treated with meropenem (2 g IV, q8 h) and vancomycin (1 g IV, q12 h). On day 4 after admission, the patient was still febrile. Meningeal syndrome was still present, and computed tomography (CT) scan showed cerebral edema and evidence of recent infarction.

On admission, blood culture, urine culture and CSF were taken for culturing. Blood culture and CSF were positive, while urine culture was sterile. Gram staining of the CSF and blood culture showed Gram-positive, short, non-sporing bacilli with diptheroid-like arrangement (**Figure 1**). For plating, Columbia agar (Torlak, Serbia) with 5% sheep blood and MacConkey agar (Torlak, Serbia) were used. No growth was seen on MacConkey agar, but large numbers of beta-haemolytic grey colonies were found on sheep blood agar. These colonies were identified as *Listeria monocytogenes* by using conventional microbiology methods and by Vitek2 System (bioMérieux, France) and confirmed by MALDI-TOF MS (Bruker Daltonics Inc, USA). The isolate was sensitive to ampicillin (minimum inhibitory concentration (MIC) $\leq$ 0.125 μg/mL), trimethoprim-sulfamethoxazole (MIC $\leq$ 0.125 μg/mL), chloramphenicol (MIC $\leq$ 0.125 μg/mL), gentamicin (MIC $\leq$ 0.064 μg/mL) and vancomycin (MIC $\leq$ 0.73 μg/mL).

After *Listeria monocytogenes* isolation on day 6, the therapy with ampicillin (2 g IV, q4 h) and gentamicin (120 mg IV) was administered. The day after, the patient was afebrile and the symptoms of meningeal syndrome started to disappear. A week after the treatment with ampicillin and gentamicin had begun, CSF analysis showed 40 WBC per mm^3 (15.5% neutrophils and 84.5% lymphocytes), proteins of 0.35 g/L and glucose of 3.4 mmol/L (glycemia 5.4 mmol/L). The control CT scan was normal. After 21 days of treatment with ampicillin and gentamicin, the patient fully recuperated and was discharged from hospital.

Discussion

Bacterial meningoencephalitis is one of the most severe conditions in medicine, with the mortality rate going up to 30% [6]. A prerequisite for the favorable outcome of this disease is early administration of adequate antimicrobial therapy, which usually indicates an empirical treatment. Recommended primary treatments for community-associated bacterial meningoencephalitis in adults include third generation cephalosporins and vancomycin, with the addition of ampicillin or amoxicillin in the circumstances implying possible *Listeria monocytogenes* cause of the infection, e.g. older age or diseases with insufficient cellular immunity [7, 8]. *Listeria monocytogenes* is one of the most common causes of acute bacterial CNS infections, after *Streptococcus pneumoniae* and *Neisseria meningitides,* and the frequency rate is 4-12% in a number of ountries in the northern hemisphere [9–11].

In accordance with the first prospective study of community-associated *Listeria monocytogenes* meningitis in adults, immunodeficiency or older age were characteristic for all patients [12]. Even with appropriate antimicrobial treatment, mortality due to *Listeria monocytogenes* meningoencephalitis is among the highest of all causes of bacterial CNS infections [13]. This patient was an old man, with insignificant medical history, and with no obvious reason for immunodeficiency except his old age. Predictably, there

were no epidemiologic clues implying *Listeria monocytogenes* infection, which is mostly sporadic and food-borne by various types of food [1].

The treatment of our patient was commenced with meropenem and vancomycin (with proven ineffectivity *in vivo* against listeriosis) [4, 14] which is a common de-escalation therapy used in the hospital. *Listeria monocytogenes* harbors five penicillin-binding proteins (PBPs), and previous investigation has shown that PBP3 is a target for beta-lactam antibiotics in this bacterium [14]. However, cephalosporins have low binding capacity for PBP3 in *Listeria monocytogenes*, even though they are proven inhibitors of other PBPs, and they are not the drug of choice for listeriosis. It has also been shown in earlier studies on imipenem-resistant *Listeria monocytogenes* strains that mutation of gene coding PBP3 is responsible for decreased susceptibility of the strains to the carbapenems [4, 14]. In addition, Stepanovic et al. reported meropenem therapy failure in *Listeria monocytogenes* infection which was explained in the same way as in the previous study, i.e. mutation of PBP3 gene [4]. This is a possible reason for the lack of success in treatment with meropenem in our patient. Ampicillin or amoxicillin is the most active antibacterial agents in treatment of *Listeria monocytogenes* meningitis and meningoencephalitis [15]. Moreover, the results from *in vitro* studies showed that aminoglycosides, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole are also effective in *Listeria monocytogenes* infections [16]. Regrettably, most antibacterial agents are not bactericidal or have low intracellular concentration capacity [1, 15, 17]. The clinical practice shows that the combination of ampicillin or amoxicillin and gentamicin is still the best option [15] and it is precisely these two antibiotics that are used to treat our patients.

Conclusion

Listeria monocytogenes is one of the most common causes of bacterial central nervous system infections in immunocompromised or elderly patients. Listeriosis still presents a diagnostic and therapeutic challenge. This case is presented to emphasize the negative outcome of empirical treatment when *Listeria monocytogenes* is not taken into consideration. Furthermore, the administration of the ampicillin and gentamicin combination for treatment should be regarded as the best therapeutic option in *Listeria monocytogenes* meningoencephalitis.

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Case report
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GIANT ANTROCHOANAL POLYP – CASE REPORT AND LITERATURE REVIEW

DŽINOVSKI ANTROHOANALNI POLIP – PRIKAZ SLUČAJA I PREGLED LITERATURE

Karol V. ČANJI[1] and Slobodan M. MITROVIĆ[1,2]

Summary

Introduction. Antrochoanal polyp is a benign tumor of the maxillary sinus mucosa passing through the sinus ostium into the nasal cavity. Nasal obstruction is the most common symptom in all patients. **Case Report.** The authors present a case of a 28-year old female who was admitted to hospital with breathing difficulty, unilateral nasal secretion, headache and deformity of the nasal pyramid. Computerized tomography examination of the nose and paranasal sinuses indicated a possibility of giant antrochoanal polyp. The antrochoanal polyp was extirpated completely using forceps, under general endotracheal anesthesia. The length of the giant polyp was 16 cm. A follow-up examination of the nose and the right maxillary sinus was performed using a rigid endoscope, but no remains of the polyp were found. **Conclusion.** The authors believe that this is probably the first or a very rare published case of complete extirpation of a giant antrochoanal polyp of this size.

Key words: Nasal Polyps; Maxillary Sinus; Nasal Obstruction; Adult; Female; Nasal Mucosa; Tomography, X-Ray Computed; Endoscopy; Signs and Symptoms

Sažetak

Uvod. Antrohoanalni polip je benigni tumor sluznice maksilarnog sinusa koji prolazi kroz ostijum sinusa u nosnu šupljinu. Nazalna opstrukcija je najčešći simptom kod svih pacijenata. **Prikaz slučaja.** Autori prikazuju slučaj dvadesetosmogodišnje žene koja je primljena u bolnicu zbog otežanog disanja, unilateralne nazalne sekrecije, glavobolje i deformacije nosne piramide. Ispitivanje nosa i paranazalnih sinusa kompjuterizovanom tomografijom upućivalo je da se radi džinovskom antrohoanalnom polipu. Antrohoanalni polip je odstranjen u celini hvatalicom, u opštoj endotrahealnoj anesteziji. Dužina džinovskog polipa bila je 16 cm. Pregledom nosa i desnog maksilarnog sinusa rigidnim endoskopom ostaci polipa nisu nađeni. **Zaključak.** Autori veruju da je ovo verovatno jedan od retkih objavljenih slučajeva džinovskog antrohoanalnog polipa ove veličine, odstranjenog u celini.

Ključne reči: Polipi nosa; Maksilarni sinus; Nazalna opstrukcija; Odrasli; Žensko; Nosna sluznica; CT; Endoskopija; Znaci i simptomi

Introduction

Antrochoanal polyp is a benign change of the mucous membrane of the maxillary sinus. During its growth, it passes through the sinus ostium and extends into the nasal cavity and through choana, into the gullet [1]. It represents 4 to 6% of all nasal polyps, and it was first described by G. Killian in 1906. Antrochoanal polyp often occurs in children and young adults and it is almost always unilateral [2]. Nasal obstruction is a symptom found in all patients. This symptom is sometimes accompanied by headaches, foreign body sensation, unilateral rhinorrhea, and snoring [1–4]. Recurrent epistaxis is a problem occurring in angiomatous polyps which have highly vascular stoma with multiple dilated blood vessels in their pathohistological structure [5]. Before a diagnosis is made, the mass may disguise the real origin of the polyp. For this reason, it is necessary to consider possibility of antrochoanal polyp formation in patients who have undergone a surgery or had a trauma and computerized tomography indicates a mass inside the maxillary sinus [6, 7]. Diagnosis of the disease is made using anterior rhinoscopy, nasal endoscopy, standard radiography, computerized tomography and magnetic resonance [8, 9]. Pathohistological diagnosis is mandatory after polyp extirpation, especially because inverted papilloma may present as a polyp [10].

Case report

A 28-year old female reported to an ear, nose and throat (ENT) specialist complaining of breathing difficulty, unilateral nasal secretion, headache and deformity of the nasal pyramid, which she had been experiencing for one year. Examination of the nose showed that the right side of the nose was obstructed by a large polypous formation, moving the nasal pyramid to the left. The same change was found during oropharynx examination. Preoperative computerized tomography indicated a complete obstruction of the right side of the nose and maxillary sinus due to a soft tissue tumor (**Figure 1**). Anterior rhinoscopy and endos-

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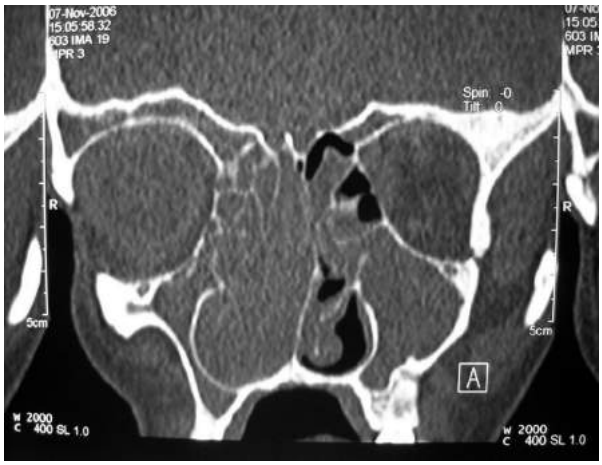

Figure 1. Computerized tomography finding
Slika 1. *Nalaz kompjuterizovane tomografije*

copic examination of the left side of nose indicated a prominent swelling of the mucous membrane of the nose without polypoid changes. Significant mucosal thickening in all paranasal sinuses and complete occlusion of the ostiomeatal complex on the left were confirmed by computerized tomography of the nose and paranasal cavities. Under general endotracheal anesthesia, endoscopy of the nose was performed which indicated that the medial wall of the right maxillary sinus was destroyed by the mass of a giant polyp. A short separation from the neighboring structures was made. The antrochoanal polyp was extirpated completely through the oropharynx using forceps. The length of the giant polyp was 16 cm (**Figure 2**). A follow-up examination of the nose and right maxillary sinus was performed using a rigid endoscope (0, 40 and 70 degrees), but no remains of the polyp were found. After that, the septum was medially located and tamponaded to fix the septum in the medial line. The postoperative course was uncomplicated. Pathological findings proved that the formation was an antrochoanal polyp.

Discussion

Many pathological masses in the nose may look like antrochoanal polyp, for example dermoid cysts, meningoencephaloceles, teratomae or sphenochoanal polyps [8]. Diagnosis by clinical examination, computerized tomography and magnetic resonance imaging before extirpation of pathological masses, sinuses and nose is of utmost importance [1, 8, 9]. Giant antrochoanal polyps can become dangerous in case of auto amputation [11]. Obstruction of breathing may require urgent tracheostomy before the polyp removal [12]. Preoperative airway maintenance was performed by endotracheal intubation. Prevention of antrochoanal polyp recurrence was enabled by endoscopy of the nose and maxillary sinus. According to Freitas et al. [13], recurrence after polypectomy was

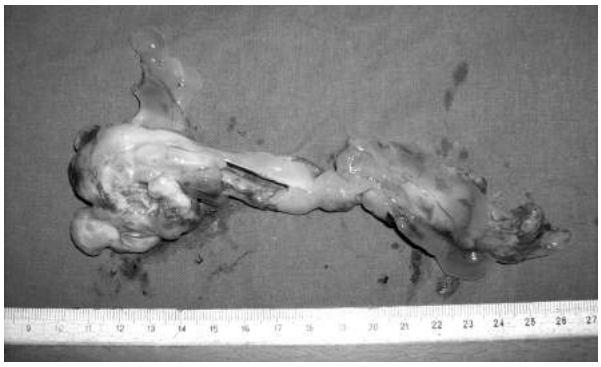

Figure 2. Extirpated giant antrochoanal polyp, 16 cm in size
Slika 2. *Odstranjeni džinovski antrohoanalni polip, dužine 16 cm*

up to 12.5%. Prevention of serious postoperative bleeding was carried out using tamponade of the nose. In the case presented here, the symptoms were common to nasal polyp, but the clinical findings and computerized tomography findings indicated existence of a giant antrochoanal polyp.

By searching published articles in the PubMed database, using keywords "antrochoanal polyp" for the ten year period (October 2004 – July 2014), 74 articles were found which contain terms "nasal", "choanal" and "antrochoanal" polyp in their headlines. They are related to children, young adults and adults. By adding terms "giant" for the same period, only five articles were found.

Živić et al. [14] extirpated a polyp of an "unusual size" of 8x5 cm by removing antral portion of the polyp applying the Caldwell-Luc procedure, and the epipharyngeal portion was extirpated by using forceps, through oropharynx. Yaman et al. [3] present a 9-cm polyp removed completely by functional endoscopic sinus surgery, which they believe is a safe and efficient method for antrochoanal polyp removal. Cetinkaya [15] reports a case of a giant antrochoanal polyp of 14 cm, removed completely through oropharynx using forceps, while the inferior portion was ligated with 1/0 silk. Kolwadkar et al. [12] removed a giant antrochoanal polyp of 15x4 cm completely through oropharynx. Bhat et al. [16] report a case of a giant antrochoanal polyp of 15 x 6 cm in an adult person, which was removed by endoscopy.

In the case presented in this paper, the polyp of 16 cm probably represents the longest giant antrochoanal polyp extirpated completely and without postoperative complications.

Conclusion

In cases of tumor of the nose and maxillary sinus, the existence of a giant antrochoanal polyp should be considered. The authors believe that this is a rare published case of a giant antrochoanal polyp extirpated completely and without postoperative complications.

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INDEKS AUTORA

INDEKS KLJUČNIH REČI

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UPUTSTVO AUTORIMA

Časopis objavljuje sledeće kategorije radova:

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

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

Priprema rukopisa

Propratno pismo

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

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

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

Rukopis

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

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

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

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

– prikaz slučaja: uvod, prikaz slučaja i zaključak;

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

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

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

4. Tekst rada

– Tekst originalnih članaka mora da sadrži sledeće celine:

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

– Tekst prikaza slučaja treba da sadrži sledeće celine: Uvod (sa jasno definisanim ciljem rada), Prikaz slučaja, Diskusija i Zaključak.

– Tekst treba da bude napisan u duhu srpskog jezika, oslobođen suvišnih skraćenica, čija prva upotreba zahteva navođenje punog naziva. Skraćenice ne upotrebljavati u naslovu, sažetku i zaključku. Koristiti samo opšte prihvaćene skraćenice (npr. DNA, MRI, NMR, HIV,...). Spisak skraćenice koje se navode u radu, zajedno sa objašnjenjem njihovog značenja, dostaviti na poslednjoj stranici rukopisa.

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

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

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

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

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

numeracija treba da odgovara redosledu pominjanja u tekstu.

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

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

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

Članci u časopisima:

∗ *Standardni članak*

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

∗ *Organizacija kao autor*

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

∗ *Nisu navedena imena autora*

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

∗ *Volumen sa suplementom*

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

∗ *Sveska sa suplementom*

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

∗ *Sažetak u Časopisu*

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

Knjige i druge monografije:

∗ *Jedan ili više autora*

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

∗ *Urednik(ci) kao autor*

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

∗ *Poglavlje u knjizi*

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

∗ *Rad u zborniku radova*

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

∗ *Disertacije i teze*

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

Elektronski materijal

∗ *Članak u Časopisu u elektronskoj formi*

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

∗ *Monografije u elektronskoj formi*

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

∗ *Kompjuterski dokument (file)*

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

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

Dozvoljeno je najviše šest priloga!

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

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

– Slike pripremiti u JPG, GIF TIFF, EPS i sl. formatu

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

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

– Svi prilozi biće štampani u crno-beloj tehnici. Ukoliko autori žele štampanje u boji potrebno je da snose troškove štampe.

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Prijem rukopisa vrši se u elektronskoj formi na stranici: **aseestant.ceon.rs/index.php/medpreg/**. Da biste prijavili rad morate se prethodno registrovati. Ako ste već registrovani korisnik, možete odmah da se prijavite i započnete proces prijave priloga u pet koraka.

8. Dodatne obaveze

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

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

INFORMATION FOR AUTHORS

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This journal publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

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3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 auto-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practise.

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The covering letter:

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– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine)

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

– original and professional papers should have the introduction (with the objective of the paper), material and methods, results and conclusion

– case reports should have the introduction, case report and conclusion

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

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

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– The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

– The text should be written in the spirit of Serbian language, without unnecessary abbreviations, whose first mentioning must be explained by the full term they stand for. Abbreviations should not be used in the title, summary and conclusion. Only commonly accepted abbreviations (such as DNA, MRI, NMR, HIV…) should be used. The list of abbreviations used in the text, together with the explanation of their meaning, is to be submitted at the last page of the manuscript.

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

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

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

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

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

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

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

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

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

Articles in journals

∗ *A standard article*

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

∗ *An organisation as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *Acomputer file*

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

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

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

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

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– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English language.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, ||, ¶, **, † †, ‡ ‡ .

– State the type of colour used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

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