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CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

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PREOPERATIVE HEMOGLOBIN AND URIC ACID LEVELS AS RISK FACTORS FOR ACUTE KIDNEY INJURY IN CARDIAC SURGERY PATIENTS

PREOPERATIVNE VREDNOSTI HEMOGLOBINA I MOKRAĆNE KISELINE KAO FAKTORI RIZIKA ZA AKUTNO OŠTEĆENJE BUBREGA KOD KARDIOHIRURŠKIH PACIJENATA

Miodrag GOLUBOVIĆ[1, 2], Andrej PREVEDEN[1, 2], Ranko ZDRAVKOVIĆ[2], Jelena VIDOVIĆ[1], Bojan MIHAJLOVIĆ[1, 2] and Jovan RAJIĆ[2]

Summary

Introduction. Acute kidney injury associated with cardiac surgery is a common and significant postoperative complication. With a frequency of 9 - 39% according to different studies, it is the second most common cause of acute kidney injury in intensive care units, and an independent predictor of mortality. This study aimed to investigate the importance of preoperative hemoglobin and uric acid levels as risk factors for acute kidney injury in the postoperative period in cardiac surgery patients. **Material and Methods.** The study included a total of 118 patients who were divided into two groups. Each group included 59 patients; the fist group included patients who developed acute kidney injury and required renal replacement therapy, and the second included patients without acute kidney injury. Types of cardiac surgery included coronary, valvular, combined, aortic dissection, and others. All necessary data were collected from patient medical records and the electronic database. **Results.** A statistically significant difference was found between the groups in preoperative hemoglobin levels (108.0 vs. 143.0 g/l, p = 0.0005); postoperative urea (26.4 vs. 5.8 mmol/l, p = 0.0005) and creatinine (371.0 vs. 95.0 μmol/l, p = 0.0005), acute phase inflammatory reactants C-reactive protein (119.4 vs. 78.9 mg/l, p = 0.002) and procalcitonin (7.0 vs. 0.2 ng/ml, p = 0.0005), creatine kinase myocardial band isoenzyme (1045.0 vs. 647.0 mg/l, p = 0.014); duration of extracorporeal circulation (103.5 vs. 76.0 min, p = 0.0005) and ascending aortic clamp during cardiac surgery (89.0 vs. 67.0 min, p = 0.0005). The exception was the preoperative uric acid level, where there was no statistically significant difference (382.0 vs. 364.0 μmol/l, p = 0.068). There was a statistically significant correlation between the use of inotropic agents and acute kidney injury development. **Conclusion.** There is a correlation between the preoperative low hemoglobin levels and postoperative acute kidney injury. There is no statistically significant correlation between the preoperative levels of uric acid and postoperative acute kidney injury.

Key words: Acute Kidney Injury; Cardiac Surgical Procedures; Preoperative Care; Hemoglobins; Uric Acid; Risk Factors; Renal Replacement Therapy; Postoperative Complications

Sažetak

Uvod. Akutno oštećenje bubrega udruženo sa kardiohirurškom operacijom česta je i značajna postoperativna komplikacija i sa učestalošću 9–39%, prema različitim studijama, predstavlja drugi najčešći uzrok akutnog zatajivanja bubrega u jedinicama intenzivne nege i nezavisan prediktor mortaliteta. Cilj ovog rada bio je da se ispita značaj preoperativnih vrednosti hemoglobina i mokraćne kiseline kao faktora rizika za razvoj akutnog oštećenja bubrega u postoperativnom periodu kod kardiohirurških pacijenata. **Materijal i metode.** Istraživanjem je obuhvaćeno ukupno 118 pacijenata, koji su podeljeni u dve grupe. Prvu grupu činilo je 59 pacijenata koji su razvili akutno oštećenje bubrega i koji su zahtevali terapiju zamene bubrežne funkcije, a drugu grupu je činilo 59 pacijenata bez akutnog oštećenja bubrega. Tipovi hirurgije bili su koronarna, valvularna, kombinovana, disekcija aorte i druge. Svi potrebni podaci o pacijentima su uzeti iz medicinske dokumentacije i iz elektronske medicinske baze podataka. **Rezultati.** Pronađena je statistički značajna razlika između grupa u odnosu na vrednosti preoperativnog hemoglobina (108 vs 143 g/l, p = 0,0005); postoperativnih vrednosti uree (26,4 vs 5,8 mmol/l, p = 0,0005) i kreatinina (371 vs 95 μmol/l, p = 0,0005), reaktanata akutne faze zapaljenja – C-reaktivni protein (119,4 vs 78,9 mg/l, p = 0,002) i prokalcitonina (7 vs 0,2 ng/ml, p = 0,0005), izoenzima CK-MB (1045 vs 647 mg/l, p = 0,014); kao i u vremenskom trajanju ekstrakorporalne cirkulacije (103,5 vs 760 min, p = 0,0005) i kleme ascendentne aorte (89 vs 67 min, p = 0,0005) tokom kardiohirurške operacije. Izuzetak su preoperativne vrednosti mokraćne kiseline, gde između ispitivanih grupa nije pronađena statistički značajna razlika (382 vs 364 μmol/l, p = 0,068). Otkrivena je statistički značajna korelacija između upotrebe inotropnih lekova i razvoja akutnog oštećenja bubrega. **Zaključak.** Postoji visok stepen korelacije između preoperativnih niskih vrednosti hemoglobina i posleoperativne pojave akutnog oštećenja bubrega. Ne postoji statistički značajna korelacija između preoperativnih vrednosti mokraćne kiseline i posleoperativne pojave akutnog oštećenja bubrega.

Ključne reči: akutno oštećenje bubrega; kardiohirurške procedure; preoperativna priprema; hemoglobin; mokraćna kiselina; faktori rizika; supstituciona terapija bubrežne funkcije; postoperativne komplikacije

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Abbreviations

CRP – C-reactive protein
CK-MB – creatine kinase myocardial band
KDIGO – Kidney Disease Improving Global Outcomes
BMI – body mass index
CSA-AKI– cardiac surgery-associated acute kidney injury

Introduction

Cardiac surgery, including coronary artery bypass grafting and valvular surgery, are among the most common surgical procedures, with over 2 million procedures performed annually throughout the world [1]. Postoperative mortality after cardiac surgery has declined significantly over the last two decades [2]. However, despite the advancement in operative techniques, peri- and postoperative care, postoperative complications have remained a significant problem. This is mainly due to an always-increasing number of older and high risk patients who are being selected for surgery [3], which is associated with a higher multiorgan dysfunction and extensive vascular disease. Postoperative complications affect the length of postoperative recovery, require greater staff engagement and significantly increase the overall treatment cost. Cardiac disorders, respiratory, neurological and hemorrhagic/thromboembolic complications, and liver and kidney failure are the most common complications [4, 5]. Furthermore, intra-hospital infections are also becoming increasingly reported as important complications [6].

Acute kidney injury and its poorly understood etiopathogenesis represents a significant problem in the treatment of cardiac surgery patients. The etiology is most likely influenced by multiple factors and depends on both the patient's general condition and the operative and postoperative treatment factors, including pharmacological therapy. Possible specific risk factors for the development of acute kidney injury include anesthesia-related problems, type of surgery and use of cardiopulmonary bypass, treatment in intensive care unit using nephrotoxic drugs and contrast agents, sepsis development and prolonged hemodynamic instability followed by hypotension [7]. The persistent risk factors associated with acute kidney injury are chronic kidney disease, albuminuria, hypertension, diabetes mellitus, advanced life age, and obesity.

The primary objective of this study was to investigate the significance of preoperative hemoglobin and uric acid levels as risk factors for the development of acute kidney injury after cardiac surgery.

The secondary objectives of the study were to test the hypothesis that certain postoperative values of renal function parameters (urea, creatinine), acute-phase inflammatory reactants (procalcitonin, c-reactive protein (CRP)), creatine kinase myocardial band (CK-MB) isoenzyme, as well as application of specific pharmacological therapy (inotropic and vasopressor agents) and extracorporeal circulation and ascending aortic clamps duration may represent risk factors for the development of acute kidney injury.

Material and Methods

A retrospective study was conducted at the Institute of Cardiovascular Diseases of Vojvodina using the medical records and hospital information system. A total of 118 patients, who underwent open heart surgery between January 2014 and December 2018, were included in the study. The first group included patients who developed acute kidney injury and required renal replacement therapy (group I, n = 59), while the patients without acute kidney injury in the postoperative period were in the second group (group II, n = 59). The patients were matched by age, gender, type of surgery and operative risk calculated by EuroSCORE II.

The diagnosis of acute kidney injury in group I was established according to Kidney Disease Improving Global Outcomes (KDIGO) criteria. Indications for continuous renal replacement therapy in the first group included uremia, circulatory blood volume overload, biochemical abnormalities, and acute kidney injury resistant to diuretics. Other indications for continuous renal replacement therapy were severe hemodynamic instability, severe systemic inflammatory response, cardiac decompensation, and any other organic disorder followed by acute kidney injury.

The main exclusion criteria for participation were:
– patients with incomplete medical history data;
– patients undergoing another type of cardiac surgery that did not require the use of extracorporeal circulation;
– patients who died intraoperatively.

Data analysis included:
• Medical history data:
– demographic characteristics (age and gender);
– cardiovascular risk factors (positive family history, smoking, arterial hypertension, hyperlipoproteinemia, diabetes mellitus);
• Physical examination:
– body height (cm); body weight (kg); body mass index (BMI) (kg/m^2);
• Laboratory blood tests:
– preoperative hemoglobin and uric acid levels;
– parameters of renal function after surgery (urea, creatinine, uric acid), with the highest serum values measured before the start of renal replacement therapy;
– postoperative values of procalcitonin, CRP, CK-MB isoenzyme;
• Surgery related parameters:
– type of cardiac surgery (coronary, valvular, combined, aortic dissection, and others);
– the urgency of the performed surgery (emergency or elective);
– duration of the extracorporeal circulation and ascending aortic clamp during surgery;
– use of inotropes and vasopressors, as well as their doses after surgery;
– outcome, i.e. mortality during hospital treatment.

Descriptive statistical methods, methods for testing statistical hypotheses, and methods for testing

Table 1. General characteristics of the study groups
Tabela 1. *Opšte karakteristike ispitivanih grupa*

	Group I/*Grupa I*		Group II/*Grupa II*		p value/*p vrednost*
	N	%	N	%	
Family history/*Porodična anamneza*	18	30,5	24	40,7	0.336
Hypertension/*Hipertenzija*	44	74,6	42	71,2	0.836
Smoking/*Pušenje*	18	30,5	22	37,3	0.560
Hyperlipoproteinemia/*Hiperlipoproteinemija*	17	28,8	21	35,6	0.554
Diabetes mellitus/*Šećerna bolest*	25	42,4	12	20,3	0.017
Type of surgery/*Tip hirurgije*					0.004
I – coronary/*I – koronarna*	16	27,1	32	54,2	
II – valvular/*II – valvularna*	16	27,1	15	25,4	
III – combined/*III – kombinovana*	15	25,4	11	18,7	
IV – aortic dissection/*IV – disekcija aorte*	9	15,2	1	1,7	
V – other/*V – druge*	3	5,1	/	/	
Emergency/elective surgery/*Hitna/elektivna operacija*	23/36	39,0/61,0	7/52	11,9/88,1	0.001

dependence were used to analyze the primary data. Descriptive statistical methods included measures of central tendency (arithmetic mean, median), measures of variability (standard deviation), and relative numbers (indicators of structure). To test the statistical hypotheses the following methods were used: t-test for two independent samples, and χ^2 test. From dependence analysis methods the following were used: Pearson's linear correlation coefficient and Spearman's rank correlation coefficient. Statistical hypotheses were tested at the level of statistical significance of 0.05.

Results

The study included 82 (69%) male and 36 (31%) female patients. The gender distribution in groups was as follows: in group I, there were 35 male (59%) and 24 female patients (41%), while in group II there were 47 male (80%) and 12 female patients (20%).

The average age of all participants was 64 years, while in the groups it was 65 (45 - 80) in group I, and 63 (35–78) in group II. There were 57 (48%) patients younger than 65 years, and 61 (52%) older than 65.

The following preoperative risk factors for the development of cardiovascular diseases were identified in group I: 30.5% of patients had a positive family history; 74.6% hypertension; 30.5% were smokers; 28.8% had hyperlipoproteinemia; while 42.4% of patients had diabetes mellitus. There were 39% of patients who underwent an emergency operation and 61% of patients underwent an elective surgery. The average BMI in this group was 28.63 kg/m^2.

In group II, the prevalence of these risk factors was: 40.7% of patients had a positive family history; 71.2% hypertension; 37.3% were smokers; 35.6% had hyperlipoproteinemia; 20.3% of patients had diabetes mellitus. There were only 11.9% of emergency operations versus 88.1% of elective. The average BMI in this group was 28.73 kg/m^2. None of the patients from group II developed acute kidney injury **(Table 1)**.

Table 2 shows the mean values of the numerical variables in both groups of the participants. There

Table 2. Comparison of the tested values of the numerical factors
Tabela 2. *Poređenje ispitivanih vrednosti numeričkih faktora*

	Group I/*Grupa I*	Group II/*Grupa II*	p value/*p vrednost*
Preoperative hemoglobin (g/l) *Preoperativni hemoglobin (g/l)*	108,0 (97,5 - 125,5)	143,0 (138,0 - 153,0)	0,0005
Uric acid (µmol/l)/*Mokraćna kiselina (µmol/l)*	382,0 (304,5 - 469,5)	364,0 (302,5 - 415,5)	0,068
Extracorporeal circulation (min) *Vantelesni krvotok (min)*	103,5 (86,0 - 143,0)	76,0 (60,5 - 100,5)	0,0005
Ascending aortic clamp (min) *Klema ascendentne aorte (min)*	89,0 (73,0 - 119,0)	67,0 (52,0 - 86,5)	0,0005
Urea (mmol/l)/*Urea (mmol/l)*	26,45 (19,0 - 37,6)	5,8 (4,5 - 7,3)	0,0005
Creatinine (µmol/l)/*Kreatinin (µmol/l)*	371,0 (256,0 - 485,0)	95,0 (81,0 - 119,0)	0,0005
Procalcitonin (ng/ml)/*Prokalcitonin (ng/ml)*	6,98 (1,95 - 22,33)	0,22 (0,11 - 0,63)	0,0005
CK-MB (mg/l)/*CK-MB (mg/l)*	1045,0 (498,0 - 2783,0)	647,0 (377,5 - 1275,0)	0,014
CRP (mg/l)/*CRP (mg/l)*	119,4 (69,9 - 213,0)	78,9 (43,8 - 108,15)	0,002

Legenda: CK-MB – kreatin kinaza MB frakcija, CRP – C-reaktivni protein

Table 3. Frequency of inotrope and vasopressor drugs use and acute renal failure development
Tabela 3. *Učestalost upotrebe inotropnih i vazopresornih lekova sa razvojem akutne bubrežne insuficijencije*

		Group I/*Grupa I*	Group II/*Grupa II*	Total/*Ukupno*
Vasopressors *Vazopresori*	Yes *Da*	40 67.8%	39 66.1%	79 66.8%
	No *Ne*	19 32.2%	20 33.9%	39 33.2%
Inotropes *Inotropi*	Yes *Da*	52 88.1%	36 61%	88 74.6%
	No *Ne*	7 11.9%	23 39%	30 25.4%

was a statistically significant difference between the mean values of preoperative hemoglobin, the postoperative values of renal function parameters (urea, creatinine), acute-phase inflammatory reactants (CRP and procalcitonin), CK-MB isoenzyme as well as the duration of both extracorporeal circulation and ascending aortic clamp during cardiac surgery ($p < 0.05$). The exception was the preoperative mean value of uric acid, where there was no statistically significant difference between the studied groups ($p = 0.068$).

The area under the receiver operating characteristic (ROC) curve showed that preoperative hemoglobin represents a good marker of postoperative acute kidney injury, with a cut-off value of 131.5 g/l with sensitivity and specificity of 83.1% and 94.9%, respectively.

Out of 118 participants, 66.8% received vasopressor medications and 74.6% inotropic medications. Using the χ^2 test for nonparametric data testing, an association between acute kidney injury after the postoperative therapeutic administration of vasopressor and inotropic drugs was investigated in both study groups **(Table 3)**.

In group I, 67.8% of patients received vasopressors, but there was no statistically significant association between vasopressor use and the occurrence of acute kidney injury. In the second group, 66.1% ($p = 1.000$) received vasopressors.

In group I, there was an association between the use of inotropes and the onset of acute kidney injury ($p = 0.001$). The percentage of patients who did not receive inotropes and developed acute kidney injury was 11.9%, while there were 88.1% of patients who developed acute kidney injury after receiving inotropes. The use of inotropes in group II was 61%.

Table 4 shows whether there was a statistically significant correlation of the listed numerical variables in the examined groups with the occurrence of acute kidney injury in the postoperative period. By calculating the Pearson's correlation coefficient, the following results were obtained:

– There is a statistically significant strong negative correlation of preoperative hemoglobin levels with the occurrence of acute kidney injury ($r = -0.740$; $p = 0.0005$), meaning that participants with lower preoperative hemoglobin levels were more likely to experience acute kidney injury after cardiac surgery.

– Acute kidney injury development was in a slight positive correlation with preoperative uric acid values ($r = 0.244$; $p = 0.008$), where higher uric acid values were a possible indicator of the development of acute kidney injury.

– There was a moderate positive correlation between the extracorporeal circulation and ascending aortic clamps time during cardiac surgery with acute kidney injury ($r = 0.339$; $r = 0.328$; $p = 0.0005$).

– Postoperative values of renal function parameters, urea and creatinine, are strongly positively

Table 4. Correlation between numerical variations within the group II
Tabela 4. *Ispitivanje korelacije između numeričkih varijabi unutar grupe II*

	Pearson's correlation coefficient *Pirsonov koeficijent korelacije*	p value *p vrednost*
Hemoglobin/*Hemoglobin*	-0,740	0,0005
Uric acid/*Mokraćna kiselina*	0,244	0,008
Extracorporeal circulation time/*Trajanje vantelesnog krvotoka*	0,339	0,0005
Ascending aortic clamps/*Kleme ascendentne aorte*	0,328	0,0005
Urea/*Urea*	0,828	0,0005
Creatinine/*Kreatinin*	0,708	0,0005
Procalcitonin/*Prokalcitonin*	0,416	0,0005
CK-MB/*CK-MB*	0,230	0,012
CRP/*CRP*	0,334	0,0005

Legenda: CK-MB – kreatin kinaza MB frakcija, CRP – C-reaktivni protein

Table 5. Risk factors for impaired renal function
Tabela 5. *Faktori rizika za oštećenje bubrežne funkcije*

Preoperative factors *Preoperativni faktori*	Intraoperative factors *Intraoperativni faktori*	Postoperative factors *Postoperativni faktori*
Chronic renal failure *Hronična bubrežna insuficijencija*	Duration of cardiopulmonary bypass *Trajanje kardiopulmonalnog bajpasa*	Cardiogenic shock *Kardiogeni šok*
Older age/*Starost*	Hypotension and vasopressors	Need for inotropes and vasopressors
Previous surgery	*Hipotenzija i potreba za vazopresorima*	*Potreba za inotropima i vazopresorima*
Prethodna operacija	Hypothermia/*Hipotermija*	Use of diuretics/*Primena diuretika*
Endocarditis/*Endokarditis*	Combined surgery	Bleeding/*Krvarenje*
Left ventricular dysfunction	*Kombinovana operacija*	Transfusion/*Transfuzija*
Disfunkcija leve komore	Systemic inflammatory response	Venous congestion
Endothelial dysfunction	*Sistemski inflamatorni odgovor*	*Venska kongestija*
Endotelna disfunkcija		Embolization
Diabetes mellitus		*Embolizacija*
Šećerna bolest		Sepsis/*Sepsa*
Anemia/*Anemija*		Respiratory failure and hypoxia
Exposure to contrast agents as part of the preoperative procedure		*Respiratorna insuficijencija i hipoksija*
Izlaganje kontrastnim sredstvima u sklopu pripreme za operaciju		

correlated with the occurrence of acute kidney injury (r = 0.828; r = 0.708; p = 0.0005).

– Acute-phase inflammatory reactants values, procalcitonin and CRP, measured postoperatively, were moderately positively correlated with the development of kidney injury (r = 0.416; r = 0.334; p = 0.0005).

– Finally, there is a small positive correlation between the measured postoperative values of CK-MB isoenzyme and the development of acute kidney injury (r = 0.230; p = 0.012).

In Group I, 25 (42.4%) of all participants had a fatal outcome within 60 days after surgery.

Discussion

Cardiac surgery-associated acute kidney injury (CSA-AKI) is a common and significant postoperative complication, with an incidence that varies between 9 – 39% [8, 9]. Among the causes of acute kidney injury in intensive care units, cardiac surgery is in the second place, just behind sepsis [10]. On the other hand, acute kidney injury represents one of the most common severe complications that occur after cardiac surgery and leads to a significant increase in morbidity and mortality, prolongs the length of stay in the intensive care and significantly increases the treatment cost [11, 12].

Studies have shown an association between acute kidney injury and subsequent development of chronic kidney disease, even though many cases of acute kidney injury are reversible within days or weeks after the onset. Although CSA-AKI is more common in patients with clearly diagnosed preoperative kidney disease, patients with preserved renal function are also at high risk [13]. Patients requiring some form of renal replacement therapy are at three times higher risk of developing end-stage chronic renal failure compared with those who do not require renal replacement therapy [14, 15]. The mortality rate in patients with renal replacement therapy is as high as 40–70% and is directly dependent on the number of episodes of acute renal failure during hospital treatment [16].

Although many different diagnostic algorithms and criteria have been used so far for the diagnosis of acute kidney injury, determining the actual incidence is difficult. Most of the existing criteria for the diagnosis of acute kidney injury are considered not sensitive enough, and therefore this significant entity remains under-recognized [17]. In clinical practice, the most commonly used algorithms for the diagnosis of acute kidney injury are Acute Kidney Injury Network, Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease, and KDIGO [18].

In cardiac surgery patients, KDIGO criteria are recommended for the diagnosis of acute kidney injury [19]:

Increase in serum creatinine ≥ 26.5 μmol/l within 48 hours;

Increase in serum creatinine ≥ 1.5 – 1.9 times baseline within 7 days;

Diuresis < 0.5 ml/kg/h for 6 hours.

The optimal moment for the initiation of renal replacement therapy in CSA-AKI is not clearly defined. The decision is based on patient's clinical characteristics, primarily hemodynamic status, volume overload, and biochemistry findings (azotemia, hyperkalemia, and acidosis). In our center, renal replacement therapy was used in patients with severe kidney injury (KDIGO stages 2 and 3).

The exact pathophysiology of CSA-AKI has not been clearly understood yet. It encompasses multiple factors that, through different mechanisms and to varying degrees, lead to damage. Factors related to cardiac surgery include kidney hypoperfusion, reperfusion injury after ischemia, neurohumoral activation, inflammation, oxidative stress, as well

as exposure to exogenous (nephrotoxic drugs) and endogenous (hemoglobin) nephrotoxins [10, 12].

Renal hypoperfusion is one of the most important factors responsible for the damage. The use of extracorporeal circulation during cardiac surgery uses non-pulsatile blood flow with low blood pressure and poor tissue perfusion and is also associated with hemodilution and sudden changes in body temperature. All these conditions have a cumulative negative effect on renal tissue and lead to structural damage to the renal tubules [20]. After extracorporeal circulation is stopped and normal circulation is established, after a period of ischemia, the kidneys regain increased blood volume, which may lead to reperfusion cell injury and cell death [21].

The use of extracorporeal circulation is associated with intravascular hemolysis, leading to an acute increase in the concentration of free hemoglobin in the blood, which can cause injury to the tubular epithelium [22]. Patients undergoing cardiac surgery are most often exposed to nephrotoxic drugs such as antibiotics (aminoglycosides and glycopeptides), non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors [23] and angiotensin receptor blockers.

In addition to all the above mechanisms of renal parenchyma injury, it was found that risk factors were contributing to the degree and severity of CSA-AKI **(Table 5)**. These factors can be divided into preoperative, intraoperative and postoperative, and each of them contributes differently to the damage. Preoperative risk factors mostly depend on the patient and cannot be modified. On the other hand, intraoperative and postoperative risk factors are variable and can be influenced. Altering the surgical procedure and type of surgery, avoiding nephrotoxic agents and blood transfusions can reduce the incidence of acute kidney injury [24].

Anemia is one of the most significant risk factors for the development of acute kidney injury. Anemia has negative effects on kidneys through several different mechanisms. The decreased oxygenation of the renal parenchyma, especially tubular epithelium, is the most important mechanism. Anemia, on the other hand, is associated with the use of erythrocyte transfusion, which itself is associated with several complications, including kidney injury [25]. The results of our study showed that there was a statistically significant difference in hemoglobin levels between patients with kidney injury and patients with preserved renal function. Such results are consistent with other studies [26, 27], which found that anemia significantly increases the incidence of acute kidney injury, and also prolongs the time spent in the intensive care and increases morbidity and mortality.

The association between low hematocrit and low hemoglobin levels during extracorporeal circulation with the development of kidney injury during the postoperative course has also been established [27]. Although anemia increases the risk of kidney injury in patients undergoing heart surgery, erythrocyte transfusion has been shown not to lead to improvement, but in some cases even to worsening of the clinical outcome [28, 29]. Khan and et al. [30] have concluded that both anemia and erythrocyte transfusions represent independent risk factors that increase the incidence of CSA-AKI. A study conducted by Karkouti et al. [31] showed that the optimal hemoglobin concentration during extracorporeal circulation, which maintains a balance between the benefits of hemodilution and lower release of free hemoglobin which is nephrotoxic on one hand and the risk of inadequate renal parenchyma oxygenation on the other, is 85 g/l.

The results of our study show that after cardiac surgery there is no statistically significant difference in uric acid levels between patients with kidney injury and patients with preserved renal function. This is in disagreement with previous studies [32, 33] which showed that uric acid represents a risk factor for worsening of renal function after coronary surgery and that this parameter can be used as a predictor of acute renal impairment after surgery. Lee et al. [34] came to the same conclusions and confirmed uric acid to be a risk factor and predictor of acute renal impairment after coronary surgery. The limit value for uric acid concentration above which a significant increase in the incidence of acute renal impairment was observed to be 330 μmol/l in males and 300 μmol/l in females.

Conclusion

There is a high degree of correlation between preoperative low hemoglobin levels and the postoperative onset of acute kidney injury. There is no statistically significant correlation between preoperative uric acid levels and the postoperative onset of acute kidney injury.

There is a moderate to a strong positive correlation between the postoperative levels of urea, creatinine, C-reactive protein, and procalcitonin with the occurrence of acute kidney injury after cardiac surgery.

The duration of the aortic clamp and extracorporeal blood pump, as well as the administration of inotropic drugs, also correlate with the onset of acute kidney injury in the postoperative period.

The results of this study indicate that measures should be taken to prevent acute kidney injury after cardiac surgery by adequate selection and preparation of patients needing surgery.

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THE EFFECTS OF AGE AND GENDER ON THE QUALITY OF LIFE AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

UTICAJ ŽIVOTNE DOBI I POLA NA KVALITET ŽIVOTA NAKON REKONSTRUKCIJE PREDNJEG UKRŠTENOG LIGAMENTA

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Summary

Introduction. The aim of the study was to determine whether there is was statistically significant difference in patients of different age and gender regarding quality of life and causes of postoperative knee instability after anterior cruciate ligament reconstruction. **Material and Methods**. The study included 776 subjects, aged 15 to 59 (average age 27 years), who we divided into groups by age and gender. All volunteered to complete the Knee injury and Osteoarthritis Outcome Score questionnaire. We examined the postoperative instability using clinical tests in order to determine its causes and incidence among the groups of patients. **Results and Discussion**. The average values related to pain during sports activities were the highest in the youngest group of patients, up to 18 years of age (81 points). Postoperative instability was reported by 27 patients (3.5%), more frequently by males. Among the causes of instability, the most common was a new sports injury, in 11 cases (40%), and inappropriate tunnel positioning in 7 (26%). The highest incidence of postoperative instability was found in the youngest group of patients, under the age of 18 years, (5%), and no such cases were found in patients aged over 41 years. **Conclusion**. There was no statistically significant correlation between the quality of life of younger and older patients after anterior cruciate ligament reconstruction, but there were differences in individual segments of the questionnaire. Postoperative instability was not common, but it was more common in males and in the youngest examinees. Inappropriate tunnel positioning and new sport injuries were the most common reasons for dissatisfaction after the primary surgery.

Key words: Anterior Cruciate Ligament Reconstruction; Quality of Life; Recovery of Function; Joint Instability; Age Factors; Sex Factors; Surveys and Questionnaires; Postoperative Complications; Reoperation; Athletes

Sažetak

Uvod. Cilj studije je da se utvrdi da li postoji statistički značajna razlika u kvalitetu života kod pacijenata različitog životnog doba i pola i da se analiziraju uzroci postoperativne nestabilnosti kolena, nakon rekonstrukcije prednjeg ukrštenog ligamenta. **Materijal i metode.** Ispitivanje je obuhvatilo 776 ispitanika starosti od 15 do 59 godina (prosečno 27), koje smo podelili u grupe prema životnom dobu i polu. Svi su dobrovoljno popunili *Knee injury and Osteoarthritis Outcome Score* upitnik. Postoperativnu nestabilnost objektivizirali smo kliničkim testovima i utvrdili njene uzroke i učestalost među grupama pacijenata. **Rezultati i diskusija.** Prosečne vrednosti upitnika u vezi sa tegobama tokom sportskih aktivnosti bile su najbolje u najmlađoj populaciji uzorka, do 18 godina starosti (81 poen). Postoperativnu nestabilnost smo zabeležili kod 27 pacijenata (3,5%), učestaliju kod muškog pola. Među uzrocima nestabilnosti, najčešća je bila nova sportska trauma, u 11 slučajeva (40%) i neadekvatan položaj tunela kod sedam (26%). Najmlađa starosna grupa, ispod 18 godina, imala je najviše postoperativnih nestabilnosti (5%), a nismo zabeležili nijedan takav slučaj kod pacijenata starijih od 41 godine. **Zaključak.** Ne postoji statistički značajna korelacija kvaliteta života pacijenata mlađeg i starijeg životnog doba, nakon rekonstrukcije prednjeg ukrštenog ligamenta, ali postoje razlike u pojedinim segmentima upitnika. Postoperativna nestabilnost nije česta, ali je učestalija kod muškog pola i u najmlađim starosnim grupama. Nezadovoljavajući položaj kalema i nova trauma predstavljaju najčešće razloge nezadovoljstva nakon primarne operacije.

Ključne reči: rekonstrukcija prednjeg ukrštenog ligamenta, kvalitet života, dob, pol, artroskopija, upitnici, nestabilnost, revizija, operacija

Introduction

Sports activities improve the overall health, both physical and mental. However, the negative aspects of sports are sports injuries that may result in early termination of sports careers and a lower general quality of life (QOL) in athletes. The issue of the QOL in the modern society is complex and includes multiple factors [1]; in recent decades, there has been a growing trend of knee joint sports injuries [1–7]. Sports-related injuries affect the adolescents, because elite sports demand early involvement of children in frequent and intense training process, but they also affect the increasingly active older population, because recreational sports may lead to joint degeneration [1, 4, 7].

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Abbreviations
QOL – quality of life
ACL – anterior cruciate ligament
OA – osteoarthritis
KOOS – Knee injury and Osteoarthritis Outcome Score
ADL – activities of daily living

The incidence of anterior cruciate ligament (ACL) injury in children and adolescents is constantly increasing, especially in those involved in competitive sports [2, 3, 5]. There is also an increasing number of reported injuries and ACL reruptures in children under 15 years of age [2, 3, 5]. Although it is well documented that postoperative instability is more common in adolescent girls and that women have a significantly higher risk of ACL injury [2–5], the dilemmas remain: why are men more prevalent in study samples; why does not ACL reconstruction prevent the development of arthrosis, as it provides stability of the knee joint and helps to restore proprioception; and when is the right time to return to sports activities. It is also not clear why active athletes have a higher incidence of postoperative complications, such as infections, than recreational athletes [8, 9]. Contemporary dilemmas are also related to the development of surgical techniques, especially in terms of whether additional anterolateral ligament reconstruction contributes to better stability [9] and if tibial osteotomy reduces the risk of arthrosis and rerupture [10].

The purpose of the ACL surgical reconstruction is not only to restore the knee joint function, but also to provide optimal QOL. Today, self-perceived health is considered as a reliable estimate of one's health [11].

The aim of this study was to determine the effects of age and gender on the QOL of patients after ACL reconstruction, as well as to analyze the causes of postoperative instability in our sample.

Material and Methods

A retrospective-prospective study was conducted at the Clinic of Orthopedic Surgery and Traumatology, Clinical Center of Vojvodina, with the prior approval of the Ethics Committee. The research included 776 patients with ACL injury who were surgically treated between March 2013 and January 2018. The sample included 626 male (80.7%) and 150 female (19.3%) patients (**Table 1**), aged from 15 to 59, average age 26.84 (SD = 7.90), who were divided into several age groups. The first group included 61 patients up to 18 years of age (7.9% of the sample), the second group from 18 to 25 years, 335 subjects (43.2%), the third group from 26 to 30 years, 153 patients (19.7%), the fourth group from 31 to 40 years, 175 (22.6%), and the fifth group of 52 patients aged 41 and over (**Table 2**).

Most of the sample included recreational athletes, 434 (56%), followed by professional athletes 303 (39%), and finally non-athletes, 39 (5%). In relation to sports activity: 337 (43%) of the respondents were amateur athletes; 157 (20%) were at the regional, 156 (20%) at the republic, 91 (12%) at international level, and only 35 (5%) were non-athletes. The highest percentage of patients suffered injuries during sports activities (728 i.e. 95%), due to falls 24 subjects (3%), and in 14 subjects (2%) the injury occurred as a result of traffic accident.

Patients who signed a written consent to participate in the study were sent the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire [12]. In addition to data on patients' postoperative QOL, general data were collected on the etiology of the injury, if they were professional or recreational athletes, as well as about their daily activities and potential knee instability. Data capture for each patient included the following parameters: gender and age distribution, cause of injury, type and level of sports activity, laterality, associated injuries, time elapsed: from injury to diagnosis, from injury to surgery, postoperative values of all 5 segments of the KOOS questionnaire, including symptoms, pain, activities of daily living (ADL), sports-related questions and QOL. The KOOS questionnaire is available, free of charge, on the Internet [12], translated from English and adapted to research. The questionnaire consists of 5 sections, which are scored separately. All items have 5 possible responses (Likert scale) with a score range from 0 to 4. The total score for each subscale ranges from 0 to 100 points, where 0 means severe problems, and 100 indicates problem-free functioning.

Two years after surgery, on average, the patients were invited for a final clinical examination, when X-ray checks were made of the operated knee joints. The postoperative instability was determined using Lachman and pivot shift tests. Control radiographs determined the position of the bone tunnels, containing graft fixed with interference screws. The correct position on profile X-rays was considered if the tunnels were located in the posterior quarter of the Blumensaat`s line of the femur (at the ACL`s footprint site) and if they were 65 ± 10 degrees in relation to the tibial plateau [13].

We have identified the causes of instability in gender and age groups and analyzed their incidence. The results of reoperations are also presented, revision arthroscopic ACL reconstructions (with bone–patelar tendon–bone graft, harvested from the opposite leg and fixed by interference screws), and in the end we analyzed return to sports activities after reoperations.

Table 1. Sex distribution and activities of daily living scores
Tabela 1. *Polna struktura i skor "aktivnosti dnevnog života"*

	N/*Broj*	Average/*Prosek*	SD/*Standardna devijacija*	Min./*Min.*	Max./*Maks.*
Male/*Muško*	626	93.2947	8.17541	32.50	100.00
Female/*Žensko*	150	94.6000	6.89129	57.50	100.00
Total/*Ukupno*	776	93.5470	7.95597	32.50	100.00

Table 2. Age groups distribution and sport and recreation scores
Tabela 2. *Vrednosti skora "sport i rekreacija" i slučajevi nestabilnosti među starosnim grupama*

Age/*Uzrast*	N/*Broj*	Average/*Prosek*	SD/*Stand. dev.*	Instability/*Nestabilnost*	Percentage/*Procenat*
≤ 18	61	80.74	16.42	3	4.9
18-25	335	79.10	19.96	12	3.6
26-30	153	74.42	21.12	6	3.9
31-40	175	70.87	23.40	6	3.4
41 ≥	52	74.00	22.30	0	0
Total/*Ukupno*	776	76.11	21.17	27	3.5%

The study excluded subjects who were not volunteers, those who filled out the KOOS questionnaire incompletely, and those who have not responded to the final examination, which included clinical tests and radiographic evaluation of the position of the bone tunnels in the femur and tibia.

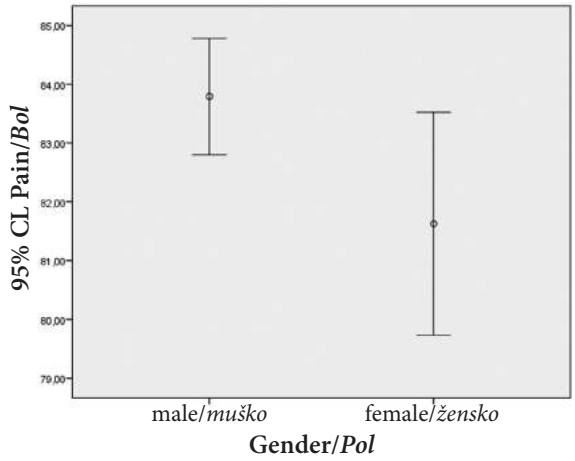
Statistical Package for Social Sciences (SPSS 21) software was used for statistical data processing. Numerical features were presented using mean values (arithmetic mean) and measure of variability (range of values, standard deviation), and attributive features using frequencies and percentages. The comparison of numerical values between the two groups was performed using the nonparametric Mann-Whitney test, while the nonparametric Kruskal-Wallis test was used to compare values between the data of three or more groups. The test of the correlation between the two characteristics was performed using Pearson's correlation coefficient. The $p < 0.05$ was considered statistically significant. The results are presented in tables and graphs.

Results

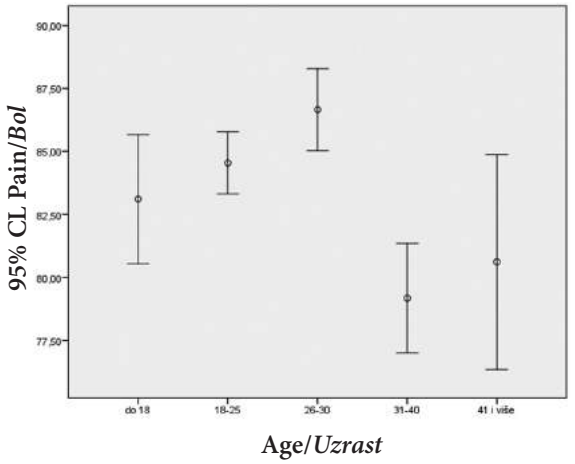
No statistically significant difference was found between the genders on the Symptoms subscale (Mann-Whitney test; U = 43454.500; p = 0.151) and difficulties with knee movement, crepitus, swelling and joint stability.

However, there was a statistically significant difference between age categories on the Symptoms subscale (Kruskal-Wallis test; H = 12.503; p = 0.014). Subjects aged 18 – 25 years and 26–30 had statistically significantly lower scores than those aged 31–40 years (Mann-Whitney test; U = 25346.500; p = 0.011; U = 11501.500; p = 0.026). Respondents aged 31–40, had statistically significantly higher scores than those aged 41 and over (Mann-Whitney test; U = 3303.000; p = 0.002).

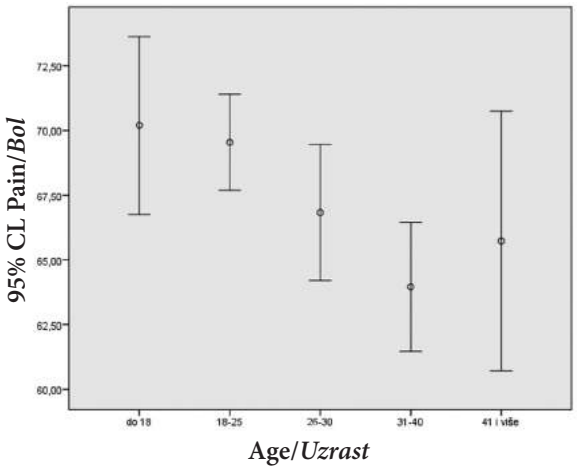
Men showed a statistically significantly higher scores on the Pain subscale and tolerated pain better than women (Mann-Whitney test; U = 40458.500; p = 0.008) **(Graph 1)**. There was also a statistically significant difference between the age categories on the Pain subscale (Kruskal-Wallis test; H = 44.499; p = 0.000) (**Graph 2**). Subjects aged up to 18 years and subjects aged 18–25 had a statistically significantly lower scores than those aged 26–30 (Mann-Whitney test; U = 3494.500; p = 0.004 and U = 22653.500; p = 0.039). Respondents aged 18–25 years and 26–30 years had a statistically significantly higher scores than those aged 31–40 years (Mann-Whitney test; U = 21327.500; p = 0.000 and U = 8159.500; p = 0.000). Respondents aged 26–30 years had a statistically significantly higher scores than



Graph 1. Sex distribution and the total pain subscale scores
Grafikon 1. *Ukupne vrednosti podskale "bol" prema polnoj strukturi*



Graph 2. Age distribution and the total pain subscale scores
Grafikon 2. *Ukupne vrednosti podskale "bol" prema starosnoj strukturi*



Grafikon 3. Age groups distribution and quality of life scores
Grafikon 3. *Kvalitet života među uzrasnim kategorijama*

those aged 41 years and over (Mann-Whitney test; U = 2891.500; p = 0.003) **(Graph 2)**.

We found that women had a statistically significantly higher scores in ADL than men (Mann-Whitney test; U = 42193.000; p = 0.049) **(Table 1)**, but there were no statistically significant differences in the scores of ADL between age groups (Kruskal-Wallis test; H = 9.178; p = 0.057).

There was no statistically significant difference in the scores of the Sport/Recreation subscale between males and females (Mann-Whitney test; U = 43841.500; p = 0.207), but a significant difference was found between the age groups (Kruskal-Wallis test; H = 18.934; p = 0.001) **(Table 2)**. The average values related to difficulties during jumps, squats, take off, sprints, landing and gym exercises, related to training and competitions were the best in the youngest group, up to 18 years of age (81 points) and they were better than in groups 18–25 years (79), 26–30 and over 41 (both 74), whereas patients in the fourth decade of life had the worst results (71 points).

Subjects under the age of 18 years had statistically significantly better scores than those aged 31 - 40 (Mann-Whitney test; U = 4160.500; p = 0.010). Those aged 18–25 had statistically significantly better scores than those aged 26–30 (Mann-Whitney test; U = 21591.000; p = 0.005), and subjects aged 18–25 years had statistically significantly better scores than those aged 31–40 years (Mann-Whitney test; U = 23616.500; p = 0.000).

In regard to the QOL subscale, females had statistically significantly higher scores than males (Mann-Whitney test; U = 40480.500; p = 0.009). There was a statistically significant difference in QOL scores between different age categories (Kruskal-Wallis test; H = 15.934; p = 0.003) **(Graph 3)**. The results showed that subjects under the age of 18 years and between 18 and 25 years had a statistically significantly higher QOL scores than those aged 31–40 (Mann-Whitney test; U = 4182.000; p = 0.012; U = 23471.000 ; p = 0.000).

By analyzing the scores between 5 age groups in each subscale of the KOOS questionnaire, we observed a statistically significant correlation where predominantly younger respondents showed higher scores, except in the subscale ADL.

However, the overall correlation of age in the total number of respondents (not by age groups) and the obtained scores, we found that age statistically significantly correlated with the following scores: Pain (Pearson's correlation; r = -0.129; p = 0.000); Sport/Recreation (R = -0.143; p = 0.000) and QOL (r = -0.122; p = 0.000). Nevertheless, the statistically significant correlations were very small (r < 0.3), so no significant association between age and scores was established. The other two subscale scores showed no statistically significant correlation; Symptoms (r = -0.032; p = 0.380) and ADL (r = -0.024; p = 0.498) scores showed that there was no statistically significant correlation in the QOL of younger and older patients.

Postoperative instability was observed in 27 patients out of 776 (3.5%). In the total sample of primary ACL reconstruction there were more men 662 (81%) than women (19%), and the repeat instability was more frequent in males, because out of the 27 patients, 24 were men (88.9%) and only 3 women (11.1%).

Among the causes of postoperative instability, the most common were new sports injuries, in 11 cases (40.1%) and inadequate tunnel positioning in 7 (26.0%). Premature return to competition was the cause of instability only in the youngest groups, under 25 years of age, because three of these young athletes returned to competing too early (3 – 6 months after surgery, against the doctor's orders). A deep infection was the cause of one case of postoperative instability and the graft was replaced by another. In five cases the causes remained unknown (correct tunnel positioning, no other postoperative complications, no new trauma, and returned to competition in the prescribed 6 – 9 months after the primary surgery).

The highest incidence of postoperative instability was found in the youngest age group under the age of 18 years (4.9%) and there were no such cases in older patients over 41 years **(Table 2)**.

Reoperations were performed in all cases with new sports injuries, all too anteriorly placed femoral tunnels (technically the best for reoperation) and in one case of infection. Out of 27 cases with postoperative instability, 19 underwent reoperation. So, out of a total of 776 patients, revision surgery was performed in 19 patients (2.5%). Eleven athletes have returned to sports activities (57.9%).

Discussion

According to various studies, the annual incidence of ACL injury in the general population varies between 0.01 and 0.08% [14–16], but the incidence is significantly higher among the population engaging in sports (1.5–1.7%) [5, 14, 16]. These injuries are generally more common in the male population [17–24], as was also the case in our sample, which included four times more males than females. The average age

of our patients was 26.8 years, which is consistent with other studies [17–24]. In our study, the biggest group included patients aged between 18 and 25 years, and that is the age when ACL injuries are most common [17–24]. The incidence of ACL injury has also increased in females, especially those younger than 20 and over 40 years of age [1, 5]. By analyzing ACL reconstructions in American high school basketball players, as many as 61% were girls and 39% were boys [25]. However, in the same group, after high school graduation (> 23 years), young men experienced ACL almost 7 times more frequently [25].

The incidence of ACL injury has led to the need to determine risk factors, causes of injury, and to develop preventive measures, in order to reduce the incidence of injuries. The risk factors are divided into internal and external, as well as to variable and invariable [26], so preventive measures are mainly aimed at correcting variable conditions in non-contact ACL sports injuries [27]. External factors include the characteristics (quality) of the playing surface, weather characteristics, type of sports footwear, and shoe-surface interaction [26, 28]. There is evidence that ACL injuries on natural grass are more frequent in dry conditions than wet field injuries [29], and that it is safer to play soccer on natural grass than on artificial ground [26]. Footwear may also be a potential risk factor, because it modulates foot fixation during the sport activity [30]. Due to the absence of surface friction, there are virtually no ACL injuries among hockey players, skaters, and ballerinas [20, 22, 23].

Internal factors are divided into anatomical, hormonal, neuromuscular, and genetic [25]. The most frequently studied anatomical factors are obesity, muscular contraction and specificities of the anatomical body structure [26, 28]. Women have, on average, a larger Q angle, a wider pelvis, a narrower intercondylar notch of the femur, lower leg muscle strength, increased knee valgus and more elastic ligaments, due to hormonal effects [5, 16, 25–29, 31]. The aggressive contraction of the thigh muscle in the slightly bent knee is also considered to be an important internal factor, especially in non-contact ACL injuries, as it leads to significant anterior tibial movement [32]. Increased posterior tibial slope angle over 10 degrees can also be a risk factor for ACL injury [10]. Because of this, by comparing genders at the same elite-level of sports, female basketball players have a 3.5 times increased risk of ACL injury than male basketball players, while female soccer players have 2.67 times more chance for ACL injury than male soccer players [33]. In our sample, the female patients showed a better QOL on average than male, since they are more engaged in the ADL after surgery, although postoperative pain is on average better tolerated by male subjects.

There are numerous disagreements about the impact of these isolated factors, but in our earlier study [23], we concluded that ACL injury in 450 operated patients was not significantly influenced by footwear type, warming, genetic predisposition, and daily therapy. Injuries occurred more frequently at competitions, at the end of matches, due to a landing or change of direction, without contact with other competitors, on dry terrain, in insufficiently prepared athletes [23].

The incidence of ACL injury in children and adolescents among the total injuries is not high (up to 3%) but is constantly increasing, especially in those involved in competitive sports [34]. In the Australian population, an increasing number of registered ACL ruptures are reported in children under the age of 15 years, and most commonly, in the period after five years or more, recurrent or contralateral ACL ruptures occur [34]. Recent studies have shown that the overall standard ACL reinjury rate is 21%, of which 10% are injuries on the ipsilateral side in individuals younger than 25 years [34]. Patients under 20 years of age were found to have an 8.7–14.3% higher risk of ACL re-reconstruction, as early as 6 months after the primary reconstruction [35, 36]. Despite this risk, the younger patients in our sample are more satisfied with their postoperative sports performance than the older patients.

The athletes who return to high-risk sports at a professional level, which involve rapid changes of direction and pivoting (soccer, basketball, handball, American football, skiing), especially those younger (under 25 years), have a significantly higher risk of recurrent ACL rupture. Among all ACL reconstructions, only 7% of operated patients experience rerupures, while among professional athletes younger than 25 years, as many as 23% [37]. Contact sports are more likely to cause injuries in the younger population (< 39 years), while alpine skiing and other non-specific activities are the cause of injury at older age (> 40 years) [38]. Korean authors [38] recommend ACL reconstructions even in patients older than 50 years, especially in case of a major functional impairment. Although their values of functional scoring scales are lower, older patients have similar results in knee stability and ligament strength relative to younger patients [40]. Thus, in our sample, patients in the fourth decade of life tolerated postoperative symptoms better than younger patients.

Revision surgery after ACL reconstruction is no longer a rarity. Reruptures, after primary reconstruction, occur in 1–13% of cases [2–8, 16, 20]. We registered 3.5% of them. Comparing the results of ACL reconstruction between adolescents and those over 20 years of age, better functional results were observed in adolescents after returning to sports after 8 months, but after 12 months after surgery, the difference was not significant [2]. However, due to the early return of adolescents to competitive sports, they were at 30% higher risk of revision surgery, as soon as two years after the primary reconstruction [2–4]. We concluded the same. The causes were adolescent age, inadequate muscle strength (below 90% of strength of the operated in relation to the healthy leg) and premature return to competition, which may need to be delayed for at least 9 months after surgery [2–7]. Individuals with a predicting factor engaged in professional sport are three times more likely to undergo revision surgery during adolescence [4]. Comparing female soccer players with ACL injury and uninjured players from the same football team, it was concluded that female play-

ers with ACL reconstruction had almost 5 times higher rates of new ACL injuries and 2–4 times higher risk of other new knee injuries [5].

In our study, postoperative instability was more common in males, because 24 of the 27 patients were male (89%), and only 3 female (11%). Other authors have also had more male patients in the sample of revision surgery [2–4, 20]. Although ACL revisions provide better knee stability, the results of the KOOS questionnaire after revision are significantly worse than results after the primary surgery [35, 36]. It is a similar case when it comes to returning to sports activities; only 58% of athletes successfully return to competitions after ACL revisions and 53% after bilateral ACL reconstructions [40].

The orthopedic surgeons are most commonly asked on the time their patients can go back to training. The assessment is individual and there is no consensus. Return to the previous competitive level occurs in 65 – 88% of operated patients [15–21]. The majority of authors today suggest granting return to sporting activities 9 to 12 months after surgery [2–7]. Others, like us, if there are no complications allow athletes to return to training 6 to 9 months after surgery [19–21, 41]. There are also those who claim that it takes more than 12 months to safely regain knee stability [42–44]. Our operated patients returned to sports competitions (matches) in 75% of cases, 8 months after ACL reconstruction on average, unless serious complications occurred (such as rerupture, deep infection [8], cyclops lesion [46]), when adequate muscle strength was achieved (at least 90% the strength compared to the uninjured leg) [22].

Premature return to competition was recorded only in the youngest groups of our sample, under the age of 25 years, because some of these young athletes competed 3 – 6 months after surgery, although they were aware of the possible consequences. Among the causes of postoperative instability in our sample, the most common were new sports injuries and inadequate tunnel positioning (66% of revision cases). Milankov et al. [20] also concluded that an unsatisfactory position of the bone tunnels and new trauma are the most common causes of post-operative knee instability, and that the mean Lysholm score after reoperation was only 88 points (65 – 90), which is a much poorer result in comparison to primary reconstruction, ranging from 92 to 98 points, according to various studies [8, 19, 21]. In our study, there were cases where causes of postoperative knee instability remained unknown, especially in patients with correct tunnel positioning, who had no postoperative complications, no new trauma, and returned to competition in the prescribed 6–9 months after the primary surgery.

Less than 50% of all athletes achieve the same level of sports performance after the revision surgery [45]. Milankov et al. [20] had slightly better results of reoperations, as only 18% of patients stopped training. Of our 19 patients who had revision surgery, only 58% returned to sports activities, which is a significantly worse result compared with the primary ACL reconstruction (60–81%) [8, 19, 21]. Our eight surveyed athletes explained their ending of sports career by waiting too long for two operations, long-term rehabilitation, dissatisfaction with the results of reoperation, inability to regain muscle strength, athletic form and place in the team, as well as changing their lifestyle (age for starting a family).

Investigating the impact of gender on QOL in 16.930 people with ACL injury showed that female patients had worse preoperative and two-year postoperative KOOS scores than men [4]. Also, women are more likely to experience recurrent ACL injury than men and find it more difficult to return to the level of sports performance they had before surgery compared to men of the same age and same level of performance [36]. In contrast, there are studies, including ours, that found that overall QOL, 1–2 years after ACL reconstruction, did not differ between the sexes [19].

In order to understand the pathomechanisms of ACL injury and offer effective prevention programs, understanding the biomechanics of movement during activity is necessary [46]. It is postulated that flexion, adduction, internal hip rotation, knee valgus position, anterior translation, and external rotation of the tibia may put ACL at increased risk for rupture [29, 30, 47]. It is called a "no return" position [46]. Video analysis of ACL sports injuries concluded that they occur most often without contact with other players, on jump or step when the athlete's knee is flexed less than 30°, with increased valgus and internal rotation of the tibia, and at the time of injury women presented with significantly higher values of these angles than men [47]. In order to prevent knee injury, especially ACL, identification of athletes at-risk may be the first step before the creation and implementation of specific training programs aimed at modifying identified risk factors, reducing injury rates, and preventing osteoarthritis [41–44, 48]. Plyometric exercise, balance training, core stabilization and neuromuscular controls have been shown to play a role in the prevention of injury [46]. These workouts lead to a decrease in knee valgus and increase knee flexion during landings, reducing sudden changes in direction of movement and jumping. Training designed in this way should be applied 2 to 3 times per week, for at least one month, to achieve expected kinematic changes [49]. The annual incidence of ACL injuries is most common among professional athletes, where it is 0.15–3.7% [14, 15]. So, if in one major sports association, with about 300 professional athletes of all categories, only 1% of members are injured annually, three athletes have ACL injury during a season. There is evidence that by implementing adequate preventive training, as many as two injuries may be avoided [49]. Such preventive training may significantly (67–84%) reduce non-contact injuries, especially among female soccer players [6, 46, 49, 50].

The disadvantages of this study are related to the subjective judgment of patients on their own health, which may not always be valid, because patients often try to "please" the physicians, social and family expectations, or may be hypochondriacs [51]. Therefore, clinical evaluation and the patient's personal assessment of health and QOL often do not correlate

[51]. In order to have a complete picture of how the injury affects all spheres of life, additional objectification of the patient's QOL is required, using measurement instruments. Also, given the risk of degenerative changes, it may be helpful to perform X-rays or second look arthroscopy 10–20 years after the primary operation, which could also be a task for some other researches in the future. Already mentioned unknown causes of postoperative instability of patients who had correct tunnel positioning, no other postoperative complications, no new traumas and who returned to training at a proper time, are challenges for future researches, because if we find all the causes, we will know how to treat them.

Conclusion

There is generally no statistically significant correlation between the quality of life of younger and older patients after anterior cruciate ligament reconstruction, but there are differences in some segments of the questionnaire.

Women have a better quality of life concerning activities of daily living than men, because they are more engaged with them after surgery. Patients in the fourth decade of life tolerate postoperative symptoms better than younger ones. Generally, postoperative pain is better tolerated by men than women. Younger patients, over the age of 25 years, are more satisfied with their participation in sports activities than older patients. Postoperative instability was not common and it was found in 3.5% of cases, predominantly in males. It is generally more common in the youngest patients, under the age of 25 years, and there were no such cases in the fifth decade of life.

Incorrect bone tunnel positioning and new traumas were the most common causes of recurrent knee instability. Causes of increased risk of reinjury and surgery revision in adolescents were inadequate muscle strength and premature return to competition. Results of anterior cruciate ligament reoperations were significantly worse than of the primary reconstructions.

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REVIEW ARTICLES
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TRANSCRANIAL DOPPLER METHODS IN THE ASSESSMENT OF CEREBRAL VASOMOTOR REACTIVITY

METODE TRANSKRANIJALNOG DOPLERA U PROCENI CEREBRALNE VAZOMOTORNE REAKTIVNOSTI

Aleksandra LUČIĆ PROKIN[1, 2], Petar SLANKAMENAC[1, 2] and Pavle KOVAČEVIĆ[2, 3]

Summary

Introduction. Transcranial Doppler is the only non-invasive neuroimaging modality in the diagnosis and monitoring of various neurovascular diseases. Apart from assessing cerebral hemodynamics of blood flow in the basal brain arteries, transcranial Doppler provides physiological data and anatomical images. **Quantification analysis of vasomotor reactivity.** Various transcranial Doppler methods evaluate cerebral vasomotor reactivity, providing important information on the properties of arterioles under induced hemodynamic conditions. Exogenous and endogenous vasoactive stimuli of different potency (apnea, acetazolamide, carbon dioxide, L-arginine) are most commonly used, making transcranial Doppler a prognostic indicator of future ischemic events. This article reviews principles of various transcranial Doppler methods in the evaluation of vasomotor reactivity, emphasizing their advantages and disadvantages. **Transcranial Doppler in the field of reduced vasomotor reactivity.** Evaluation of vasomotor reactivity has a role in the prediction of future ischemic events, evaluation of revascularization effect after carotid endarterectomy, but also in the increasingly significant choice of the right time to perform it. In recent years, transcranial Doppler methods have found application in other areas of dysfunctional cerebral hemodynamics: dementia, hypertension, migraines, and sepsis. **Conclusion.** Due to an excellent temporal resolution, non-invasive approach, good cost-benefit ratio, bedside monitoring, relative simplicity in terms of interpretation and performance, and portability, transcranial Doppler in vasomotor reactivity may be the ideal tool in the evaluation of cerebral hemodynamics, arterial perfusion integrity and collateral capacity.

Key words: Ultrasonography, Doppler, Transcranial; Cerebrovascular Circulation; Vasomotor System; Hemodynamics; Prognosis; Brain Ischemia

Sažetak

Uvod. Transkranijalni dopler jedini je neinvazivni neuroimidžing modalitet u dijagnostici i praćenju različitih neurovaskularnih bolesti. Procenjujući cerebralnu hemodinamiku analizom protoka krvi u bazalnim arterijama mozga, transkranijalni dopler dodaje fiziološke podatke anatomskim slikama. **Kvantifikaciono ispitivanje vazomotorne reaktivnosti.** Pomoću različitih metoda transkranijalnog doplera procene cerebralne vazomotorne reaktivnosti, dobijaju se važne informacije o sposobnosti arteriola u indukovanim hemodinamičkim uslovima. Najčešće se koriste egzogeni i endogeni vazoaktivni stumulusi različite potentnosti (apnea, acetazolamid, ugljen-dioksid, L-arginin) čime transkranijalni dopler dobija ulogu prognostičkog pokazatelja budućih ishemijskih događaja. U članku će biti prikazan princip izvođenja različitih metoda transkranijalnog doplera u evaluaciji cerebralne vazomotorne reaktivnosti, uz isticanje prednosti i nedostataka svake od navedenih. **Transkranijalni dopler u oblasti redukovane vazomotorne reaktivnosti.** Procena cerebralne vazomotorne reaktivnosti ima ulogu prognostičkog pokazatelja budućih ishemijskih događaja; u proceni revaskularizacionog efekta nakon karotidne endarterektomije, ali i sve značajnijeg odabira pravog vremena za njeno izvođenje. Poslednjih godina, metode transkranijalnog doplera našle su primenu i u drugim sferama afunkcionalne cerebralne hemodinamike: demencija, hipertenzija, migrena, sepsa. **Zaključak.** Sa dobrom vremenskom rezolucijom, neinvazivnim pristupom, dobrim odnosom troškova i koristi, transkranijalni dopler u evaluaciji cerebralne vazomotorne reaktivnosti može biti, skoro pa idealno, sredstvo za procenu intrakranijalne hemodinamike, integriteta arterijske perfuzije i indeksa kolateralnog kapaciteta.

Ključne reči: transkranijalni dopler ultrazvuk; cerebrovaskularna cirkulacija; vazomotorni sistem; hemodinamika; prognoza; moždana ishemija

Introduction

Since its introduction in 1982, transcranial Doppler (TCD) has become an important tool in the diagnostics and monitoring of various vascular diseases. As the only non-invasive neurovisual modality, it found its place in the assessment of cerebral hemodynamics by analyzing blood flow in the basal arteries of the brain [1–3].

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Abbreviations

TCD – transcranial Doppler
VMR – vasomotor reactivity
CO_2 – carbon dioxide
$PaCO_2$ – arterial partial pressure of carbon dioxide
PaO_2 – partial pressure of oxygen
CBF – cerebral blood flow
MCA – middle cerebral artery
BFV – blood flow velocity
PET – positron emission tomography
ACZ – acetazolamide
BHI – breath holding index
TIA – transient ischemic attack
L-A – L-arginine
ICA – internal carotid artery
PIO_2 – inspired PO_2
CEA – carotid endarterectomy

Cerebral vasomotor reactivity (VMR) describes the cerebral autoregulatory mechanism, following changes in induced hemodynamic challenges. It belongs to the category of chemoregulation and it is an indirect indicator of cerebral autoregulation (Class of Recommendation - II; Level of Evidence - B). This term has numerous synonyms in the literature, like autoregulatory reserve, cerebrovascular reactivity, capacity of cerebrovascular reserve, carbon dioxide (CO_2)-reactivity, vasomotor reserve, cerebrovascular resistance [3–5].

As a ratio between the percentage change in blood flow and arterial partial pressure of carbon dioxide ($PaCO_2$), VMR is defined as the range between maximal dilation and constriction of arterioles, in response of the basal artery to specific vasoactive stimuli [4–9].

Numerous studies have identified hypercapnia as a potent vasoactive stimulus for a significant increase in cerebral blood flow (CBF). In this sense, elevations in CBF with hypercapnia "wash out" of CO_2 from brain tissue attenuate the rise in central $PaCO_2$. In contrast, hypocapnia causes cerebral vasoconstriction, which reduces CBF and attenuates the fall of brain tissue $PaCO_2$. An emerging concept, therefore, is that cerebrovascular reactivity and ventilatory response to $PaCO_2$ are tightly linked, so that regulation of CBF has an important role in stabilizing breathing during fluctuating levels of chemical stimuli [10–12].

The multifactorial effects of extracellular pH on VMR correlate with CO_2 activity, but in the opposite direction [13]. The role of partial pressure of oxygen (PaO_2) in the day-to-day regulation of CBF seems to be minor. However, although hypoxia per se is a cerebral vasodilator, reflected in a rise in CBF in proportion of the isocapnic hypoxia severity, under normal conditions hypoxia leads to hyperventilation-induced lowering of $PaCO_2$ and subsequent cerebral vasoconstriction. The role of PaO_2 becomes important during hypoxemia associated with chronic lung disease [12–15].

These vasoactive stimuli affect changes in the diameter of the arterioles, while the basal arteries change only the blood flow velocity (BFV). This fact confirms the resistance of the middle cerebral artery (MCA), allowing the TCD-measured BFV in the MCA to be used as a valid surrogate in the detection of CBF changes [16–19].

Quantification analysis of vasomotor reactivity

In addition to modern, functional technological methods for brain visualization, xenon-enhanced computed tomography (Xe/CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI), the conventional TCD method is often used in clinical work. The TCD measures BFV using it as an index of relative change in blood flow. Four TCD tests use selective vasoactive stimuli:

1. Apnea test (breath holding test),
2. CO_2 inhalation/Re-inhalation,
3. Acetazolamide (ACZ) test,
4. L-arginine test.

Apnea test (breath holding test)

The apnea test is one of the earliest applied methods first presented by Ratnatunga and Adiseshiah (1990), while the clinical use started in 1992 [20]. The apnea test is based on balancing alveolar PaO_2 and $PaCO_2$ in venous blood. After achieving a balance, all changes that occur are aimed at the metabolic production of CO_2. As a stimulus, apnea reduces the elimination rate of CO_2 to zero. As a result, CO_2 accumulates in the blood and its fractional concentration and partial pressure rise [21]. Markus et al. described this non-invasive, simple and reproducible method for assessing VMR [22].

When conducting a breath holding test, the patient should be sitting upright, if possible. A monitoring headband is placed around the patient's head and securely tightened. Bilateral MCAs are insonated using the transtemporal approach with 2-MHz ultrasound probe, at a depth of 45 or 54 mm and baseline velocity is recorded. After a normal inhalation, the subject should hold his/her breath for 30 seconds. Deep breathing is not allowed before apnea begins. When the patient starts holding his breath, the neurosonologist starts recording the flow velocity. Recording is stopped as soon as the patient exhales. This procedure is repeated twice and, after that, the average breath holding index (BHI) is calculated. If the patient is not able to achieve the given period of apnea, the achieved time apnea is accepted and entered into the formula. If the device has no automatic calculator for BHI **(Figure 1)**, the following formula is used:

$$BHI = \frac{BFV_{max} - BFV_{et\ rest}}{BFV_{et\ rest}} \times \frac{100}{sec.}$$

(BFV max stands for the value at the end of apnea, and BFV et rest for the value before apnea) [22–25].

The BHI is calculated as the percentage increase in BFV that occurs during apnea (in the time achieved) or as a percentage deviation of the BFV from the zero line divided by the apnea time (30 seconds). The following criteria can be used to evaluate the BHI results: normal VMR ≥ 0.69; impaired

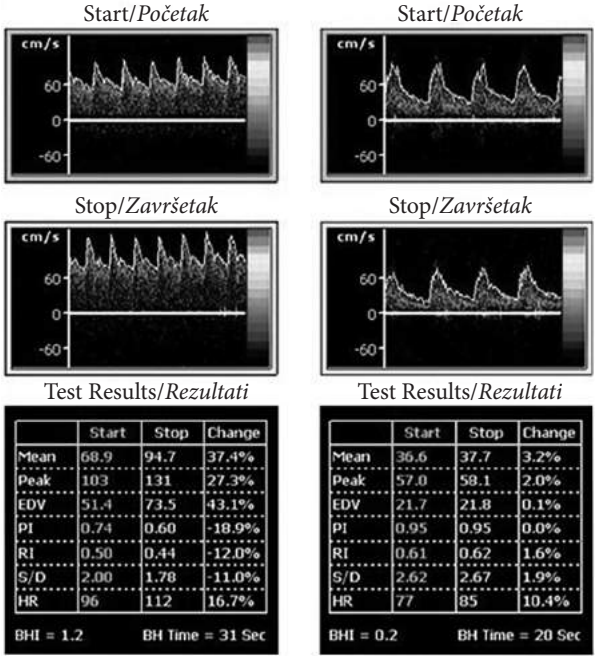

Figure 1. Apnea test: a) normal findings; b) pathological BHI in significant internal carotid artery (ICA) stenosis
Slika 1. *Apnea test: a) uredan nalaz; b) patološki nalaz zadržavanja dana kod signifikantne stenoze unutrašnje karotidne arterije*

VMR 0.21–0.69; significantly impaired VMR ≤ 0.20 [13, 23, 24]. Reduced BHI represents a failure of collateral blood flow to maintain adequate cerebral perfusion in response to hypercapnia [22–24].

Advantages:
– natural endogenous stimulus,
– no external gas sources are needed,
– good tolerance and repeatability,
– short method implementation,
– safety, economy, simplicity.

Disadvantages:
– Cannot measure end-tidal CO_2 ($PETCO_2$) and end-tidal oxygen tension ($PETO_2$),
– The rates of change in PaO_2 and $PaCO_2$ are relatively slow and vary from subject to subject based on the circulation time required for blood to travel from the lungs to the tissues and then back to the lungs,
– The CO_2 capacitances of the body are very large relative to metabolic changes, resulting in a buffering of end tidal partial pressure changes relative to content changes and thereby limiting the change in $PaCO_2$ from those at steady state,
– The $PaCO_2$ and PaO_2 change continuously in opposite direction,
– The changes in $PaCO_2$ and PO_2 are not linear therefore very sensitive to time of breath-hold,
– The length of the stimulus is limited by the subject's ability to hold his breath [21].

Some research centers also examine the control of the sympathetic nervous system. Following hyperventilation to achieve hypocapnia, a decrease in flow velocity in the MCA is recorded as a result of vasoconstriction. The combination of hypercapnia (holding breath) during hypocapnia (hyperventilation) is used to calculate VMR:

$$VMR = \frac{\text{BFV hypercapnia} - \text{BFV hypocapnia}}{\text{BFV et rest}} \times 100\ [\%].$$

The value over 65% indicates normal VMR, while the value less than 33% reflects an exhausted VMR. The VMR between 33% and 65% represents borderline impaired autonomic control [25].

Carbon-dyoxide inhalation method

The CO_2 inhalation method is the second most commonly used test involving the inhalation of a gas mixture (2–5% CO_2 and 95–98% O_2) via ventilation mask, which is connected to a respiratory balloon. The nose is closed for better inhalation. The patient's blood pressure, heart rate as well as an $PETCO_2$ are recorded using capnography. Bilateral MCAs are monitored at a depth of 45–54 mm. After that, a 2–5 minute period of gas mixture inhalation begins [26–28]. The capnograph is monitored to ensure that the $PETCO_2$ level increases to at least 10 mmHg above baseline value. After 3 minutes, the gas mixture inhalation is stopped and after one minute the patient's level of $PETCO_2$ is near the baseline.

Next, the patient is required to hyperventilate (fast and deep breaths). Monitor the capnograph until $PETCO_2$ level decreases to at least 10 mmHg below the baseline value. After 1 minute, record three bilateral MCA velocities, $PETCO_2$ and a blood pressure. The patient is required to breathe normally and continue monitoring the MCA velocities and $PETCO_2$ until they return to near baseline values.

Calculate average MCA velocities by selecting 10 cardiac cycles from the TCD trend function. The average of three velocities of MCA, $PETCO_2$ for baseline, hypercapnia (breathing of CO_2 mixture), and hypocapnia (hyperventilation) are included in the formula:

$$VMR = \frac{\text{BFV (hypercapnia)}}{\text{BVF et rest)}} \times 100 - \frac{\text{BFV hypocapnia}}{\text{BVF et rest}} \times 100\ [\%].$$

The following criteria are used to evaluate the CO_2 challenge results:
1. Normal VMR: 86% ± 16%,
2. Mild to moderately reduced VMR: 69% to 39%,
3. Severely reduced VMR: 38% to 16%,
4. Exhausted VMR ≤ 15% [22, 27–29].

Advantages:
– The precise concentration of gas mixture is known,
– Inspiring gas of known composition will result in a particular PaO_2 and $PaCO_2$ based on the metabolic parameters and alveolar ventilation in a given subject. The composition of the inhaled mixture can be varied to give the required arterial concentrations of O_2 and CO_2,
– Inspired PO_2 (PIO_2) and PCO_2 ($PICO_2$) can be varied independently.

Disadvantages:

– $PaCO_2$ and PaO_2 are not a direct function of the $PICO_2$ and PIO_2 (possible variation of $PaCO_2$ and PaO_2 as a consequence of individual variation of $PICO_2$ in minute ventilation),

– Changes in arterial gases (low PaO_2 and/or high $PaCO_2$) stimulate peripheral and central chemo-receptors, altering ventilation,

– Complex calculations are required to measure individual resting $PETCO_2$ and $PETO_2$,

– Breath-by-breath variability in tidal volume, and hence alveolar ventilation, results in variation in $PETCO_2$ and $PETO_2$ [29].

2a. Carbon-dyoxide re-inhalation method

In this method, the subject re-breathes from a bag primed with a concentration of CO_2 and O_2 forming a semi-closed system, where the $PaCO_2$ rises progressively as a result of the addition of metabolically produced CO_2 into the system. The PaO_2 is kept constant by an infusion of O_2 from an external source equal to the O_2 consumption.

When a comfortable condition is obtained, the time course of blood flow velocity is simultaneously recorded from both MCAs by a TCD with 2-MHz fixed probes. The patient breathes room air only, through a ventilation mask connected with a sampling line to the capnograph to allow continuous recording of the expiratory flow ($PETCO_2$).

Hypocapnia is obtained by asking the patient to reach a maximal hyperventilation during 15 seconds. The empty 1-liter breathing bag is connected to the ventilation mask with a T-shaped tube which is then filled with its own exhaled gas mixture. Then, the patient is instructed to re-breath the same exhaled air from the bag. Re-breathing is continued for 5 min, until a stable, high level of $PETCO_2$ concentration is recorded for a few seconds, and before returning to room air for recovery (4 min) **(Figure 2)** [30, 31].

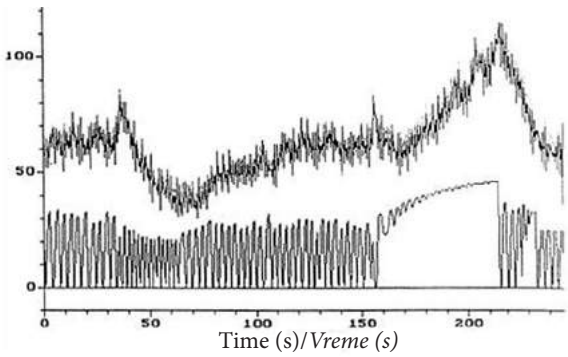

Figure 2. CO_2 reactivity test in a healthy subject; Both MCAs velocity (cm/sec) (upper trace, superimposed) and $PETCO_2$ pressure (mmHg) (lower trace) trends are simultaneously displayed. The superimposition of both MCAs velocity trends (upper trace) prove hemispheric flow symmetry
Slika 2. *Inhalacija ugljen-dioksida kod zdrave osobe; Simetrija protoka (cm/sec) u obe srednje moždane arterije (gornja linija) i parcijalni pritisak ugljen-dioksida (mmHg) (donja linija). Superpozicija protoka leve i desne srednje moždane arterije u gornjoj liniji dokazuje simetriju hemisfernog protoka*

Advantages:

– This method allows studies of physiological responses to a steadily increasing $PaCO_2$ with simultaneous control of PaO_2 levels,

– Breath-by-breath changes in tidal volume have little effect on the observed $PETCO_2$.

Disadvantages:

– This method results only in a slow steady increase in $PETCO_2$ with or without a steady level of $PETO_2$,

– Requires custom equipment consisting of mixed gases, computer control, gas analyzers (capnograph), along with all precautions in order to use this method.

2b. Inspired concentrations of O_2 and CO_2 – modified prospective end tidal targeting (MPET) breathing circuit

This novel method of manipulating $PaCO_2$ and PaO_2 independently and with fine control is the only method that correlates $PETCO_2$ values with $PaCO_2$ [32]. Based on the use of sequential re-breathing circuits, the exact targeting and control of $PaCO_2$ and PaO_2 is achieved by delivering a volume of fresh gas into alveoli on each breath [33]. The fresh gas is a composition of the following three gases 1) 100% Oxygen, 2) 10% Oxygen, balance Nitrogen (90%) and 3) 10% Oxygen, 20% CO_2, balance Nitrogen (70%) – the mixture of which is determined by taking into account the patient's metabolic O_2 consumption (VO_2) and metabolic CO_2 production (VCO_2) and the target of $PaCO_2$ and PaO_2 that is to be achieved [33]. All this is performed through an automated gas delivery system.

Advantages:

– Only method whereby $PETCO_2$ has been shown to be equal to the independent variable, $PaCO_2$,

– Independent control of $PETCO_2$ and $PETO_2$ and thereby $PaCO_2$ and PaO_2,

– Control of $PETCO_2$ and $PETO_2$ is independent of patient's respiratory rate or breathing pattern,

– Delivery of a standardized and repeatable stimulus,

– Requires minimal cooperation.

Disadvantages:

– The use of a face mask which may prohibit use in subjects with claustrophobia or a beard,

– Requires sufficient cooperation to provide a minimal minute ventilation,

– Requires custom equipment consisting of an automated gas blender, sources of O_2, air and two specialty mixed gases, computer control, special program for blender control and gas analysis, capnograph along with all precautions, in order to administer this method.

Acetazolamide method

Acetazolamide (ACZ) or Diamox is a more potent cerebral vasodilator than induced hypercapnia and it is particularly useful in non-cooperative patients [34]. This dilatory effect appears to be mediated by an increase in CO_2 concentration secondary to an inhibition of erythrocyte carbonic anhydrase [34]. According to its mech-

anism, ACZ is a competitive inhibitor of carbonic anhydrase. Carbonic anhydrase is a zinc-containing enzyme that catalyzes the reversible reaction between carbon dioxide hydration and bicarbonate dehydration:

$$CO2 + H_2O = H_2CO_3 = H^+ + (HCO^{-3}).$$

In the presence of ACZ, the carbonic anhydrase is inhibited from catalyzing the aforementioned reaction resulting in an increase in $PaCO_2$ [35, 36].

An intravenously administered dose of 1000 mg ACZ results in a significant increase in BFV in the insonated MCA, after 2 minutes. The vasodilatory effect reaches its maximal level at 10–12 min. after injecting the drug. Some investigators observed a plateau between 10 and 30 min., while others found the maximal increase in BFV at approximately 15 min., followed by a moderate decrease [37–39].

A symmetric increase of BFV by about 30–60% is accepted as physiological. The criteria used for defining the pathological responses to ACZ include < 10% increase in the absolute or absolute change in CBF < 10 ml/100 g/min. [36, 37, 39].

$$VMR = \frac{BFVacz - BFV\ et\ rest}{BFV\ et\ rest} \times 100\ [\%].$$

Advantages:

– Administration does not alter the systemic blood pressure making it a good surrogate for measuring VMR in the presence of hypotension,

– It is safe,

– Independence from the respondents' cooperation.

Disadvantages:

– Must be injected intravenously rendering its use somewhat invasive,

– The time course of response to oral administration is highly variable and $PaCO_2$ changes in response to changes in ventilation are superimposed on those of ACZ,

– The mechanism of effect of ACZ does not allow a quantifiable measure of change in CO_2, thereby making each application an independent stimulus that is non standardized [35, 36],

– Hypersensitivity to sulfonamides, electrolyte disturbances, marked kidney and liver disease, adrenocortical insufficiency and long-term use in chronic noncongestive angle-closure glaucoma [36, 37, 39].

L-arginine method

In the last two decades, the use of L-arginine (L-A) in the evaluation of VMRs has become an established method for the evaluation of cerebral endothelial function. Infusion of L-A, an amino acid involved in endogenous nitric acid synthesis, induces transient vasodilation in the microcirculation. Nitric oxide (NO) plays a key role in regulation of vascular tone [40, 41]. This method is based on a non-invasive monitoring of BFV (most commonly in MCA) according to the equation:

$$CBF = vm \times \text{cross-sectional area of the blood vessel}$$

In addition to bilateral TCD insonation of the MCA, blood pressure and heart rate on the left radial artery as well as $PETCO_2$ (in exhaled air) are monitored simultaneously with a ventilation mask connected to the capnograph. Through the pump, a 30-minute infusion of 100 ml 30% L-A chloride is administered intravenously, during which the patient is instructed to breathe normally. The subjects are followed in a 15-min. interval after L-A application [40, 41]. All measurements are performed at 3 continuous times:

1. at rest (first 15 minutes)
2. during the 30-minute L-A infusion
3. after the end of the infusion (15–20 minutes).

The mean arterial velocity (*vm*) is recorded bilaterally in MCAs by the procedure that is described earlier. Throughout the procedure, the mean arterial blood pressure and heart rate are measured continuously using non-invasive plethysmography which is connected directly to the TCD. The $PETCO_2$ is measured with a capnograph, which is connected to a ventilation mask and to the TCD [40, 41].

The TCD software is used to determine *vm* during the 10-min. rest and during the 10-min. interval after L-A infusion. Data are calculated according to the formula:

$$vm = \int \frac{vdt}{(t0 - t10)}$$

vm – average blood flow velocity in MCA.

Arterial pressure, heart rate, CO_2 and $PETCO_2$ must be calculated at the same intervals as *vm* (using TCD software), while a paired T-test should be used to compare these parameters before and after intravenous L-A infusion [41, 42].

The VMR for L-arginine in MCA is calculated according to the following formula [41–43]:

$$\Delta v = \frac{vm\ (\text{during L-A infusion}) - vm\ (\text{et rest})}{vm\ (\text{et rest})}$$

The physiological value of VMR with L-A is 21.3±10.9% or 30–60% increase in BFV is achieved in healthy subjects [43]. Criteria that have been used to define an abnormal response to ACZ include < 10% increase in the absolute CBF or an absolute change of < 10 mL/100 g/min. [36, 42–45].

Advantages:

– Safe and well tolerated,

– Blood pressure, heart and respiratory rates, arterial pH, $PaCO_2$ are unaffected,

– A gold standard for systemic endothelial function and intima-media thickness as a marker for morphological changes.

Disadvantages:

– Standardization of the L-A dose is required,

– Necessary complex equipment and the most advanced software.

Transcranial Doppler in reduced vasomotor reactivity

In measuring VMR, the TCD methods have a wide clinical range of applications, especially in steno-occlusive carotid diseases. The VMR is a prognostic indicator of future ischemic events [1, 46, 47]. Using the TCD method of CO_2 inhalation, Blasser et al. have found VMR to be an important independent predictor of stroke in symptomatic carotid stenosis with a risk of as much as 27% monthly, compared with 5.4% in those with preserved VMR [48]. Using the TCD apnea test, Silvestrini et al. defined the annual risk of ipsilateral stroke in asymptomatic carotid stenosis (> 70%) - 4.1%, in patients with physiological VMR, compared to 13.9% in those with reduced VMR [24]. Vernieri et al. determined that in the severe carotid artery disease, an impaired VMR was associated with an increased probability of stroke of 32.7%/yr compared to 8%/yr if VMR was normal [49]. Similarly, Kleiser et al. measured the VMR in 85 patients with ICA occlusions using TCD as an indicator of CBF. In follow-up studies over 38 ± 15 months, they found that in the group with greater VMR, none developed a stroke, whereas in the group with diminished VMR, 32% suffered ipsilateral events including transient ischemic attacks (TIAs) and strokes [50].

Evaluation of VMR before and after carotid endarterectomy (CEA) is significant because of the evaluation of the revascularization effect on cerebral hemodynamics, as well as the increasingly important choice of the right time to perform it. The CEA contributes to the normalization of collateral circulation and restitution of cerebral hemodynamics in symptomatic [47, 48, 52–56] and asymptomatic carotid disease [57–61]. Reduced VMR may encourage consideration of CEA or stenting in asymptomatic carotid disease or extra/intracranial bypass surgery in patients with recurrent hemodynamic stroke or TIA [3, 4, 52, 62].

The TCD methods for measuring VMR have also found application in other areas of dysfunctional cerebral hemodynamics. Studies have shown that microangiopathy in vascular and degenerative dementia can lead to arteriolosclerotic processes and vasoconstriction, resulting in reduced VMR [63, 64]. Reduced VMR has been reported in patients with traumatic brain injuries, sleep apnea, hypertension, migraines, sepsis, diabetes mellitus, systemic lupus erythematosus [65–67]. Due to the reported contradictory results, further prospective studies are needed to delineate their clinical significance.

In the selection of particular vasoactive stimulus for evaluation of VMR, a great variety of defects are noted in the mechanism of action and mode of administration. It can be concluded that none of them is a standard repeatable stimulus that is comparable between individuals or in the same person over time. However, numerous studies show that CO_2 can be considered the most appropriate vasoactive stimulus, despite the fact that exact mechanism by which CO_2 affects vascular tone is still not well understood [68]. As the measurement of $PaCO_2$ is invasive, requiring an arterial blood sample, $PETCO_2$ is most frequently used as a suitable surrogate [69].

The TCD in VMR assessment has several limitations:

– limited spatial resolution provides one value for each hemisphere,

– focal impairments secondary to downstream branch vessel pathology may be undetecable,

– highly operator-dependent method,

– patients must have a "temporal bone window" (almost 10% of individuals have absolutely no sonic windows),

– velocity changes in basal arteries are surrogates of changes in blood flow in the downstream cerebral arterioles [1, 3, 4].

In our environment, given the fact that most neurology departments have a TCD, the apnea test becomes available to any neurologist, especially in situations where other, more costly methods may remain unavailable. It seems that the TCD apnea test would be useful as a first step in the assessment of cerebral VMR.

Conclusion

Recognizing the value of transcranial Doppler methods in the evaluation of vasomotor reactivity is very important, especially in monitoring intracranial hemodynamic consequences of cervical artery steno-occlusive lesions. This contributes to the evaluation of the mechanism of stroke, planning the therapeutic approach and prognosis of operated patients. Therefore, the vasomotor reactivity tests may play a role in the therapeutic decision making for patients with carotid disease, where the benefit-to-harm ratio for revascularization procedures is seemingly low. However, with a good temporal resolution, non-invasive approach, good cost-benefit ratio, bedside monitoring, relative simplicity in terms of interpretation and performance, as well as portability, transcranial Doppler in vasomotor reactivity may almost be the ideal tool for evaluating cerebral hemodynamics, arterial perfusion integrity and collateral capacity.

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IMAGING TECHNIQUES IN THE ASSESSMENT OF ENDOVASCULAR INFRARENAL ABDOMINAL AORTIC REPAIR

VIZUELNE METODE U PRAĆENJU USPEHA ENDOVASKULARNOG TRETMANA ANEURIZME INFRARENALNE AORTE

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Summary

Introduction. Imaging is essential in the assessment of endovascular infrarenal abdominal aortic repair results. Complications include endoleaks, graft migration, kinking and infolding, stenosis, occlusion, and secondary ruptures. **Examination Modalities.** Contemporary imaging strategies are based on using noninvasive imaging modalities. After endovascular infrarenal abdominal aortic repair, the standard evaluation modality is computed tomography angiography, whereas additional modalities include magnetic resonance imaging, ultrasonography, and radiography. However, although an invasive imaging method, digital subtraction angiography is still performed in some patients. Computed tomography angiography provides excellent contrast, spatial resolution, and exact measurements of structures of interest, which is essential in the follow-up. **Follow-up Protocol.** Currently recommended follow-up protocol in the first year is contrast-enhanced computed tomography imaging at 1 and 12 months after the procedure. **Conclusion.** Due to its characteristics, reproducibility and availability, computed tomography angiography remains the cornerstone diagnostic modality of post-procedural assessment in patients with endovascular infrarenal abdominal aortic repair.

Key words: Aortic Aneurysm, Abdominal; Blood Vessel Prosthesis; Postoperative Complications; Diagnostic Imaging; Computed Tomography Angiography; Endovascular Procedures

Sažetak

Uvod. Vizuelna dijagnostika je ključna u praćenju uspeha endovaskulanog tretmana aneurizme infrarenalne aorte. Komplikacije su endoleak, migracija grafta, presavijenost i uvučenost grafta, suženje, okluzija i rupture. **Modaliteti ispitivanja.** Savremena strategija vizuelne dijagnostike se bazira na upotrebi neinvazivnih modaliteta. Standardni modalitet je angiografija kompjuterizovanom tomografijom, a dodatni modaliteti su megnenta rezonancija, ultrasonografija i radiografija. Kod biranih pacijenata invazivna digitalna suptrakciona angiografija se još uvek izvodi. Odlična kontrastna i spacijalna rezolucija angiografije kompjuterizovanom tomografijom omogućavaju tačno merenje mesta od interesa, što je ključno za praćenje uspeha procedure. **Protokol praćenja.** Trenutno je preporučena angiografija kompjuterizovanom tomografijom jedan i dvanaest meseci nakon procedure. **Zaključak.** Zbog svojih karakteristika, reproducibilnosti i dosupnosti, angiografija kompjuterizovanom tomografijom ostaje osnovni dijagnostički modalitet postproceduralnog praćenja kod endovaskularnog tretmana aneurizme infrarenalne aorte.

Ključne reči: aneurizma abdominalne aorte; vaskularna proteza; postoperativne komplikacije; dijagnostički imidžing; CT angiografija; endovaskularne procedure

Introduction

Radiology is a fast-growing discipline of medicine and thanks to the implementation of new technologies [1] accompanied by the technical development of materials necessary for endovascular therapy of abdominal aortic aneurysms (AAA), it has become a vital factor affecting the beginnings of endovascular aortic repair (EVAR). However, production of these personalized stent-grafts, preparations for their deplopment and follow up would be impossible without highly sophisticated imaging modalities. Three-dimensional images of pathological tissues, with millimeter and sub-millimeter spatial resolution, are the cornerstone in the contemporary treatment of AAAs; they may prevent their rupture, which is a surgical emergency with a high rate of mortality before the patients are admitted for treatment [2].

Practically, all available imaging modalities have their place in a dynamic protocol of pre- and post-procedural imaging. This paper reviews imaging assessment after EVAR. In the pre-procedural part, parameters that are evaluated are patient selection and procedure planning. In the post-procedural part, the most common complications and suggested follow-up protocols are discussed.

Post-Procedural Imaging

The EVAR is a relatively novel procedure [3], and as with the majority of other novel procedures, the post-procedural protocol is quite rigorous. The

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Abbreviations

US – ultrasound
MRI – magnetic resonance imaging
AAA – abdominal aortic aneurysm
EVAR – endovascular aortic repair
OAS – open aortic surgery
CTA – computed tomography angiography
DUS – Doppler ultrasonography
CEUS – contrast-enhanced ultrasound
MRA – magnetic resonance angiography
HU – Hounsfield units

optimism associated with lower early morbidity rates compared to open aortic surgery (OAS) [4] was replaced with caution after analysis of data gathered in a longer period of time. Comparison of endovascular and open surgical approach repairs showed that a significant number of patients treated with EVAR underwent reintervention. It is also the main disadvantage of EVAR, considering that a 6-year follow-up showed a reintervention rate of 29.6% for EVAR, compared to 18.1% for OAS [5]. These data require detailed and mandatory postoperative surveillance with a goal of screening the postoperative status and especially reporting pathological phenomena leading to reintervention.

At the moment, computed tomography angiography (CTA) is the most common modality used for the assessment of EVAR, but Doppler ultrasonography (DUS), contrast-enhanced ultrasound (CEUS), magnetic resonance angiography (MRA) and plain radiography, also have their place in the follow up protocol.

Complications

Pathological phenomena, regardless of the imaging modality used, are common, so we will discuss them first.

Endoleaks

Endoleak is defined as a persistent blood flow in the aneurysm sac. It is the most common EVAR complication, and its consequences differ widely, depending on the type of endoleak [6]. Endoleaks are divided into five types, according to their location **(Figure 1).**

Type 1. Endoleaks at attachment sites of stent-graft. It is further subdivided into the following subtypes: 1a: proximal (aortic infrarenal), 1b: distal (iliac), 1c: on the level of the iliac occlusion.

The incidence of type Ia, proximal endoleaks, increases in anatomically difficult situations, such as short (< 15 mm) neck, large neck diameter (> 32 mm), tapered necks, increased angulations (> 60°), and landing zones with calcification, thrombus, or uneven size with incidence being reported in 0–10% of EVAR procedures [7].

Type 2. Type 2 endoleaks occur from collateral or retrograde filling the aneurysm sac by one or more of the lumbar, hypogastric, or inferior mesenteric arteries. Given the occlusion or at least partial occlusion of the aortic sac, the pressure in the sac decreases, thus enabling retrograde flow through these arteries, which are branches of the part of aorta covered with stent graft material. They are divided into two subtypes: 2a leaks through a single vessel, 2b leaks through multiple vessels. Type 2 endoleaks account for approximately 40% of all endoleaks and are reported in 20–30% of EVAR cases after 30 days, 18.9 % after 1 year, and 10% over 1 year [7].

Type 3. Endoleaks develop from the graft fabric defect. They are subdivided into: 3a midgraft defects, 3b develop at junctions. Their incidence is considered to be 4% over one year [7]. Like Type 1 and Type 3 endoleaks are considered high-pressure, high-risk leaks, and always warrant urgent management [6].

Type 4. Endoleaks due to a porous endograft which is detected < 30 days after graft placement, due to fabric porosity. They present at the time of the operation on completion aortograms, when patients are fully anticoagulated [6].

Type 5. Endoleaks that are referred to as endotension which may be associated with persistent or recurrent systemic pressure in the aneurysm sac without an identifiable Type 1–4 endoleak on imaging.

Migration

Strong and pulsatile nature of aortic arterial flow combined with a pathologically changed arterial wall is ground for the development of stent-graft migration, usually toward distal parts of aorta. This can lead to endograft migration and consequent development of dangerous Type 1 endoleak and other complications, including rupture.

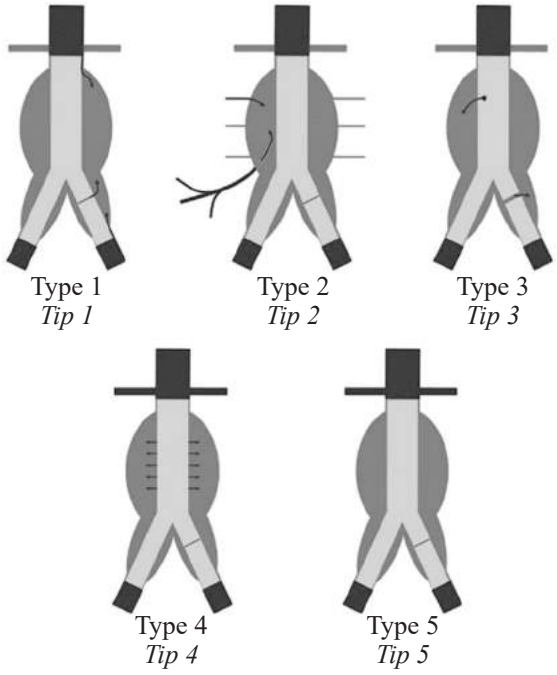

Figure 1. Types of endoleaks
Slika 1. *Vrste propuštanja*

Kinking and Infolding

Change of graft material structure, may result in more or less pronounced stenosis and reduced blood flow; the reported incidence in the literature is 57% during a 4-year follow up [8] and 3.7% on average follow up after 22 months, in the more recent publications [9].

Stenosis and occlusion

Whether as a consequence of impaired structural integrity or non-adherence to the postoperative medical protocol, stenosis and occlusion may lead to acute ischemia in the affected lower extremity which is a state of severely impaired vitality of lower limbs due to acute occlusion of the arterial blood vessel [10].

Secondary rupture

Aortic rupture after EVAR is a rare, but a devastating event. It may occur due to a technical error or the inability of devices to accommodate changes in anatomy over time, or due to graft material fatigue leading to a failure [11]. Early rupture happens within 30 days after the procedure, and late more than 30 days after the procedure. Early ruptures can be avoided by meticulous pre-operative planning, improved technical performance, and the introduction of new graft designs [12]. Late ruptures are connected with endoleaks or aneurysm expansion without a detectable endoleak [11].

Examination Modalities

Computed tomography angiography

High spatial resolution, excellent contrast resolution of crucial anatomical and graft structures, and broad availability of CT machines, make CTA the most common modality in follow-up of EVAR at the moment. The difference in Hounsfield units (HU) of contrast-filled active lumen, thrombosed lumen of the aortic sac, metallic endograft structure, and the surrounding retroperitoneal fat, enable clear distinction between these structures and their detailed analysis. As with all imaging methods, there is a limit in blood vessel diameter which is detectable by CTA. Therefore, small vascular structures cannot be visualized [13]. The lack of temporal resolution can be solved by an additional later phase of contrast examination or more contemporary CT machines and dynamic CT applications [14]. Lower operator dependence, high reproducibility with the possibility to compare previous results, and no influence of patient's body habitus make CTA optimal for EVAR follow-up.

However, exposure to ionizing radiation remains an issue, especially in the case of repeated control examinations, thus adding to the burden of patient exposure. Given that imaging is essential for EVAR follow-up, radiation exposure should be limited, particularly in light of carcinogenic potential, to a cumulative lifetime total of 400 millisieverts (mSv) [15].

Taking into account the high incidence of diabetes and renal insufficiency in patients with AAA, and the fairly high dose requirement of iodinated contrast with CTA, contrast-induced nephropathy is a major concern with these patients [16].

On non-contrast CT examination, it is possible to perform an analysis of aneurysm sac dimensions and volume. Simple measurement can give information on the maximal sac diameter **(Figure 2)** which in comparison to previous imaging, gives information of the aortic volume dynamics. Criteria for AAA rupture prediction are based only on the diameter of AAA [17]. Also, it is possible to use contemporary software analysis to perform volumetric analysis of the aneurysm sac by its segmentation to compare it with previous measurements. Single volume or diameter can be indirect signs of endoleak presence (in the case of diameter/volume increase) or absence (in the case of diameter/volume decrease).

Boos et al. reported that measurement of the centerline diameter and volume from the renal arteries to the iliac bifurcation of the abdominal aortic aneurysm is sufficient for the follow-up of patients who underwent EVAR, because the correlation between the measurements was nearly perfect. But in conclusion, the optimal volume (renal arteries to the iliac bifurcation) and diameter (centerline) have only moderate sensitivity (57–64%) and good specificity (64–82%) for detection of endoleaks; therefore, if possible, intravenous contrast material should be used in all patients after EVAR to achieve early detection of endoleaks [18].

Intravenous contrast administration enables analysis of arterial lumen dimensions and endoleak detection. The contrast material demonstrates endoleaks in the aneurysm sac beyond stent-graft **(Figure 3).** Parts of thrombus inside the sac can be partially calcified. Usually, the density of calcium formations is much higher than that of contrast, but in its development, it can be in HU range of contrast material. In that case, comparison with the precontrast images enables differential diagnosis of calcifications from endoleaks **(Figure 4).**

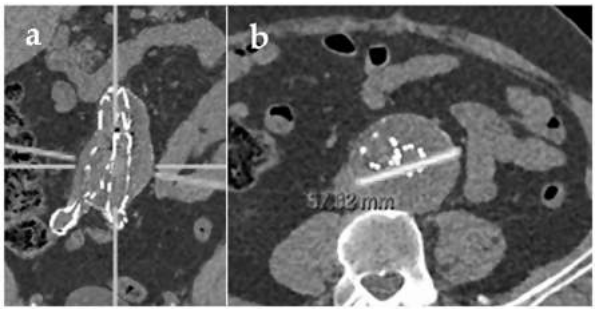

Figure 2. a) Perpendicular alignment to the longitudinal axis of the aorta; b) Measurement of the maximum aortic diameter

Slika 2*. a) podešavanje linije preseka na ravan normalnu na pravac pružanja aorte; b) merenje najvećeg prečnika aorte*

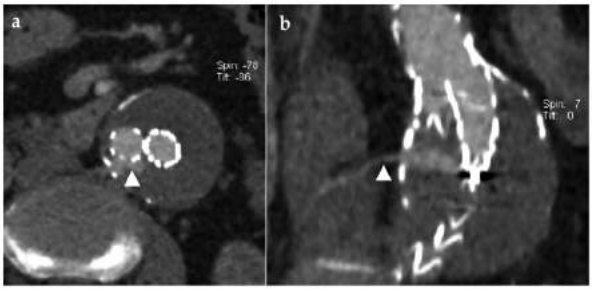

Figure 3. Endoleak type 2a; a) Contrast media in the aneurysm sac outside the stent graft (arrow head); b) Origin of the contrast flow from the right lumbar artery is easily seen on coronal reconstruction (arrow head)
Slika 3. *Propuštanje tip 2a; a) postojanje kontrasta u sakusu aneurizme van stent grafta (glava strelice); b) poreklo kontrastnog protoka iz desne lumbalne arterije je bolje prikazano na koronalnim rekonstrukcijama (glava strelice)*

The timing of the contrast phase of the examination is essential in the detection of endoleak. Arterial enhancement of the endograft lumen and arterial leak to the aneurysm sac do not have to be visible at the same moment, either because of slower accumulation of contrast in the aneurysm sac, sometimes beneath possibility of recognition in the early arterial phase in endoleaks Type 1 and 3, or retrograde flow through collateral arteries in endoleak Type 2.

In the analysis of Lemkuhl et al. the maximum endoleak enhancement was reached at 22 seconds after the bolus-tracking threshold, during the phase when the bolus of contrast material had already passed the aorta. The highest endoleak detection rate was achieved later, at 27 seconds after the bolus-tracking threshold, during the phase when the mean peak enhancement of the aorta and the endoleak had already passed, and maximum contrast could be achieved between the remaining endoleak enhancement and the rapidly de-enhancing aorta. These authors reported that scan phases 3 and 6, at

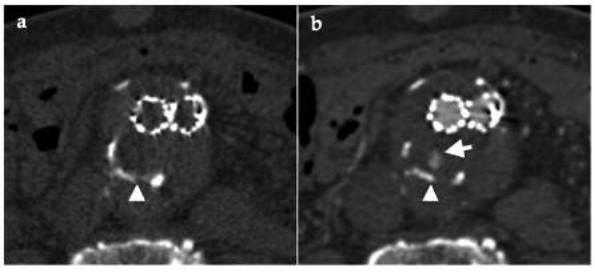

Figure 4. Endoleak in the calcified aneurysm sac lumen; a) Non contrast examination reveals calcifications in the aneurysm sac; b) Post-contrast examination reveals a calcification (arrow head) a hyperdensity which corresponds to an endoleak (arrow)
Slika 4. *Propuštanje u kalcifikovanom sakusu aneurizme; a) nativni pregled detektuje kalcifikaciju u sakusu aneurizme; b) postkontrastni pregled detektuje zonu hiperdenziteta (strelica) koja odgovara propuštanju uz ranije prikazane kalcifikacije (glava strelice)*

12 and 27 seconds after the bolus-tracking threshold, respectively, were the most useful scan phases in patients who have undergone EVAR. The first of these phases shows the highest aortic enhancement and should be used to evaluate the aorta and its branches, detect early endoleaks, and assess the endograft anchoring and position. The second of the two phases is used to detect endoleaks [14].

Indirect methods for endoleak detection are above-mentioned analysis of aneurysm sac diameter and volume, and analysis of proximal and distal endograft junction. In an attempt to decrease the number of follow up imaging sessions, Goncales et al. found that although large-scale confirmation of this concept is required, they firmly believe that when sufficient effective proximal and distal seal are achieved in the primary procedure, direct (Type 1 or 3) endoleaks and migration are exceedingly rare. However, the presence of an effective seal may not prevent complications in the long term (> 5 years) due to late degeneration of the aortic wall [19].

Besides the evaluation of endoleaks due to excellent contrast resolution between metallic graft component and the surrounding structures, CT is used for the analysis of the structure and position of the stent-graft.

Stent migration is a complication that can lead to above mentioned dangerous high flow endoleaks Type 1 and 3. The CTA analysis of graft position and comparison with the previous findings enables migration detection and prompts to further patient management. Studies have shown that one of the main predictors of stent migration is the proximal fixation length, with each millimeter increase in length fixation, reducing the hazard of migration by 2.5% [20].

Reduced blood flow in distal arteries can be the consequence of kinking, fracture, stenosis or occlusion of endograft with each of these phenomena readily evident on CTA examination. Endograft migration, tortuosity of the aorta and iliac arteries are risk factors for these complications. Accumulation of thrombotic material on the endoluminal side of endograft is possible and leads to stenosis of the active lumen. This can be demonstrated as laminar thrombosis on the endograft material next to the bloodstream **(Figure 5).**

Endograft infection is a complication with an incidence of 0.5–1% of performed stent-graft procedures [21]. Infection can be diagnosed by clinical examination and laboratory analysis. On CTA images, periaortic stranding, aortic wall thickening and formation of organized collections next to the aortic wall can be appreciated. The presence of gas inside the aneurysm sac can be a sign of infection, but also in the early post-interventional imaging, it is possible to be iatrogenically deployed gas into the aneurysm sac during the intervention. In this case, analysis of inflammatory factors and if necessary a control CT are mandatory **(Figure 6).**

Magnetic Resonance Imaging

Just as CT, magnetic resonance imaging (MRI) also enables a three-dimensional analysis of the

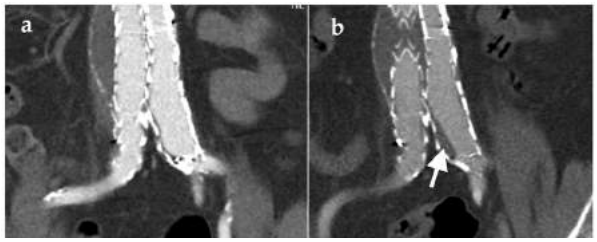

Figure 5. Endograft stenosis: a) Two years after EVAR, there are no signs of peri-mural thrombus; b) Four years after EVAR, there is a thrombus on the medial surface of the left iliac graft (arrow)
Slika 5. *Stenoza endografta; a) dve godine nakon endovaskularnog tretmana aneurizme aorte nema perimuralne tromboze; b) četiri godine nakon tretmana postoji perimuralni tromb na medijalnom aspektu levog ilijačnog grafta (strelica)*

postoperative status. Axial T1W gradient-echo sequence, followed by pre- and post-contrast T1W single-shot fast spin-echo sequence protocol, can be used. Analysis of tomograms is, as with CTA examination, performed by multiplanar reconstruction, maximum intensity projections, and by using volume rendering technique. Absence of ionizing radiation, no dependence on the operator skills and patient body habitus, with high reproducibility are obvious advantages of MRA method. However,

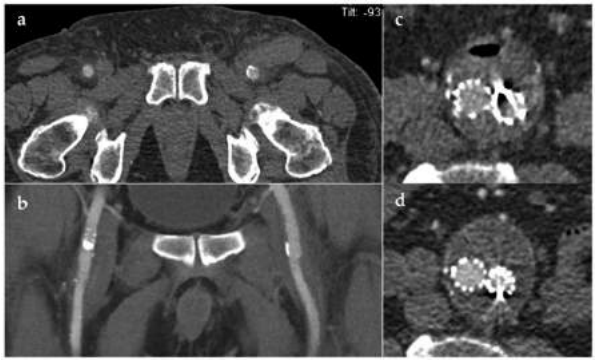

Figure 6. Hematoma in the left Scarpa's region and air in the aneurysm sac on early post-operative imaging two days after procedure; a and b) Hematoma in the left Scarpa's region with preserved blood flow through common femoral artery; c) Gas in the aneurysm sac, most probably due to perioperative manipulation; no other signs of infection and patient was discharged from hospital with no signs of inflammation; d) On examination two years later, there is no gas in the aneurysm sac and reduced latero-lateral sac diameter
Slika 6. *Hematom u levoj Skarpovoj regiji i vazduh u aneurizmatskom sakusu na ranom postoperativnom pregledu dva dana nakon intervencije. a) i b) hematom u levoj Skarpovoj regiji sa očuvanim protokom krvi kroz femoralnu arteriju; c) gas u aneurizmatskom sakusu najverovatnije kao posledica peripriceduralnog ulaska. Nije bilo drugih znakova infekcije i pacijent je otpušten iz bolnice u dobrom opštem stanju; d) na pregledu dve godine nakon intervencije detektuje se smanjenje dijametra sakusa, bez prisustva gasa.*

poor demonstration of the metallic endograft components and no available MRI machines in some regions are disadvantages of this modality.

In analyzing endoleaks, MRA is equivalent to CTA in sensitivity, and in Type II endoleaks, it is superior in patients with newer generation nitinol EVAR grafts [22]. Also, in patients with impaired renal function, it is possible to perform time-of-flight imaging, but the specificity of these examinations has been documented as low as 54% in endoleak detection [23]. MRA is particularly useful in patients with nitinol stent-grafts; stainless steel and nickel alloy grafts cause a large amount of susceptibility artifact that precludes optimal evaluation by MRI [24].

Ultrasound

Ultrasound (US) is a non-ionizing and relatively cheap imaging modality that is an alternative to CTA and MRA. In detection of endoleaks, there are various modes, beginning with standard B-mode for diameter evaluation of the aneurysm sac, Doppler ultrasound, and contrast-enhanced ultrasound. Ultrasound has a high specificity for endoleaks in up to 95%, but the overall sensitivity is as low as 70%, compared to CTA as a gold standard [25, 26].

Cumulative radiation dose and renal injury associated with CTA are an issue that is being attempted to overcome by CEUS. This modality found the greatest use in detection and follow up of endoleaks. A meta-analysis that has evaluated seven trials comparing CEUS with CTA found a sensitivity of 0.98 and specificity of 0.88 of CEUS [27]. CEUS has several advantages compared to CT, including lower cost, shorter scanning time, and most importantly, absence of nephrotoxicity and radiation exposure. However, at times, the poor performance of CEUS in endoleak detection may be due to patient factors [28]. Lack of a suitable panoramic stent visualization with CEUS brings about the need to obtain concurrent radiographs when performing surveillance using CEUS, for the diagnosis of stent migration and kinking [29].

On the other hand, direct comparison with previous images is severely diminished in US evaluation compared with three-dimensional modalities such as CT and MRI, which offer multiplanar comparison and evaluation of millimetric changes in diameters of vessels analyzed. Also, there can be a discrepancy in diameters measured by different modalities with reported overestimation of the aorta diameter on ultrasound compared to CT [30], which implies surveillance using a single imaging modality.

Also, operator and patient habitus dependence and poor visualization of endograft structure are disadvantages of US as an EVAR surveillance modality.

Radiography

Radiographic analysis of blood flow is not possible, but excellent spatial resolution and possibility of analyzing the metallic structure of endograft give place to standard radiography in surveillance protocol. Stent graft kinking, fracture, migration,

and change of diameter are readily demonstrated. Even minimal endograft migration can be detected on consequently performed standard radiographs, with image overlapping and analysis of the relationship to bony landmarks. However, the impossibility of lumen analysis and three-dimensional imaging determines radiography as a supplementary surveillance modality.

Intrasac Pressure Measurement

The theoretical premise of EVAR is that a successful treatment will exclude the AAA sac from the systemic arterial pressure and circulation [31]. Imaging modalities cannot measure the pressure inside the sac, and the rationale for the success of the intervention is the measurement of diameters. It is possible to measure the intra-sac pressure with guide wire mounted tip-pressure sensors. Expansion of the sac may occur in the absence of intra-sac fluid accumulation and is associated with higher and more pulsatile intra-sac pressure. However, in patients with intra-sac fluid, expansion can occur with low intra-sac pressure as well [31].

Follow-up Protocol

Currently, the recommended surveillance protocol is contrast-enhanced CT imaging performed 1 and 12 months after the procedure. Should CT imaging at one month after EVAR identify an endoleak or other abnormality of concern, postoperative imaging at six months should be added to further evaluate the proper exclusion of an aneurysm. If neither an endoleak nor aneurysm enlargement is documented during the first year after EVAR, color duplex ultrasonography may be a reasonable alternative to CT imaging for postoperative surveillance [32]. In addition to ultrasonographic surveillance, standard anteroposterior and lateral radiography should be performed; if the patient's body habitus preclude an adequate DUS, then a non-contrast CT with plain radiographs can be substituted [33]. In case of US or radiography proven increase in the size of the aneurismal sac or signs of endoleaks, CTA evaluation is mandatory.

The extended follow-up period has led to a significant drop from the follow-up regimen, but annual imaging follow up compliance post-EVAR in the United States is significantly below recommended levels [34]. Devastating complications must be acknowledged, and their consequences treated before fatal events. Development and broader application of aneurysm sac pressure sensors are expected to enhance the safety of follow-up without CT, but after confirmation [35].

The regular follow-ups do not affect the surveillance or prevent complications [36], and despite clear guidelines, follow-up routines vary significantly between centers. A stratified follow-up protocol can be suggested based on the risk of complications. Most complications appear early after the procedure, so early CT is beneficial. Also, patients at risk (early endoleak, hostile anatomy, etc.) would require more frequent follow-ups.

Conclusion

Imaging is essential in the pre- and post-procedural evaluation of patients treated with endovascular aortic repair. Multiple modalities enable evaluation of morphology, selection of patients, and detection of complications. Due to its characteristics and availability, computed tomography angiography remains the cornerstone of the pre- and post-procedural assessment in patients with infrarenal abdominal aortic aneurysm.

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NON-HODGKIN LYMPHOMA IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY SYNDROME

NE-HOČKINOV LIMFOM KOD LJUDI KOJI ŽIVE SA SINDROMOM STEČENE IMUNODEFICIJENCIJE

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Summary

Introduction. Even in the era of combined antiretroviral therapy, the mortality rate in patients with human immunodeficiency virus infection remains high, especially with a contributing diagnosis of a malignant disease, such as non-Hodgkin lymphoma. Given the previous, the goal of this research was to establish the incidence of non-Hodgkin lymphoma in human immunodeficiency virus positive patients, as well as to determine their clinical characteristics and mortality in regard to patients with human immunodeficiency virus only. **Material and Methods.** The retrospective study included 396 human immunodeficiency virus-positive patients. Medical records were reviewed to analyze the average age, duration of infection, average duration of therapy, nCD4+ T-cell count, human immunodeficiency virus viral load, as well as the number and types of malignant diseases. **Results.** The average age of the patients was 44.2 years; the average nCD4+ T-cell count was 296.94 cells/μL, while the mortality rate was 14.65%. The leading causes of death were non-Hodgkin lymphoma and acquired immunodeficiency syndrome. The most frequently diagnosed malignancy was non-Hodgkin lymphoma, where the average count of nCD4+ T-cells was 162.29 cells/μL. Patients with human immunodeficiency virus and non-Hodgkin lymphoma had significantly lower nCD4+ T-cell count, in regard to patients with human immunodeficiency virus only, and the mortality rate in this group of patients was 85%. **Conclusion.** The incidence of non-Hodgkin lymphoma in human immunodeficiency virus-positive patients represents a growing threat, given the exceptionally high mortality. The nCD4+ T-cell count may indicate acquired immunodeficiency syndrome and late diagnosis of human immunodeficiency virus together are predictors for non-Hodgkin lymphoma and its poor outcome. It points to the importance of increasing the scope of human immunodeficiency virus testing, as well as finding a better treatment approach.

Key words: Lymphoma, Non-Hodgkin; HIV Infections; Acquired Immunodeficiency Syndrome; Mortality; CD4-Positive T-Lymphocytes; CD4 Lymphocyte Count; Early Diagnosis

Sažetak

Uvod. Uprkos uvođenju kombinovane antiretrovirusne terapije, stopa smrtnosti kod pacijenata sa infekcijom virusom humane imunodeficijencije i dalje je visoka, naročito ukoliko imaju istovremeno dijagnostikovano maligno oboljenje, a posebno ne-Hočkinov limfom. Cilj ovog istraživanja bio je da se utvrdi učestalost ne-Hočkinovog limfoma kod pacijenata pozitivnih na virus humane imunodeficijencije, njihove kliničke karakteristike i smrtnost u odnosu na pacijente bez ne-Hočkinovog limfoma. **Materijal i metode.** Retrospektivna studija je obuhvatila 396 ispitanika sa potvrđenom infekcijom virusom humane imunodeficijencije. Uvidom u medicinsku dokumentaciju, analizirane su prosečne godine života, prosečna dužina trajanja infekcije i terapije, broj nCD4+ T-limfocita, broj virusnih kopija, kao i broj i vrsta malignih oboljenja. **Rezultati**. Prosečna starost pacijenata u našoj studiji iznosila je 42,2 godine, a prosečan broj nCD4+ T-limfocita 296,94 ćelija/μL, dok je stopa smrtnosti bila 14,65%. Vodeći uzroci smrti su bili ne-Hočkinov limfom i sindrom stečene imunodeficijencije. Najučestaliji malignitet bio je takođe ne-Hočkinov limfom, pri čemu je prosečna vrednost nCD4+ T-limfocita u ovoj grupi iznosila 162,29 ćelija/μL. Pokazana je statistički značajno manja vrednost nCD4+ T-limfocita u grupi pacijenata sa infekcijom virusom humane imunodeficijencije i ne-Hočkinovim limfomom u odnosu na pacijente koji su imali samo virus humane imunodeficijencije, pri čemu je smrtnost u ovoj grupi iznosila 85%. **Zaključak.** Ne-Hočkinov limfom kod pacijenata sa infekcijom virusom humane imunodeficijencije predstavlja sve veći problem, s obzirom na visoku stopu smrtnosti. Budući da su broj nCD4+ T-limfocita i kasna dijagnoza infekcije virusom humane imunodeficijencije najveći faktori rizika za razvoj ne-Hočkinovog limfoma, neophodno je povećati obim testiranja na virus humane imunodeficijencije, te pronaći adekvatniji pristup u lečenju ovih pacijenata.

Ključne reči: non-Hočkinov limfom; HIV infekcije; AIDS; mortalitet; CD4+ T limfociti; CD4 broj limfocita; rana dijagnoza

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Abbreviations
HIV – human immunodeficiency virus
AIDS – acquired immunodeficiency syndrome
ADI – average duration of infection
ADT – average duration of therapy
ART – antiretroviral therapy
DLBCL – diffuse large B-cell lymphoma
SPSS – Statistical Package for the Social Sciences
PLWH – people living with HIV
NHL – non-Hodgkin lymphoma

Introduction

With improvements in antiretroviral therapy (ART), the incidence of acquired immunodeficiency syndrome (AIDS) related opportunistic infections and tumors has decreased. However, the mortality rate in this group of patients remains high [1]. Over the last few decades, many of the most common causes of death in patients with human immunodeficiency virus (HIV) infection have changed its course, and due to ART they became significantly less prominent, However, malignant diseases remain in the focus of interest, considering that they are responsible for the largest number of deaths in HIV-infected patients [2]. Due to the decline in the immune system in HIV-infection, it is comprehensible how these patients are more susceptible to the development of malignant diseases. A large number of recent studies have shown that non-AIDS-defining malignancies are increasingly prevalent in the world, most likely as a result of prolongation of life, thus creating the basis for the onset of comorbidities [3]. Contrary to the previous, in our population AIDS-defining malignancies are highly present and the incidence is still increasing. Among them, the most common is non-Hodgkin lymphoma (NHL), in its three subtypes - diffuse large B-cell (DLBCL), Burkitt, and central nervous system lymphoma. The presumed pathogenic mechanisms for the onset of lymphomas in HIV-infected population imply the effect of oncogenic viruses, excessive secretion of cytokines, as well as chronic antigenic stimulation. Some recent studies also indicate the possible influence of HIV-proteins secreted mostly in germinal centers of lymph nodes, as a contributing factor for the development of lymphomas [4]. As a consequence of immunodeficiency, combined with immune suppression due to chemotherapy, the treatment of these patients is extremely hard and often has a fatal outcome [5]. Several studies have so far identified common characteristics of HIV-positive patients diagnosed with NHL, all relying on the late presentation of HIV as the crucial problem [6, 7]. The goals of this research were to establish the incidence of NHL in this group of patients, determine their immunological parameters and differences compared to HIV-positive patients without NHL in the Autonomous Province of Vojvodina, as well as to determine the most common cause of death in these patients. Given the fact that NHL is AIDS-related malignancy, we hypothesized that patients with HIV and NHL have a significantly lower immune system compared to patients with HIV only. Considering the fact that regular HIV testing is still neglected in our country, we also hypothesized that the majority of patients were diagnosed at a late stage of HIV-infection, and that the most common cause of death in these patients was AIDS-related.

Material and Methods

This retrospective study was performed at the Clinic of Infectious Diseases, Clinical Center of Vojvodina, from January 2007 to December 2019. The Ethics Committee of the Clinical Center of Vojvodina has reviewed and approved the study. The study included a total of 396 patients with HIV diagnosis, confirmed by Western-Blot analysis and PCR assay. Patients aged younger than 18 years were excluded.

The medical records were reviewed to collect information on demographic, clinical characteristics and laboratory test results. We analyzed the following variables: demographics (age, gender) and clinical (average duration of infection (ADI), average duration of therapy (ADT), nCD4+ T-cell, and HIV viral load

Table 1. Demographic and clinical characteristics of HIV-infected patients (2007–2019)
Tabela 1. *Demografske i kliničke karakteristike pacijenata sa HIV infekcijom (2007–2019)*

Patients/*Pacijenti*		N (%)/*Broj (%)*	396 (100%)
Gender/*Pol*	Male/*Muški*		364 (91.92%)
	Female/*Ženski*		32 (8.08%)
Age/*Starost*		Years/*Godine*	42.20
ADI		Months/*Meseci*	78.52
ADT		Months/*Meseci*	66.23
CD4+ nadir	Min/*Minimum*	Cells/μL/*Ćelije/μL*	1
	Max/*Maksimum*		1265
	Median/*Medijana*		296.94
Mortality rate/*Stopa smrtnosti*		N (%)/*Broj (%)*	58 (14.65%)

Legend: ADI – average duration of infection, ADT – average duration of therapy
Legenda: ADI – Prosečna dužina trajanja infekcije, ADT – Prosečna dužina trajanja terapije, HIV – virus humane imunodeficijencije

Table 2. Distribution of malignant diseases in patients with HIV infection
Tabela 2*. Distribucija malignih bolesti kod pacijenata sa HIV infekcijom*

Total number of patients with malignancies *Ukupan broj pacijenata sa malignitetima*				N (%)/*Broj (%)*	21 (100%)
Type of malignant disease *Tip malignitet*a	AIDS-defining tumors *AIDS definišući tumor*i	NHL	DLBCL	N (%)/*Broj (%)*	14 (66.67%)
		Kaposi's sarcoma *Kapošijev sarkom*	Cutaneous/*Kutani*	N (%)/*Broj (%)*	2 (9.52%)
			Visceral/*Visceralni*	N (%)/*Broj (%)*	3 (14.29%)
	non-AIDS-defining tumors *ne-AIDS-definišući tumori*	Lung cancer/*Karcinom pluća*		N (%)/*Broj (%)*	1 (4.76%)
		Colon cancer/*Karcinom kolona*		N (%)/*Broj (%)*	1 (4.76%)

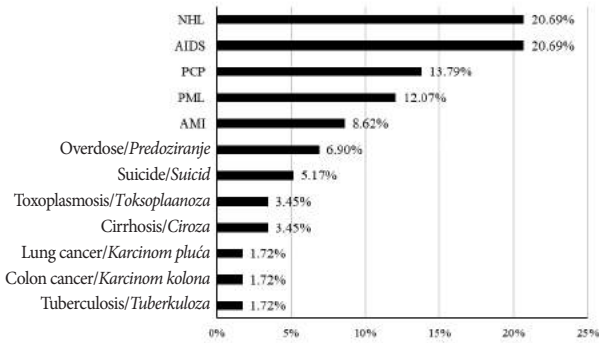
All the values were calculated relative to a total number of patients/ *Sve vrednosti su izračunate u odnosu na ukupan broj pacijenata*
Legend: DLBCL – diffuse large B-cell lymphoma
Legenda: DLBCL – difuzni B-krupnoćelijski limfom, AIDS – sindrom stečene imunodeficijencije, NHL – ne-Hočkinov limfom

determined by polimerase chain reaction assay). All diagnoses of malignant diseases were histopathologically confirmed.

Statistical data processing was done using the software program Statistical Package for the Social Sciences (SPSS) version 21.0. The descriptive analysis included calculating mean, minimum, maximum and median values. The normality of the distribution and the homogeneity of the variance were tested for each group, and afterwards difference between variables was determined by Student's t-test. The values of $p < 0.05$ were considered statistically significant.

Results

In total, 396 patients (92% male and 8% female) with HIV infection were included in the study, whose mean age was 42.2 years **(Table 1)**. The ADI from 2007 to 2019 was 78.52 months, while the ADT was 66.23 months. The lowest nCD4+ T-cell count was 1, the mean was 296.94, and the highest nCD4+ T-cell count was 1265 cells/μL, respectively. The mortality rate was 14.65% by the end of the study, during the period of 12 years. The leading causes of death were NHL (20.69%) and AIDS (20.69%), followed by pneumocystis carinii pneumonia (PCP) (13.79%), progressive multifocal leukoencephalopathy (PML) (12.07%), acute myocardial infarction (AMI) (8.62%), overdose (6.90%) and suicide (5.17%) **(Graph 1)**. AIDS was considered as the cause of death in patients who died before being definitively diagnosed with an opportunistic infection or malignant disease. The least common causes of death were tuberculosis (1.72%) and non-AIDS defining tumors – lung and colon cancer (both 1.72%). A total of 21 malignant diseases were diagnosed in 396 HIV-infected patients **(Table 2)**. The predominant malignancies were AIDS-defining tumors (90.48%), while non-AIDS-defining accounted only for 9.52%. The most frequent among the AIDS-defining tumors was non-Hodgkin lymphoma (66.67%), followed by visceral (14.29%) and cutaneous Kaposi's sarcoma (9.52%). Apart from Kaposi's sarcoma and non-Hodgkin lymphoma, which are considered AIDS-defining, two additional cancers - lung and colon (4.76% both), referred to as non-AIDS-defining tumors, were also recorded. All patients diagnosed



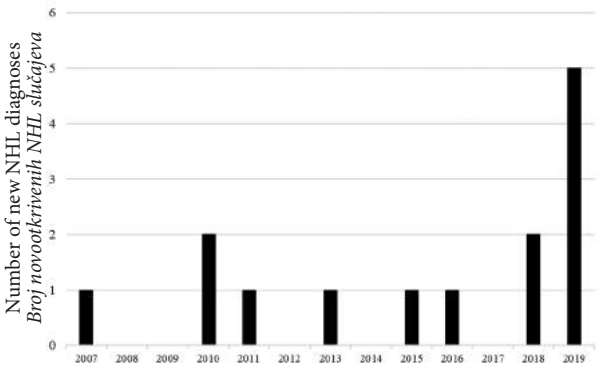
Graph 1. Distribution of patients by cause of death
Grafikon 1. *Distribucija pacijenata u odnosu na uzrok smrti*
Legend: NHL – non-Hodgkin lymphoma, PCP – pneumocystis carinii pneumonia, PML – progressive multifocal leukoencephalopathy, AMI – acute myocardial infarction
Legenda: NHL – ne-Hočkinov limfom, PCP – Pneumocystis carinii pneumonija, PML – progresivna multifokalna leukoencefalopatija, AMI – akutni infarkt miokarda, AIDS – sindrom stečene imunodeficijencije

with Kaposi's sarcoma were alive by the end of the study, and further research considered them as patients with non-Hodgkin lymphomas. The **Graph 2** shows the distribution of newly diagnosed cases of non-Hodgkin lymphoma per year. During the 13-year period, NHL incidence has been increasing. In 2007, one new case of NHL was diagnosed. After two years, two new cases were diagnosed in 2010, then one new case per year from 2011 to 2016. During 2018, two new cases of NHL were detected. A large increase in incidence was recorded in 2019, and 5 new cases of NHL were diagnosed. The **Table 3** summarizes characteristics of HIV-positive patients with NHL diagnosis. Median age of these 14 patients was 48.09 years. The lowest nCD4+ T-cell count was 16, the mean 162.29, and the highest nCD4+ T-cell count was 436 cells/μL, respectively. The average duration of infection was 62.62 months. Compared to all patients in the study, the ADT for patients with diagnosed NHL was significantly lower (10.45 months). Among the patients with diagnosed NHL, the lowest viral load was 22101 copies/mL while the highest recorded value was 4199025 copies/mL (mean was 868720.23 copies/mL). There was a statistically significant difference in the nCD4+ T-cell count between HIV-positive patients

Table 3. Characteristics of patients with HIV infection and NHL diagnosis
Tabela 3. *Karakteristike pacijenata sa infekcijom virusom humane imunodeficijencije i dijagnozom ne-Hočkinovog limfoma*

Age/*Starost*		Years/*Godine*	48.09
nCD4+ T-cells count/*broj nCD4+ ćelija*	Min/*Minimum*		16
	Max/*Maksimum*	Cells/µL/*Ćelije/µL*	436
	Median/*Medijana*		162.29
ADI		Months/*Meseci*	62.62
ADT		Months/*Meseci*	10.45
Viral load/*Broj kopija virusa u krvi*	Min/*Minimum*		22101
	Max/*Maksimum*	Copies/mL/*Kopije/mL*	4199025
	Median/*Medijana*		868720.23
Mortality rate/*Stopa smrtnosti*		N (%)/*Broj (%)*	12 (85.71%)

Legend: ADI – average duration of infection, ADT – average duration of therapy
Legenda: ADI – Prosečna dužina trajanja infekcije, ADT – Prosečna dužina trajanja terapije



Graph 2. Distribution of new cases of non-Hodgkin lymphoma from 2007 – 2019
Grafikon 2. *Distribucija novootkrivenih slučajeva ne-Hočkinovog limfoma (NHL) od 2007. do 2019. godine*

and HIV-positive patients with diagnosed NHL – significantly lower nCD4+ T-cell count was present in patients with NHL. In regard to the ADT, there were statistically significant differences between groups. Furthermore, although age, ADI and viral load have been considered as clinical parameters of HIV-positive patients with diagnosed NHL, in our study they were not statistically significant **(Table 4)**.

Discussion

The low difference in the ADI and ADT in our study can be explained by changes in 2016 European AIDS Clinical Society (EACS) guidelines, which recommend starting combined antiretroviral therapy (cART) as soon as possible, irrespectively of CD4 T-cell count [8]. Analyzing the clinical characteristics of patients with HIV infection, we found that the average count of nCD4+ T-cell was 296.94 cells/µL. Given the fact that nadir CD4+ T-cell count below 300 cells/µL represents AIDS, this result speaks in favor of the fact that the majority of HIV-positive patients are diagnosed at a late stage of infection, which confirmed our hypothesis. Recent studies have also shown that over 50%

Table 4. Comparison of characteristics between two subgroups of patients with HIV infection
Tabela 4. *Poređenje kliničkih parametara između dve podgrupe pacijenata sa infekcijom virusom humane imunodeficijencije*

Parameter/*Parametar*		Mean/*Srednja vrednost*	p*
Age (PLWH)/*Starost (HIV-pozitivni pacijenti)*	Years *Godine*	42.01	0.0569
Age (PLWH+NHL)/*Starost (HIV+NHL)*		48.09	
nCD4+ T-cells count (PLWH)/*broj nCD4+ T-ćelija (HIV)*	Cell/µL *Ćelije/µL*	300.15	0.0047
nCD4+ T-cells count (PLWH+NHL) *broj nCD4+ T-ćelija (*PLWH+*NHL)*		162.29	
ADI (PLWH)	Months *Meseci*	79.08	0.5555
ADI (PLWH+NHL)		62.62	
ADT (PLWH)	Months *Meseci*	67.94	0.0000
ADT (PLWH+NHL)		10.45	
Viral load (PLWH)/*Broj kopija virusa (HIV)*	Copies/mL *Kopije/mL*	924524.61	0.9105
Viral load (PLWH+NHL)/*Broj kopija virusa (HIV+NHL)*		868720.23	

* $p < 0.05$ was considered statistically significant/* $p < 0,05$ je smatrano statistički značajno
Legend: ADI – average duration of infection, ADT – average duration of therapy, PLWN – people living with HIV
Legenda: ADI – Prosečna dužina trajanja infekcije, ADT – Prosečna dužina trajanja terapije, HIV – virus humane imunodeficijencije, NHL – ne-Hočkinov limfom, PLWH – osobe sa infekcijom virusom humane imunodeficijencije

of all HIV diagnoses were established at a late stages of infection [9, 10]. This result indicates the need to increase the extent and frequency of HIV-testing, especially in populations at high risk for this infection, in order to prevent AIDS-related complications. The mortality rate in our study was 14.65%, and the most common causes of death were non-Hodgkin lymphoma and AIDS. A number of studies have shown that despite highly effective ART, the incidence and number of deaths caused by NHL in people living with HIV (PLWH) is still increasing. For example, a study in the United States showed that among patients with NHL, especially DLBCL, the highest mortality was found in the group of patients with conjoint HIV-infection. The fact that DLBCL is one the most frequent B-cell lymphomas in PLWH, with high mortality, was also confirmed in a German cohort study with overall death rate higher than 30%, as well as in the study in Sub-Saharan Africa, where patients with HIV and NHL had a poor survival [11–13]. The assumed reason for this lies in severe immunodeficiency combined with immunosuppression in these patients, and therefore, a very complicated treatment and a specific approach are needed. Besson et al. reported that the mortality rate of patients with NHL does not change regarding whether they have HIV or not, however, the rate of NHL recurrence in HIV patients is significantly higher in regard to non-HIV populations [14]. Equal number of patients in our groups died from AIDS. The exact cause of death in this group of patients could not be established, due to the fact that they all died before being diagnosed with an AIDS-related condition. AIDS has also been confirmed as one of the most common causes of death in studies done in Asia and Canada [15, 16]. Non-AIDS defining malignancies were the least common cause of death in our study. These results differ from many recent studies in which malignancies not related with AIDS are the first or second most common cause of death in PLWH [17, 18]. In those studies, patients were diagnosed mostly in early stages of HIV infection, therefore they had a better chance for immune reconstitution, hence life prolongation, unlike patients in our study. This result again implicates the necessity of more extensive testing for HIV in our population in order to obtain early diagnosis. Considering the pathogenesis of HIV infection that leads to slow destruction of the immune system, it is understandable how these patients are more susceptible for developing a malignant disease. Therefore, it is expected that in the analysis of the prevalence of malignant diseases, non-Hodgkin lymphoma is in the first place. The second most common malignant disease was Kaposi's sarcoma accounting for 24%, while non-AIDS malignancies accounted only for 9%. The introduction of cART at the beginning and its constant improvement has led to better outcomes of many malignant diseases, as well as other AIDS-related comorbidities. However, due to a rising number of complications and lethal outcomes of patients with NHL and HIV, a number of studies have been conducted to determine the incidence and characteristics of these patients, all confirming very high incidence of NHL in HIV positive patients. Our results speak in favor of increasing NHL incidence in PLWH over time, as confirmed by Howlader, Olszewski and Ramaswami [11, 19, 20]. The question that remains is whether this increase is attributable to a real increase in incidence, or just higher HIV testing frequency in people with NHL, since it has not been a consistent practice until recently. Speaking of the factors that may contribute to the development of NHL in HIV-positive patients, we found that detection of late-stage HIV infection is one of the most significant factors. The average number of nCD4+ T-cells count in patients with NHL and HIV in our study was 162.29 cells/μL, far below the limit for the diagnosis of AIDS, whereby their count of nCD4+ T-cells was significantly lower in relation to the count of these cells in patients without NHL. The fact that the diagnosis of AIDS according to nCD4+ T-cell count is a strong predictor for NHL was confirmed in other studies as well [14, 19, 21–23]. Taking into consideration the fact that nCD4+ T-cells count is an indirect indicator of infection duration, this result was expected. Most of the patients in our study were diagnosed with AIDS and NHL at the same time, with mortality rate of 85%. Already existing immune deficiency in these patients due to HIV infection, with further immune suppression due to chemotherapy for NHL explain how the combination of these two conditions suggest poor outcome, as indicated in other studies as well [23–25]. Most of these patients died soon after diagnosing AIDS and NHL, which is why the ADI and therapy in these patients differ in regard to patients with HIV, and why they have statistically lower duration of therapy in comparison with HIV-positive patients only. Even though Hentrich and Howlader suggested that one of the contributing factors for the onset of NHL is older age, in our study we have not found a statistically significant difference between PLWH without and with NHL, but the difference is almost significant [12, 14]. This can be explained by the fact that the general population in our study was older, as well as the fact that patients with HIV and NHL died before finishing the study and calculation of the overall age of patients.

Conclusion

Even though combined antiretroviral therapy has changed the course of human immunodeficiency virus infection, the mortality rate in these patients remains high. In the Autonomous Province of Vojvodina, the most common causes of death are acquired immunodeficiency syndrome related conditions, due to insufficient scope of human immunodeficiency virus testing. As one of the most common causes of death, and also one of the most common malignant diseases, non-Hodgkin lymphoma is a growing threat. Late diagnosis of human immunodeficiency virus and CD4+ T-cell count indicative for acquired immunodeficiency syndrome are predictors of non-Hodgkin lymphoma and poor outcome. Increasing the scope of human immunodeficiency virus testing should contribute to earlier diagnosis of human immunodeficiency virus,

and therefore better outcome for these patients. In addition, even though we did not have a representative sample in our study, improvement in the field of screening for non-human immunodeficiency virus related malignancies should be in the focus of interest, as suggested in a number of researches done so far.

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COMPARISON OF SEROLOGICAL AND MOLECULAR METHODS IN THE DIAGNOSIS OF CYTOMEGALOVIRUS INFECTIONS IN DIALYSIS PATIENTS

POREĐENJE SEROLOŠKIH I MOLEKULARNIH METODA U DIJAGNOSTICI INFEKCIJA CITOMEGALOVIRUSOM KOD PACIJENATA NA DIJALIZI

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Summary

Introduction. Cytomegalovirus is the most common cause of infections in the post-transplantation period. A reliable and timely laboratory diagnosis of cytomegalovirus infection in patients on dialysis and in the post-transplantation period is significant because of the possibility of preventing or mitigating the effects of cytomegalovirus disease. The main objective of this study was to compare serological and molecular polymerase chain reaction methods to determine the presence of cytomegalovirus in the blood of dialysis patients. **Material and Methods.** The study included 28 dialysis patients, potential renal transplant recipients. All patients were evaluated for the presence of cytomegalovirus in the blood by a quantitative polymerase chain reaction method as well as in the serum for the presence of anti-cytomegalovirus Immunoglobulin G and Immunoglobulin M antibodies. **Results.** According to the comparative enzyme-linked immunosorbent assay for detecting antibodies in dialysis patients, 96.4% were once exposed to the virus, while 7.1% showed current infection not confirmed by polymerase chain reaction test. No statistically significant association was found between the positive finding of anti-cytomegalovirus Immunoglobulin M antibodies and the findings of the polymerase chain reaction cytomegalovirus method when Chi-square (χ^2) and Fisher's correlation tests were conducted ($p > 0.05$). **Conclusion.** Due to 7.1% false positives results for the presence of anti-cytomegalovirus Immunoglobulin M antibodies in the serum of immunocompromised dialysis patients, not confirmed by polymerase chain reaction test, serological techniques are not reliable in detecting active cytomegalovirus infection causing positive finding of anti-cytomegalovirus Immunoglobulin M, so confirmation of cytomegalovirus deoxyribonucleic acid by polymerase chain reaction method is required.

Key words: Cytomegalovirus; Cytomegalovirus Infections; Polymerase Chain Reaction; Diagnosis; Kidney Transplantation; Renal Dialysis; Enzyme-Linked Immunosorbent Assay; Serotyping

Sažetak

Uvod. Citomegalovirus je najčešći uzrok infekcija u posttransplantacionom periodu. Pouzdana i pravovremena laboratorijska dijagnoza infekcije citomegalovirusom kod pacijenata na dijalizi i u posttransplantacionom periodu je značajna zbog mogućnosti prevencije ili ublažavanja posledica citomegalovirusne bolesti. Glavni cilj ovog istraživanja je poređenje serološke sa molekularnom metodom lančane reakcije polimeraze za određivanje prisustva citomegalovirusa u krvi pacijenata na dijalizi. **Materijal i metode.** Ispitivanjem je bilo obuhvaćeno 28 pacijenata na dijalizi, potencijalnih recipijenata za transplantaciju bubrega. Svim pacijentima je određivano prisustvo citomegalovirusa u krvi kvantitativnom metodom lančane reakcije polimeraze, a paralelno u serumu i prisustvo antitela na citomegalovirus, imunoglobulina G i imunoglobulina M. **Rezultati.** Uporednom enzim-imunoesej metodom detekcije antitela kod pacijenata na dijalizi 96,4% je nekada bilo izloženo virusu, dok je 7,1% pokazalo trenutnu infekciju koja nije potvrđena testom lančane reakcije polimeraze. Upotrebom χ^2 i Fišerovog testa nije dokazana statistički značajna povezanost između pozitivnog nalaza antitela imunoglobulina M na citomegalovirus i nalaza citomegalovirusa metodom lančane reakcije polimeraze ($p > 0,05$). **Zaključak.** Zbog 7,1% lažno pozitivnih nalaza na prisustvo antitela imunoglobulina M na citomegalovirus u serumu imunokompromitovanih pacijenata na dijalizi koje test lančane reakcije polimeraze nije potvrdio, serološke tehnike nisu pouzdane u detekciji aktivne infekcije citomegalovirusom zbog čega pozitivni nalazi antitela imunoglobulina M na citomegalovirus zahtevaju potvrdu deoksiribonukleinske kiseline citomegaloirusa metodom lančane reakcije polimeraze.

Ključne reči: citomegalovirus; citomegalovirusne infekcije; PCR; dijagnoza; transplantacija bubrega; bubrežna dijaliza; ELISA; serotipizacija

Introduction

Cytomegalovirus (CMV) belongs to the herpes family and it is also known as human herpes virus 5 (HHV-5). The CMV can be transmitted in utero, perinatally, horizontally (direct or indirect transmission) and also by tissue or organ transplantation [1]. Peripheral blood mononuclear cells and endothelial cells are the main site of infection. The infection happens in immunocompetent as well as in immu-

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Abbreviations

CMV – cytomegalovirus
PCR – polymerase chain reaction
IgG – Immunoglobulin G
IgM – Immunoglobulin M
ELISA – enzyme-linked immunosorbent assay
EDTA – ethylenediaminetetraacetic acid
DNA – deoxyribonucleic acid
TNAI – total nucleic acid isolation

nocompromised persons and the level of viremia is not the only crucial factor for the onset of symptoms [2]. The most important interaction for the onset of symptoms is between the virus itself and the immune system. In immunocompetent people, CMV causes asymptomatic infections or infections with a mild clinical presentation, but it persists in the organism of a person who has once been infected, often for a lifetime. The newborns, immunocompromised persons, patients with transplanted organs, and persons affected by acquired immunodeficiency syndrome (AIDS) represent high risk groups for the development of serious CMV infections, leading to an increased rate of morbidity and mortality. In developed countries, 75% of adult population is CMV imunoglobulin G (IgG) positive, and in underdeveloped areas, the number rises up to 100% [3]. Severe clinical manifestations of CMV infection include retinitis, gastroenteritis, hepatitis, encephalitis, esophagitis, enterocolitis, pancreatitis and pneumonia [2].

Essentially, CMV infection development depends on two basic factors: immunosuppression intensity and transplanted organ type [4]. The CMV is the most common cause of infections in the post-transplant period [5]. In solid organs and bone marrow recipients, CMV infection can occur in several ways: as a primary infection, as a secondary infection (reactivation of latent virus) or as a superinfection (reinfection with another viral strain). Infections most commonly occur after the transplantation of heart and lungs (39%), liver and pancreas (29%), heart (25%) and kidneys (8%). The greatest risk for CMV infection is the combination of seronegative recipients and seropositive donors [6]. When donors are seropositive and recipients seronegative, the risk is enormous and it goes up to 90%.

Viral (CMV) infection does not imply its clinical presentation. In solid organ recipients, active CMV infection (virus replication) happens in 60–100% of patients, and without preventive antiviral treatment, clinical manifestations of CMV disease are present in 20–60% of patients with active infection. Recipients of solid organ transplants are the most vulnerable during two to four months after the transplantation [7]. Without preventive antiviral treatment, about half of bone marrow recipients develop active CMV infection and 20–25% of these patients develop CMV infection with clinical manifestations [8].

Detection and quantification of CMV in blood is significant for patient monitoring and making the decision when to initiate antiviral therapy [9]. Today, there are many commercial immunoenzymatic tests which detect imuoglobulin M (IgM) and IgG antibodies to CMV [10]. Serological tests are frequently used to determine patient's immune status prior to tissue and organ transplantation; however, it is insufficient and inconceivable to diagnose CMV infection with clinical presentation in immunocompromised persons without confirmatory molecular tests [11, 12]. In the case of solid organ transplant (especially renal transplants) or bone marrow transplant recipients, detection of CMV in urine may contribute to identify high-risk patients, although this is not a reliable prognostic marker for CMV disease occurrence [13]. The main aim of this research paper was to compare serological methods with molecular polymerase chain reaction (PCR) method in the context of CMV presence determination in the blood of dialysiss patients.

Material and Methods

This research was conducted during 2016, at the Laboratory for viral and PCR testing of the Laboratory Medicine Center, Clinical Center of Vojvodina in Novi Sad. The research protocol was approved by the Ethics Committee of the Clinical Center of Vojvodina in Novi Sad.

The research included 28 dialysis patients from the Dialysis Department of the Clinic of Nephrology and Clinical Immunology of the Clinical Center of Vojvodina in Novi Sad. All patients needed dialysis due to chronic kidney disease and were potential organ recipients.

Blood samples were taken from every patient and were stored in test tubes with ethylenediaminetetraacetic acid (EDTA) which served as an anticoagulant. Immediately after blood collection, the test tubes were centrifuged at 3000 – 4000 rpm for 10 minutes; blood plasma was separated and used for PCR analysis.

The CMV was determined in every patient by using quantitative PCR method, as well as by blood serum anti-CMV IgG and IgM antibodies (quantitative). Serological status of patients (anti-CMV IgG and IgM antibodies) was determined by enzyme-linked immunosorbent assay (ELISA) which was carried out using EUROIMMUN analyzer I 2-P (EUROIMMUN AG, Luebeck, Germany) according to manufacturer's protocol. The cups for anti-CMV IgG and IgM antibodies were coated by the inactivated cell lysate of Human Fetal Lung Fibroblast Cells - Medical Research Council cell strain 5 (MRC-5) that were infected by "AD169" strain of human cytomegalovirus.

COBAS AMPLICOR analyzer (Roche Diagnostics, USA) in combination with manual and automatic isolation of CMV deoxyribonucleic acid (DNA) was used for CMV viremia detection by PCR method. Total Nucleic Acid Isolation (TNAI) commercial kit (Roche Diagnostics, USA) was used for automatic nuclear CMV DNA isolation from individual patient's blood samples, which was carried out on COBAS AmpliPrep analyzer (Roche Diagnostics, USA). COBAS AmpliPrep analyzer uses magnetic particles and it consists of four basic steps: cell lysis, nucleic acid

stabilization and deproteinization, nucleic acid binding to magnetic particle surface, rinsing of unbound material, and purified nucleic acid elution.

Manual isolation of CMV DNA was done using COBAS AMPLICOR CMV MONITOR test protocol (Roche Diagnostics, USA) according to manufacturer's instructions [14]. COBAS AMPLICOR CMV MONITOR TEST is based on four basic processes: sample preparation, PCR amplification of target DNA by using specific primers complementary to CMV DNA, hybridization of amplified products and oligonucleotide probes which are specific for target sequence, and colorimetric detection of probes which are attached to the amplified product. Isolation of CMV DNA is done directly from blood plasma by viral particles lysis, which includes chaotropic agent and then the DNA is precipitated by ethanol. COBAS AMPLICOR CMV test uses biotinylated specific primers LC342C and LC383, in order to define a sequence consisting of 362 nucleotides which is located within the polymerase gene at amino terminus of CMV DNA. Target DNA fragment, located within the human CMV polymerase gene, is CMV-specific and it is not homologous to ones found in other herpes family members.

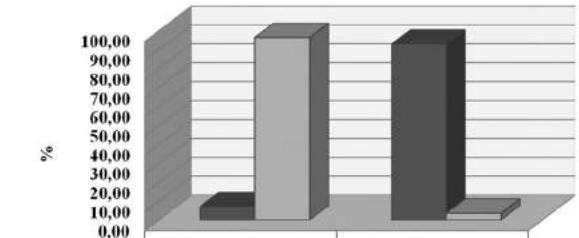
Correlation between measured parameters was determined by χ^2 and Fisher's test (Statistica software, version 8.0.). Statistical significance was set at $p \leq 0.05$. The results were presented in tables and graphs.

Results

The ELISA method of antibody detection in potential organ recipients through renal transplantation showed that there were 27 (96.4%) positive patients and 1 (3.6%) patient who was negative for anti-CMV IgG antibodies in the serum. Only 2 (7.1%) patients were positive for anti-CMV IgM antibodies, and 26 (92.9%) had negative results (**Graph 1**). Comparative analysis of results showed that the majority of patients (27) were sometimes exposed to the virus, while only a small number (2) of patients had an ongoing infection confirmed by the PCR test **(Table 1)**. The statistical processing of results by χ^2 and Fisher's correlation test showed that there was no statistically significant correlation between the findings of positive anti-CMV IgM antibodies and the PCR CMV DNA test result (Chi-square, $p = 0.1498$; Fisher exact p, one-tailed, $p = 0.2455$).

The percentage of PCR success with manual DNA isolation was 92.9%. Among 28 tested patients, 2 samples were incorrect, while the percentage of PCR success with automatic DNA isolation was 100%.

The PCR method yielded no positive results with either manual or automatic isolation of CMV DNA. According to the molecular (PCR) method with manual DNA isolation, of 28 patients 26 (92.9%) were negative for viral DNA, while the same method with automatic isolation showed that all 28 (100%) patients were negative for viral DNA.



Graph 1. Serological status (anti-CMV IgG and IgM antibodies) of dialysis patients in %

Grafikon 1. *Serološki status (anti-CMV IgG i IgM antitela) bolesnika na dijalizi izražen u %*

Legend: IgM Ab - immunoglobulin M antibodies; IgG Ab - immunoglobulin G antibodies

Legenda: IgM At – antitela imunoglobulina M; IgG At – antitela imunoglobulina G

Statistical processing of results (χ^2 and Fisher's correlation test) showed that there was no statistically significant correlation between CMV detection by serological (ELISA) method and molecular (PCR) method ($p > 0.05$) **(Table 1)**.

Discussion

Laboratory methods for the diagnosis of disseminated infections and active tissue/organ damage, caused by human CMV, include cultivation of peripheral blood leukocytes for virus isolation, pathohistological analysis of biopsy samples, and blood analysis based on pp65 antigen measuring and CMV detection by PCR [15]. Analysis of corresponding cell culture is not convenient for prognostic assessment; it takes 48 hours to 3 weeks to cultivate cells and it shows limited application especially in people with a compromised immune response. Analysis of pp65 antigen is very demanding and blood samples need to be introduced in this procedure not later than 6 hours since blood collection because the titer of this antigen decreases when blood is stored for a longer period of time.

Today, there are many commercial enzyme-linked immunosorbent assays which determine anti-CMV IgM and IgG antibodies. Serological diagnostics is used for immune status determination of dialysis patients who are potential organ recipients, while the diagnosis of CMV infection with clinical manifestations in immunocompromised persons is very insufficient and implausible without confirmatory molecular tests [16].

In our research, ELISA method for antibody detection in potential renal transplant recipients showed that there were 27 (96.4%) positive patients and 1 (3.6%) negative patient for anti-CMV IgG antibodies in the serum. Only 2 (7.1%) patients were positive for anti-CMV IgM antibodies and 26 (92.9%) were negative. Vilibic et al. [5] obtained

Table 1. Comparison of results obtained by ELISA and PCR methods
Tabela 1. *Poređenje rezultata dobijenih ELISA i PCR metodama*

Results *Rezultati*	PCR and manual isolation of CMV DNA *PCR i ručna izolacija CMV DNK*	PCR and automatic isolation of CMV DNA *PCR i automatska izolacija CMV DNK*	Results *Rezultati*	ELISA anti-IgM Ab *ELISA anti-IgM At*	ELISA anti-IgG Ab *ELISA anti-IgG At*
Positive finding (n) *Pozitivan nalaz (n)*	0 (0%)	0 (0%)	Positive finding (n) *Pozitivan nalaz (n)*	2 (7.1%)	27 (96.4%)
Negative finding (n) *Negativan nalaz (n)*	26 (92.9%)	28 (100%)	Negative finding (n) *Negativan nalaz (n)*	26 (92.9%)	1 (3.6%)
Invalid finding (n) *Nevažeći nalaz (n)*	2 (7.1%)	0 (0%)			

Legend: PCR – Polymerase Chain Reaction; ELISA – Enzyme-Linked Immunosorbent Assay; CMV – Cytomegalovirus; DNA – Deoxyribonucleic acid; IgM Ab – immunoglobulin M antibodies; IgG Ab – immunoglobulin G antibodies
Legenda: PCR – lančana reakcija polimeraze; ELISA – enzimimunoesej; CMV – citomegalovirus; DNA – deoksiribonukleinska kiselina; IgM At – antitela imunoglobulina M; IgG At – antitela imunoglobulina G

similar results – among 255 tested hemodialysis patients, 254 (99.6%) were positive for anti-CMV IgG antibodies and only 1 (0.4%) was positive for anti-CMV IgM antibodies in the serum according to ELISA method. Results of our research are in accordance with literature data, although samples were different. If the patient was anti-CMV IgM antibodies positive, that implied primary CMV infection, but these antibodies appeared rarely in dialysis patients (2/28), which can also be seen in results. A positive anti-CMV IgM antibodies test may arise suspicion and indicate that there is an active infection, although it is not a proof; it may also indicate antibody lag after recent infection considering that antibodies persist in blood serum up to 6 months after active infection and also the test can be false positive, because PCR method showed that these patients were negative for CMV DNA. A positive finding of anti-CMV IgM antibodies and negative PCR of CMV DNA in the same patient can be explained by the lagging of antibodies in the blood after the primary infection. Positive IgG antibody titer indicated that 27 out of 28 patients were exposed to CMV infection at a certain point of life. In CMV IgG seropositive recipients, there is a danger of virus reactivation (latent infection) [7]. The presence of IgG antibodies is therefore a marker of potential infection, and although a seropositive recipient is an immune person in immunological context, this term cannot be used to implicate protection from endogenous or exogenous infection. In CMV infection, IgM antibody synthesis is rare, but a rise in IgG antibodies titer is present [2]. Antibodies of IgM class are produced in primary infection, but not in recurrent infection in immunocompetent persons and they stay in the organism for 3 – 4 months. However, some immunocompetent persons may have detectable IgM antibodies during the recurrent infection. Immunocompromised persons sometimes do not produce IgM antibodies during the primary infection, and one third of them have detectable IgM antibodies in the recurrent infection [8].

When it comes to monitoring of immunocompromised potential recipients, serological techniques do not represent a method of choice because of altered and incomplete humoral immune response, late appearance of antibodies during infection, as well as the fact that their presence does not confirm an active infection, which additionally decreases the reliability of serological diagnostic results.

Numerous studies showed an association between CMV viral load and the presence of CMV disease. The CMV disease is clearly defined by a high viral load, which implicates a significant role of virus in disease pathogenesis. Studies of immunocompromised patients showed that PCR detection of CMV DNA is used for reliable prognosis of further disease course and its clinical outcome and it also enables monitoring of antiviral therapy efficacy and indirect assessment of virus resistance to given therapy. Quantitative CMV DNA level determination showed that high viral load as well as its rise in time implies bad prognosis. PCR, as a reference method, is a specific, sensitive, fast and reliable method for viremia determination and possible disease occurrence or development in immunocompromised patients. Clinical significance of PCR method lies in early detection of CMV in blood. Positive PCR test for CMV in blood has a high positive predictive value for symptomatic infection appearance, while a negative PCR test has a high negative predictive value for CMV disease development, because it excludes virus replication in blood.

Gärtner et al. evaluated TNAI method of CMV DNA isolation on COBAS AmpliPrep analyzer in contrast to COBAS AMPLICOR CMV MONITOR test [14]. Amplification was done on COBAS AMPLICOR analyzer. Specificity of these methods was assessed based on 100 healthy individuals' tests. All examinees were negative for CMV DNA presence (specificity of 100%). The sensitivity test was done by testing EDTA blood plasma of CMV positive transplant recipients. Out of a total of 30 samples, 4 were

negative with either manual or automatic DNA isolation, probably due to a known fact that CMV detection in blood plasma during CMV replication is limited to a shorter period of tome compared to CMV antigen or DNA detection in leukocytes. Three samples were positive in multiple repetitions of TNAI isolation method and negative in multiple repetitions of manual isolation. The TNAI method confirmed higher level of sensitivity in clinical samples. However, there is a strong correlation in viral titers in 23 positive samples between TNAI method and manual DNA isolation method ($r^2 = 0.98$). In our study, there is also an agreement between these methods, 92.86% and 100% success, with automatic TNAI isolation being more successful. Our results correlate with both isolation methods.

Adani et al. [17] used ELISA method of antibody detection in blood plasma samples of renal transplant recipients, and confirmed anti-CMV IgG antibodies in 100% of examinees, and only 6% of anti-CMV IgM positive examinees. Serological diagnostics is not the real indicator of CMV infection, because of frequent virus reactivation in immunocompromised people. Immunocompromised persons sometimes do not produce IgM antibodies during the primary infection, and one third has detectable IgM antibodies in the recurring infection. For CMV DNA detection, Adani et al. [17] used a real-time PCR method. Out of 98 examinees, 32 (32.7%) had a positive CMV DNA finding. A negative anti-CMV IgM antibody test and a positive PCR test in the same patient can be explained by a later production of antibodies because of immunosuppression. In 4 patients, no CMV DNA was detected, and they tested positive for IgM antibodies, which can be explained by antibodies lag after primary infection as noticed in some healthy individuals. Out of 6 patients who had high CMV DNA concentration, which was in correlation with their clinical status, two died in a year. Virus concentration in the initial phase of an active infection, as well as the rate of its increase, correlates with CMV disease in transplant recipients. Patients with a lower DNA concentration did not have disease symptoms.

If a potential recipient is PCR CMV DNA positive, the therapy should immediately be initiated and he/she cannot receive an organ until tests are negative [18]. If patients are PCR DNA negative and IgM antibodies negative, but IgG antibodies positive, as in the majority of obtained results, they are good candidates for organ transplantation. After the transplantation, or just before it, immunosuppressive therapy is given in order to avoid organ rejection, and reactivation of CMV infection is expected due to a reduced body defense mechanism.

Conclusion

Due to 7.1% of false positives for anti-cytomegalovirus immunoglobulin M antibodies in the serum of immunocompromised dialysis patients, not confirmed by a polymerase chain reaction test, serological techniques are not reliable in detecting active cytomegalovirus infection causing positive findings of anti-cytomegalovirus immunoglobulin M antibodies, so confirmation of cytomegalovirus deoxyribonucleic acid by polymerase chain reaction method is required.

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LITHIUM IN THE TREATMENT OF BIPOLAR DISORDER – MONITORING OF THE ADVERSE EFFECTS

LITIJUM U LEČENJU BIPOLARNOG POREMEĆAJA – PRAĆENJE NEŽELJENIH DEJSTAVA

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Summary

Introduction. Lithium therapy remains the gold standard in the treatment of bipolar disorder and clinical guidelines recommend it as the first choice for maintenance treatment of bipolar disorder. However, the use of lithium has decreased over the years, mainly due to the fear of its adverse effects. The aim of this paper was to review current literature data for the monitoring and overcoming side effects of lithium therapy in order to provide contemporary evidence for adequate lithium use. **Material and Methods.** A literature review of lithium therapy in bipolar disorder, using both Medline and manual searches, was performed. Classification of studies, in relation to their quality, was performed using the guidelines established by the American Academy of Neurology. **Results.** Despite methodological limitations of recent studies, there is irrefutable evidence that lithium therapy can cause toxicity and side effects related to renal, thyroid and parathyroid function, as well as weight gain. **Conclusion**. There is clear evidence that lithium can cause various side effects, but clinically significant conditions in this regard are rare and successfully treated. Literature data confirm strong efficacy of lithium and suggest its wider use in bipolar disorder. By following clinical recommendations and careful monitoring during lithium treatment, the risk of serious side effects is low compared to its efficacy.

Key words: Bipolar Disorder; Lithium; Antimanic Agents; Drug-Related Side Effects and Adverse Reactions; Risk Assessment; Kidney Function Tests; Thyroid Function Tests; Teratogens; Weight Gain; Parathyroid Glands

Sažetak

Uvod. Terapija litijumom predstavlja zlatni standard u lečenju bipolarnog poremećaja i klinički vodiči je preporučuju kao prvi izbor u tretmanu održavanja. Svedoci smo smanjene upotrebe litijuma tokom proteklih godina uglavnom zbog straha od neželjenih efekata. Cilj istraživanja je bio razmatranje aktuelnih podataka iz literature koji se odnose na monitoring i prevazilaženje neželjenih efekata nastalih zbog tretmana litijumom sa ciljem pružanja savremenih dokaza za adekvatnu primenu ovog leka. **Materijal i metode**. Korišćenjem indeksne baze *Medline* pregledana je literatura koja se odnosi na terapiju bipolarnog poremećaja litijumom. **Rezultati.** Uprkos metodološkim ograničenjima savremenih istraživanja, pronađeni su neoborivi dokazi da terapija litijumom može da izazove toksičnost i neželjene efekte koji se odnose na bubrežnu, tiroidnu i paratiroidnu funkciju, kao i povećanje telesne težine. **Zaključak.** Postoje jasni dokazi da litijum može izazvati različite neželjene efekte, ali klinički značajna stanja u tom smislu su retka i mogu SE uspešno lečiti. Podaci iz literature potvrđuju neprikosnovenu efikasnost litijuma i sugerišu njegovu širu upotrebu u lečenju bipolarnog poremećaja. Praćenjem kliničkih preporuka i pažljivim monitoringom tokom tretmana litijumom, rizik od ozbiljnih neželjenih efekata je mali kada se uporedi s njegovom efikasnošću.

Ključne reči: bipolarni poremećaj; litijum; antimanični lekovi; lekovima izazvane nuspojave i neželjene reakcije; procena rizika; testovi bubrežne funkcije; testovi funkcije štitne žlezde; teratogeni; dobijanje na težini; paratireoidne žlezde

Introduction

Mood disorders affect at least 350 million individuals worldwide. They are associated with high morbidity and mortality rate, significant disability, comorbidity with mental and physical disorders, as well as negative social and economic consequences for the patients, their families and the whole society [1, 2]. The cyclical course, as a core feature of bipolar disorder (BD), makes its treatment very challenging. Due to the heterogeneity of symptoms, the treatment of BD is complex and most commonly leads to increase in the number of prescribed medications. The treatment includes a great number of medications, biological therapies (electroconvulsive therapy or transcranial magnetic stimulation) and psychosocial techniques. Treatment tolerability has a significant impact on the long-term management of BD, because adverse effects often lead to treatment noncompliance, which is high. In addition, safety concerns associated with the disease, primarily high risk of suicide, require suitable pharmacological treatment established in the early course of the illness.

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Abbreviations
BD – bipolar disorder
TSH – thyrotropin-stimulating hormone
T4 – thyroxine

Lithium is prescribed all over the world as an effective treatment of depressive as well as manic episodes. Its anti-suicidal action is also proven as very important. Lithium is "the gold standard" among mood stabilizers and all contemporary guidelines recommend it as the first choice in the long-run treatment of BD [3, 4]. Although efficacious, lithium has some clinical disadvantages which include a narrow therapeutic range requiring monitoring of its serum concentrations as well as possible damage to the endocrine and renal systems.

The narrow therapeutic index of lithium salts requires regular monitoring of its serum concentrations and therapeutic actions in case of side effects during a prolonged treatment. Taking into account the decrease in the use of lithium due to the fear of side effects, it is important to obtain clear knowledge on them, in order not to deprive the patients of "the gold standard" in the treatment of BD, and to provide patients with an optimal treatment with maximum efficacy and best tolerability. The absence of side effects leads to a good compliance, and it is a prerequisite for a successful treatment of BD.

Even small deviations in the serum lithium levels may cause clinically significant problems. During prophylaxis therapy, the serum level of lithium under 0.6 mmol/l increases the risk of illness recurrence, and the level above 1.2 mmol/l significantly increases the risk of side effects. This is the main reason why monitoring the serum levels of lithium makes the treatment safe. Lithium ions do not bind to plasma proteins and they do not metabolize. Lithium is almost wholly excreted in the urine, so any changes in renal function, fluid balance or electrolyte levels can potentially lead to lithium accumulation and toxicity [5].

The aim of this paper was to review the current literature on the side effects of lithium during its long-term application in order to determine their frequency, intensity, as well as possible predictors of occurrence.

Material and Methods

A literature review of the lithium therapy in BD, using both Medline and manual searches, was performed. Classification of studies, related to their quality, was performed using the guidelines established by the American Academy of Neurology.

Results

The use of lithium in the treatment of BD has decreased substantially, partly due to the active marketing of alternative medications, but also because of the perceived risks associated with its use. Side effects of lithium have been in the focus of interest of researchers for years, but the interpretation of the results of decades-long research has largely been made difficult for a number of reasons. In addition to the fact that most studies were methodologically weak, different designs of studies over six decades of research made the combination of data and its synthesis difficult. Thus, the evidence considering side effects of lithium is far from ideal. Despite these limitations, investigators manage to identify five key areas in which lithium therapy produces adverse effects:

1. renal function
2. thyroid function
3. parathyroid function
4. teratogenicity
5. weight gain [6, 7].

The intensity, frequency and possible predictors of the impairment of renal function have remained insufficiently clarified [8]. The renal side-effects are of greatest concern to both clinicians and patients, and in this regard the analysis is reassuring; even with long-term lithium use, the risk of renal toxicity, specifically end-stage renal failure, is fairly low (0.53% compared to 0.2% in the general population). Chronic kidney disease is more common, but it occurs predominantly at older age and only in a small proportion of this group (2%) it progresses to end-stage renal failure. New studies identified predictors for a decline in renal function: female gender, age, comorbidities such as diabetes mellitus or hypertension, and high median serum lithium concentration. The length of lithium treatment has a negative association with side effects which suggests that the effects have rapid onset once patients start taking lithium [6, 8, 9]. The identification of the potential causal effect of lithium on renal function is difficult, because of the confounding effects of diabetes and cardiovascular disease, which may lead to decline in renal function. The literature data suggest that stable lithium maintenance therapy does not increase the risk of renal dysfunction in adult patients with affective disorders who have a baseline glomerular filtration higher than 60 ml/min. These results therefore contradict the opinion that long-term lithium therapy is associated with nephrotoxicity in the absence of episodes of acute intoxication and that duration of therapy and cumulative dose are the major determinants for toxicity [6]. If there is a decrease in renal function in patients treated with lithium, contemporary guidelines focus on active management of diabetes, hypertension and other cardiovascular risk factors, avoidance of episodes of acute lithium toxicity, dehydration and other drugs that can reduce renal clearance of lithium [10–12]. Structural changes may occur in the glomerules of lithium-treated patients, but they are nonspecific and can be seen also in patients before the start of lithium treatment. The morphological changes that are specifically associated with lithium therapy are confined to the distal tubules and collecting ducts and are reversible

[13]. The results of newer studies are encouraging because they suggest that lithium treatment is not nephrotoxic. However, substantial uncertainty remains because most of these studies were of short duration and unable to detect long-term effects. Present clinical recommendations include assessment of renal function before the beginning of lithium therapy and henceforth monitoring at intervals as short as 6 weeks. Because the absolute risk of end-stage renal failure is so low, annual testing is probably sufficient in the absence of clinical reasons for more frequent monitoring [8].

Lithium has four negative effects on thyroid function, which include inhibition of iodine uptake, inhibition of triiodothyronine (T3) secretion, alteration in thyroglobulin structure, and inhibition of thyroxine (T4) secretion. Recent studies have shown that in lithium-treated patients there is a significant rise in mean serum thyrotropin-stimulating hormone (TSH) and a significant fall in mean serum T4, 6–12 months after the start of lithium treatment. The TSH concentrations tend to increase in response to the inhibitory effect on T4 availability. Similarly to the development of renal side effects, the risk of hypothyroidism seemed to be greatest at early stages of lithium treatment (6–12 months after initiation). Reports about the incidence of this side effect range from 1% to 30%, and almost all hypothyroid patients respond to supplementary treatment with T4 [9, 13]. This side effect can be successfully treated and it does not represent a contraindication for lithium use. Risk factors for development of hypothyroidism in patients receiving lithium therapy include female gender, young age, old age, patients with diabetes and high median serum lithium concentration. Most of these patients are asymptomatic and the diagnosis is made only biochemically. It is unclear when subclinical hypothyroidism needs intervention, especially given the complex interaction between thyroid status and mood. As the symptoms of lithium-induced hypothyroidism overlap with those of depression, the underlying cause may remain undiagnosed and untreated by psychiatrists. Determination of serum TSH, a sensitive indicator of decreased thyroid function, may help resolving diagnostic doubts [8, 9]. Although with a far lower incidence, an increased risk of hyperthyroidism may be seen in patients treated with lithium [14].

There is a positive association between lithium and hyperparathyroidism. Primary hyperparathyroidism is quite frequent in patients receiving lithium: an absolute risk of 10% (vs. 0.1% in the general population) is probably attributable to lithium's inactivation of the calcium-sensing receptor and interference with intracellular second messenger signaling. This effect leads to an increased release of parathyroid hormone, which raises calcium concentrations in blood. Thyroid and parathyroid abnormalities occur in about 25% of patients receiving lithium therapy and that is why close clinical monitoring should be done. More research is needed to clarify the relation between lithium, calcium and kidney function [6, 8, 9]. Guidelines for BD make no mention of serum calcium level monitoring, which seems to be an important omission in view of the high absolute risk of hyperparathyroidism. Baseline blood tests before lithium is introduced should include TSH and serum calcium, and these tests should be repeated every year or more frequently if clinical symptoms are reported [9, 15].

Maternal physiological changes during pregnancy may necessitate drug dosage adjustments. For example, the glomerular filtration rate increases during pregnancy causing many medications to be excreted more rapidly. As a result, serum concentrations may fall and the patient may require higher doses to prevent relapse. Serum lithium levels should be maintained in the therapeutic range during pregnancy. After establishing the pre-pregnancy baseline, lithium levels should be checked monthly during pregnancy, unless it is clinically indicated to do so more often. Lithium dose usually needs to be increased over the course of pregnancy. It is recommended to check lithium levels on weekly basis in the last month of pregnancy, both because of increased clearance rates and because of the potential toxicity of lithium in complications such as pre-eclampsia. Dosage of lithium needs to be reduced one week before delivery. Clearance and fluid volume decreases after the delivery which can lead to higher serum lithium concentrations and side effects unless dose is reduced. Lithium serum level should be checked 24 hours after the delivery and after each dose adjustment [16, 17].

The association between lithium exposure and Ebstein's anomaly has been established earlier and previous analysis of the published data estimated the risk to be 1 to 2/1000 live births. Recent analysis suggests that exposure to lithium in the first trimester is actually associated with a 0.05–0.1% risk of cardiovascular anomalies (a low absolute risk, but perhaps still higher than in the general population). High-resolution ultrasound and fetal echocardiography are recommended based on the possibility that lithium increases risk of heart defects [6, 18]. The evidence that exposure to lithium is teratogenic is quite weak and authors suggested that the risk has been overestimated. However, studies have never been large enough to be decisive. Adequate control of the mood disorder is of paramount importance in order to provide the greatest benefit to the mother and child while minimizing potential risks. Clinicians should explain the uncertainty of risk to women of childbearing age considering the balance of risks between possible harm to the baby and maternal mood instability before continuation or stopping lithium therapy [9, 17, 18].

There is a positive association between lithium and weight gain and factors which may explain it are its insulin-like properties in increasing cellular glucose uptake, increased thirst, direct stimulation of the hypothalamic appetite centre, and the induction of hypothyroidism. Risk of increased weight

gain and body mass index is quite lower with lithium compared to atypical antipsychotics and other mood stabilizers [9, 19]. The increase in body weight may impair the compliance and the doctor–patient relationship and also lead to a number of other medical complications, such as high blood pressure, high triglycerides and diabetes [20].

When initiating lithium therapy, prescribers should advise the patient that poor adherence or rapid discontinuation may increase the risk of relapse, discuss lithium's side effects, monitor patient's weight, serum urea, electrolytes, creatinine, thyroid hormones, and rule out pregnancy. After starting the treatment, serum levels of lithium should be between 0.6 – 0.8 mmol/L. The optimal maintenance level is the highest dose tolerated without significant side effects and will vary from patient to patient. Lower levels may be required in the elderly, as they may experience toxicity at standard therapeutic blood levels [11, 12, 21].

Monitoring lithium:

1. Check serum lithium levels routinely every 3 months

2. Consider measuring serum lithium levels even more frequently for:

– Older people

– People taking drugs that interact with lithium (thiazides, nonsteroidal anti-inflammatory drugs, angiotensin-converting-enzyme inhibitors)

– People at risk of renal, thyroid or other complications

– People with poor symptom control

– People with poor adherence

3. Other indications for checking serum lithium levels include:

– Clinical deterioration

– Abnormal laboratory results

– Change in sodium or fluid intake

– Symptoms suggestive of abnormal renal or thyroid function

4. At every appointment check patients for symptoms of neurotoxicity (tremor, paresthesia, ataxia, cognitive impairment) which can occur at therapeutic levels, and gastrointestinal symptoms (anorexia, nausea, diarrhea) which can be signs of lithium toxicity.

5. When discussing whether to continue lithium, take into account clinical efficacy and other risk factors for renal impairment [11, 12, 22].

Conclusion

The use of lithium has decreased all over the world, mostly due to the fear of side effects. The evidence of recent studies indicate that the use of lithium should increase again, because there is no threat of more serious side effects if clinicians respect recommendations and conduct close monitoring during treatment.

Studies have shown that lithium therapy is associated with increased risk of reduced urinary concentrating ability, hypothyroidism, hyperparathyroidism and weight gain. Most of the studies showed no significantly increased risk of congenital malformations, alopecia or skin disorders and little evidence for a clinically significant reduction in renal function in most patients. The evidence that lithium can adversely affect the endocrine system is clear, but clinically significant conditions are either rare or treatable. Due to the fact that higher mean serum lithium level has been identified as a risk factor for all lithium side effects, prescribing lithium within the reference range is particularly important. Findings suggest that close monitoring of adverse effects in all patients taking lithium is essential, and that clinicians should do their best to use the lowest effective dose of lithium. Timely diagnosis and treatment of associated medical conditions (diabetes, hypertension and other somatic diseases which are more common in patients suffering from bipolar disorder than in general population) is equally important. Extra effort is required to follow the guidelines and precautions that ensure maximum efficacy and safety of lithium treatment because our patients are entitled to it.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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CARDIAC LIPOMA CAUSING OBSTRUCTIVE SLEEP APNEA – A CASE REPORT

LIPOM SRCA KAO UZROK OPSTRUKTIVNE APNEE TOKOM SPAVANJA − PRIKAZ SLUČAJA

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Summary
Introduction. Cardiac lipomas are rare benign cardiac tumors. The symptoms they cause and the diagnosis depend on their size and location. **Case Report.** We report the case of a 69-year-old male, whose main symptom was progressive dyspnea on exertion and in the supine position. The diagnosis of a large subepicardial lipoma in the wall of the right atrium, causing superior vena cava compression and consecutive obstructive sleep apnea syndrom, was made using different imaging techniques. The patient underwent open heart surgery, and the tumor was extracted with no intraoperative and postoperative complications. During a 1-year follow up, he remained asymptomatic, with no clinical signs of obstructive sleep apnea after the surgery. **Conclusion.** When it comes to the diagnosis and treatment of obstructive sleep apnea, cardiac tumors should be considered.
Key words: Sleep Apnea, Obstructive; Lipoma; Heart Neoplasms; Heart Atria; Diagnosis; Cardiac Imaging Techniques

Sažetak
Uvod. Lipomi su benigni tumori srca. Simptomi koje izazivaju, kao i dijagnoza, zavise od njihove veličine i pozicije na kojoj se nalaze. **Prikaz slučaja.** Prikazujemo slučaj 69-godišnjeg bolesnika muškog pola, čija je glavna tegoba bila progresivna dispnea pri naporu i ležećem položaju. Različitim imidžing tehnikama dijagnostikovan je subepikardni lipom u zidu desne pretkomore koji je uzrokovao kompresiju gornje šuplje vene i kao posledicu sindrom opstruktivne apnee tokom sna. Pacijent je operisan, tumor je u potpunosti uklonjen bez intraoperativnih i postoperativnih komplikacija. Pacijent nema simptome u postoperativnom periodu jednogodišnjeg praćenja, bez kliničkih znakova opstruktivne apnee tokom sna. **Zaključak.** Tumori srca moraju se uzeti u obzir kao uzrok u slučaju dijagnostike i tretmana opstruktivne apnee tokom sna.
Ključne reči: opstruktivna apnea tokom sna; lipom; neoplazme srca; srčana pretkomora; dijagnoza; imidžing tehnike srca

Introduction

Primary tumors of the heart are extremely rare, with an incidence between 0.0017% and 0.056% according to autopsy reports [1]. Lipomas account for 8.4% of benign tumors of the heart [2].

Although histologically benign, they sometimes cause serious health issues such as syncope, dizziness, sudden cardiac death, peripheral embolism, depending on the size and location. Cardiac lipomas are usually incidentally found.

Obstructive sleep apnea (OSA) is a sleep disorder defined as a complete (apnea) or partial (hypopnea) upper airway obstruction. Polysomnography (PSG) is the gold standard for the diagnosis of OSA. The most common sleep variable which is measured during PSG is apnea hypopnea index (AHI) which is defined as a sum of apneas and hypopneas per hour of sleep [3]. The AHI index of five or more sleep events per hour is consistent with the diagnosis of OSA [4].

Case Report

We report the case of a 69-year-old male who was admitted to our hospital for evaluation of fatigue and progressive dyspnea on exertion and in the supine position for the past few years. His previous medical history included OSA, hypertension and obesity. His body mass index (BMI) was 35,5 kg/m^2. The OSA was treated with continuous positive airway pressure (CPAP), but with no significant improvement of symptoms during the treatment.

On admission, physical examination, laboratory test results, vital signs and electrocardiogram (ECG) were normal. Transthoracic echocardiography (TTE) showed a mass in the right atrium, so transoesophageal echocardiography (TOE) was pre-

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Abbreviations

AHI – apnea hypopnea index
OSA – obstructive sleep apnea
PSG – polysomnography
BMI – body mass index
TTE – transthoracic echocardiography
TOE – transesophageal echocardiography
CT – computed tomography
SVC – superior vena cava
SVCS – superior vena cava syndrome
OSAS – obstructive sleep apnea synrdom

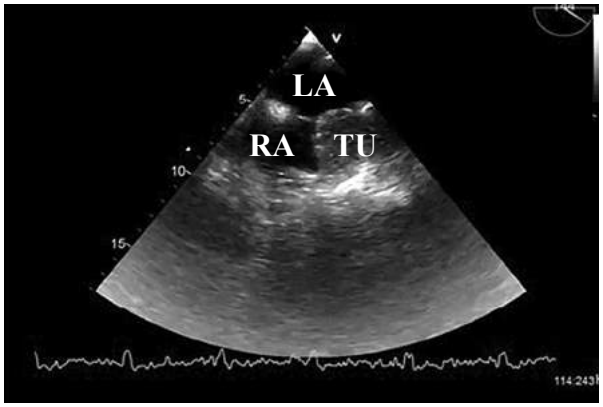

Figure 1. Transesophageal echocardiography (bicaval view)
Slika 1. *Transezofagealni ehokardiografski pregled, bikavalni presek*
Legend: LA: left atrium, TU: lipoma, RA: right atrium
Legenda: LA: leva pretkomora, TU: lipom, RA: desna pretkomora

formed for better characterization of the mass. The TOE showed a well circumscribed tumor mass, sized 39 x 30 mm in the right atrium **(Figure 1).**

In order to thoroughly evaluate the size and position of the tumor mass and its relations to neighboring structures, computed tomography (CT) was preformed, showing oval homogenous, hypoechogenic (-110 HU) mass sized 53 x 36 x 23 mm between the superior vena cava (SVC) and right and left atrium **(Figure 2).** The SVC was compressed by the tumor

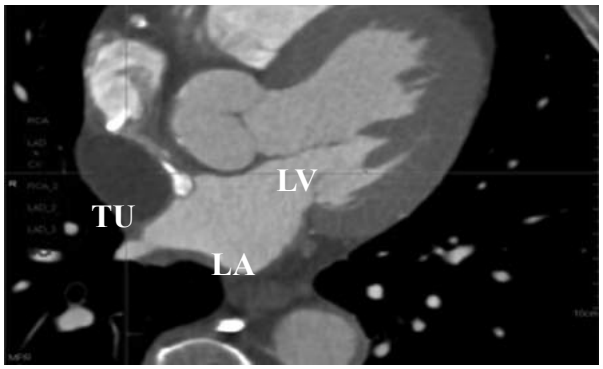

Figure 2. Computed tomography scan
Slika 2. *Kompjuterizovana tomografija – presek*
Legend: LV: left ventricle, TU: lipoma, LA: left atrium
Legenda: LV: leva komora, TU: lipom, LA: leva pretkomora

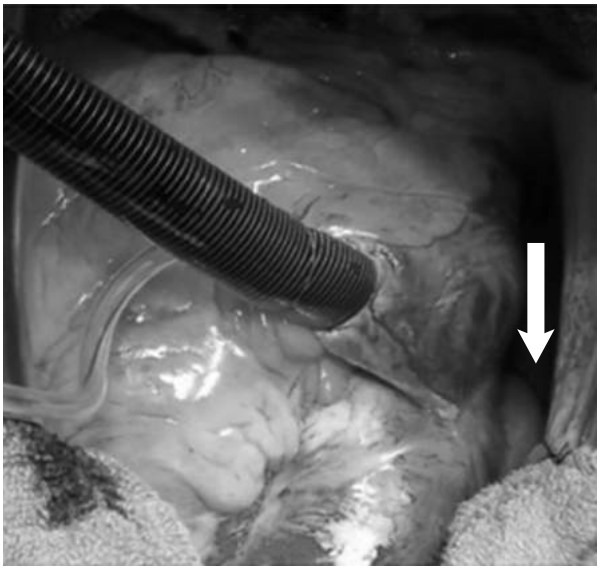

Figure 3. Intraoperative image of the lipoma (arrow)
Slika 3. *Intraoperativni prikaz lipoma (strelica)*

and narrowed to a 3 mm diameter. The CT showed no infiltration of the surrounding structures. Multislice computed tomography (MSCT) of the coronary arteries was simultaneously performed and showed no significant stenosis of epicardial coronary arteries.

A multidisciplinary team, including a cardiologist, cardiac surgeon and a thoracic surgeon, made a decision for surgical treatment. The patient underwent cardiac surgery with extracorporeal circulation, and the subepicardial tumor, located between the SVC and Sondergaard's groove, was completely extracted **(Figures 3 and 4).** There were no complications in the perioperative and early postoperative period.

The histopathological examination showed yellow groups of mature fat cells, with interlobular fibrous septa, confirming the diagnosis of a benign cardiac lipoma. The patient was discharged on the 10th postoperative day, with significant improvement of symptoms.

The PSG examination 6 months after the surgery showed that AHI was below the diagnostic value for

Figure 4. Gross appearance of the surgically resected tumor
Slika 4. *Makroskopski prikaz hirurški reseciranog tumora*

OSA, even though his BMI remained unchanged. During the one-year follow up he was with no symptoms and handling physical strains with no problems.

Discussion

Cardiac lipomas are rare benign, usually small tumors of the heart that are, in the majority of cases, incidentally found at autopsy [5]. They may cause symptoms that vary from mild fatigue to very severe medical conditions like heart failure and sudden cardiac death. We reported a rare case of cardiac lipoma that caused obstructive sleep apnea syndrome (OSAS) by compressing the SVC.

The OSAS is characterized by repetitive episodes of apnea due to upper airway collapse during sleep. Rarely, OSAS may occur when there is a narrowing of the upper airway by edema and vascular congestion resulting from superior vena cava syndrome (SVCS) [6, 7].

The proposed mechanism of OSAS in SVC compression is the increase of cervical venous pressure to 20 or even 40 mm Hg, comparing to the normal range of 2 to 8 mm Hg [8]. That increase of hydrostatic pressure is likely to cause transudation of fluid into the interstitial space surrounding the upper airway [9].

Few clinical cases have been reported about OSAS caused by SVCS in the literature. Mediastinal compression of SVC due to malignant, as well as non-malignant processes have been reported [7, 10, 11], but none of them reported about cardiac lipoma. To our knowledge, this is the first reported case of lipoma in the atries of the heart presenting as OSAS.

There are literature reports on heart hemangioma causing OSAS [12]. Total surgical extraction of the tumor causes disappearance of OSAS symptoms, which is in agreement with our findings. The etiology of cardiac lipomas still remains unclear, with no significant difference in prevalence in regard to age and gender [13]. The main difference between lipomas and lipomatous hypertrophy collection is the presence of a tiny capsule [1]. Regarding the origin, cardiac lipomas can be divided into subendocardial (the most common), subepicardial, and myocardial [5].

The diagnosis of cardiac lipoma is nowadays easier with improvement of the imaging techniques, including TTE, TOE, CT and magnetic resonance imaging, which provide not only information about the size and location, but also effects of the tumor on hemodynamics and composition of the tumor [5].

At CT, lipomas appear as homogeneous hypodense masses with negative attenuation values between −50 and −150 HU [14]. It is necessary to pay attention to differentiate low grade liposarcomas from benign lipomas. At CT, low grade liposarcomas generally appear heterogeneous with thickened septae [15]. Also, the pathological diagnosis of the tumor is usually needed to finally distinguish lipomas and well differentiated liposarcomas [1]. In our case, attenuation value of hypodense formation was typical for lipoma which was confirmed by histopathological analysis.

Although cardiac lipomas usually remain asymptomatic, they may cause a variety of symptoms, depending on the size and location. They can cause obstruction of the outflow tract, heart valve dysfunction, vena cava compression, arrhythmias, syncope, chest pain, dispnea and even sudden cardiac death [16].

The recommended treatment for symptomatic cardiac lipomas is surgery, with complete extraction of the tumor and the capsule local recurrence is quite low, even with subtotal resection [17]. We performed a total tumor extraction and during the follow-up no clinical or echocardiographic signs of local recurrence were noticed.

Conclusion

In conclusion, cardiac tumors should be considered as a potential cause of obstructive sleep apnea syndrome, especially if there is no symptom improvement using standard therapy. Imaging techniques are gold standard for the diagnosis of cardiac tumors and surgical extraction is the therapy of choice.

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CUTANEOUS MANIFESTATIONS CAUSED BY SIMULIUM ERYTHROCEPHALUM BITES IN HUMANS – A CASE SERIES

KOŽNE MANIFESTACIJE KOD LJUDI IZAZVANE UBODOM INSEKTA SIMULIUM ERYTHROCEPHALUM – PRIKAZ SERIJE SLUČAJEVA

Ljuba VUJANOVIĆ, Marina JOVANOVIĆ, Zoran GOLUŠIN, Zorica GAJINOV and Sanja JAKOVLJEVIĆ

Summary

Introduction. In contact with insects, people present a wide range of reactions, local or systemic, whether caused by insect bites or a venom injections, sucking blood or exposure to insect's body and its secretions. Simulium erythrocephalum is an aggressive anthropophilic species, and following its bite humans may develop purpuric macules, edema and erythema surrounding the bite site. Our case series indicates frequent occurrence of Simulium erythrocephalum bites in Serbia. **Case Series.** We present a series of 30 cases reviewed between April and July 2006, who developed a local reaction at the site of this insect's bite. In all patients, the skin lesions were located on the extremities, mainly the lower legs. The number of bites varied from two to more than ten. In all patients the bites occurred either during outdoor activities on the riverside or their residence was near the Danube. The therapy with antihistamines, local and/or systemic corticosteroids, as well as symptomatic therapy, with compression and limb elevation was recommended. The skin lesions regressed in one week in most patients, but in few they lasted for several weeks. **Conclusion.** In addition to its impact on human health, Simulium erythrocephalum also has a role in veterinary medicine. Insect bites in animals can cause significant livestock losses, occasionally resulting in animal mortality. Apart from this, Simulium erythrocephalum may also be a vector in the transmission of the parasite from the genus of Leucocytozoon that infests birds.

Key words: Insect Bites and Stings; Simuliidae; Insect Vectors; Dermatitis; Signs and Symptoms; Treatment Outcome; Serbia; Erythema; Edema; Purpura

Sažetak

Uvod. Pri kontaktu sa insektima, kod ljudi se razvijaju različite reakcije, lokalne ili sistemske, bez obzira na to da li je u pitanju ubod insekta i injekciranje otrova, isisavanje krvi, ili dodir sa drugim delovima tela insekta i njegovim izlučevinama. Nakon uboda insekta *Simulium erythrocephalum*, agresivne antropofilne vrste simulide, kod ljudi se pored edema i eritema mogu javiti i purpurične makule oko mesta uboda. Našim prikazom slučajeva ukazujemo na učestalu pojavu uboda insekta. *Simulium Erythrocephalum* u Srbiji. **Prikaz slučajeva.** Prikazujemo seriju pacijenata koji su pregledani u periodu od aprila do jula 2006. godine koji su razvili lokalnu reakciju na mestu uboda ovog insekta. Kod svih bolesnika ubodi insekata su bili lokalizovani na ekstremitetima, pretežno potkolenicama. Broj uboda je varirao od dva do deset pa i više uboda. U vreme nastajanja uboda bolesnici su boravili ili šetali u priobalnom području ili im je mesto stanovanja bilo u neposrednoj blizini Dunava. Kod svih pacijenata sprovedena je terapija antihistaminicima, lokalnim i/ili sistemskim kortikosteroidima, zavisno od težine kliničke slike. Promene na koži su se povukle nakon nedelju dana, a kod nekih pacijenata trajale su više nedelja. **Zaključak.** Pored uticaja na zdravlje ljudi, *Simulium erythrocephalum* ima i značaj u veterinarskoj medicini. Ubodi ovog insekta kod životinja mogu izazvati značajne gubitke u stočarstvu, ponekad izazivajući i uginuće stoke. Pored toga, mogu biti vektori u transmisiji protozoa iz roda *Leucocytozoon* koji infestuje ptice.

Ključne reči: ujedi i ubodi insekata; Simuliidae; vektori insekata; dermatitis; znaci i simptomi; ishod lečenja; Srbija; eritem; edem; purpura

Introduction

Insects are part of the phylum of animals called Arthropoda. The word insect comes from the Latin word insectum which means "separated into segments" such as head, thorax and abdomen. After getting in contact with insects, people present a wide range of reactions, local and/or systemic, whether caused by insect bites or venom injections, sucking blood or exposure to insect's body and their secretions [1, 2]. Following a bite, the venom may cause local toxic reactions at the bite site. Massive local reactions, as well as life-threatening anaphylaxis are based on the allergic reaction mediated by IgE antibodies [2]. Clinical manifestations include erythema, wheals, papules and blisters followed by intensive pruritus [1], as well as angioedema [3]. Apart from allergic reactions, severe clinical complications may occur, such as acute interstitial nephritis, acute toxic hepatitis and acute myocarditis [4], rhabdomy-

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olysis and hemolytic-uremic syndrome following fire ant bite [5, 6]. Furthermore, following insect stings people may develop some atypical reactions, for instance exotropia, diplopia, optic neuritis, neurological disorders, Myasthenia gravis, Guillain-Barre syndrome, anxiety and insomnia, or even anaplastic large cell lymphoma [1, 7–11]. Insects are also vectors in transmission of many diseases such as malaria, leishmaniasis, tularemia, typhus, trypanosomiasis [1, 12].

During the last few decades, more frequent occurrence of Simulium erythrocephalum bites have been noticed in Serbia. Simulium erythrocephalum is an aggressive, anthropophilic kind of simulium, whose bites result in many skin disorders in humans. Skin changes are mostly located on lower extremities, and besides edema and erythema lesions may have a hemorrhagic component [1, 13–15].

In order to prevent exposure to insects, protective clothing as well as physical protection with a net, insecticides and repellents are used. The treatment depends on the severity of clinical manifestations. Symptomatic therapy with local application of heat, antihistamines and corticosteroids is commonly used. In the case of anaphylaxis it is essential to monitor the patient appropriately and implement resuscitation measures [1, 2, 16].

We present a case series indicating the significance and frequency of Simulium erythrocephalum bites in Serbia, as well as its effects on human health.

Case Series

A total of 30 patients who presented a local reaction to an insect bite were examined in the period from April to July, 2006, at the Clinic of Dermatovenereology Diseases, Clinical Center of Vojvodina. The data collected from medical histories, clinical manifestations on the skin, and consultations with entomologists from the Faculty of Agriculture, revealed that the skin lesions were mainly caused by insect bites of a fly known as Simulium erythrocephalum. In all patients the skin lesions were located on the extremities, mostly on lower legs. The number of bites varied from two to more than ten. All patients were either outdoors on the riverside, or their residence was nearby the Danube river. The clinical examinations showed that the bite sites were surrounded by areas of purpura and more or less evident edema, accompanied by pain and itch **(Figure 1)**. In most cases, erythema affected a large skin area **(Figure 2).** The therapy included desloratadine tablets at a dose of 5 mg twice a day during ten days, while in more severe cases prednisone tablets were used at a dose of 20 mg once a day during five days, as well as fluocinolone acetonide; compression and limb elevation were recommended. In most cases the skin lesions regressed within a week, but in some patients they lasted for several weeks. None of the patients developed a systemic reaction, such as anaphylaxis or life-threatening conditions, so resuscitation was not necessary.

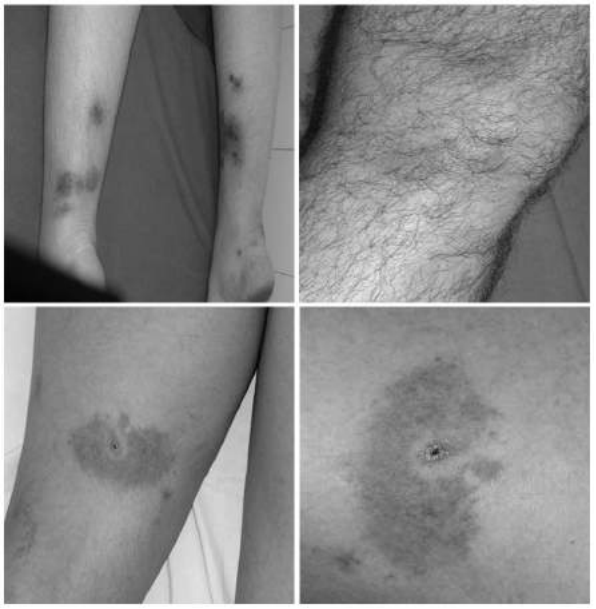

Figure 1. The bite sites surrounded by areas of purpura and more or less evident edema
Slika 1. *Mesta ugriza okružena područjima purpure i manje ili više evidentnim edemima*

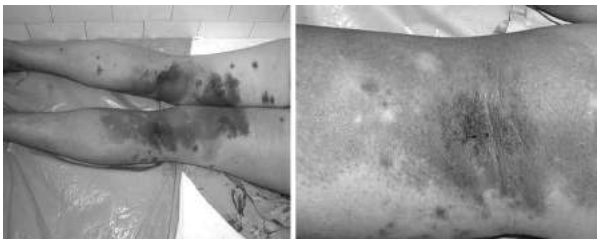

Figure 2. In most cases erythema affects a large area of the skin
Slika 2. *U većini slučajeva eritem zahvata veliki deo kože*

Discussion

Simulium erythrocephalum is an insect which belongs to the family Simuliidae (genus Simulium, order Diptera, suborder Nematocera). This group of insects is relatively small and it has 2.151 species worldwide [1, 17]. It was first described by De Geer in 1776, in Sweden, as Tipula erythrocephala [18]. During the last decades, many reports have been published that indicate its ubiquity in Europe, from Scandinavia in the north, Ireland, Great Britain, France, Spain in the west, Lithuania and Russia in the east [18–22], while in Serbia its endemic habitat is near the town of Golubac, which explains its broadly accepted name "Golubac fly" [14, 23].

Simulium erythrocephalum is a black fly with wide wings, about 3–3.5 mm in size **(Figure 3)** [13]. Its life cycle occurs in running water, such as streams, streamlets and rivers where the larva is fed by water filtration. Hydrometeorological conditions have a crucial impact on the development of these

Figure 3. Simulium erythrocephalum is a black fly with wide wings, about 3–3.5 mm in size
Slika 3. *Simulium erythrocephalum je crna muva sa širokim krilima, veličine oko 3–3.5 mm*

insects. It is known that Simulium erythrocephalum is most active in spring and summer, while high water level and low temperatures are to the benefit of their progress. Preferred nutrition differs among various species of Simulium, some of them expressing severe anthropophilia. Females typically feed on blood and their saliva contains histamine responsible for inflammatory reactions in humans. Males do not bite and they feed on nectar [13, 24].

Following a Simulium erythrocephalum bite, humans present with local and/or systemic reactions. The bite itself is painless, with gradual increase of extreme pain accompanied by itch and formation of the central crust. Furthermore, erythematous area and edema with appearance of ecchymosis, nummular eczema and nodules on lower extremities may also appear [1, 14, 15]. Our patients had erythema and edema as well as punctiform and confluent hemorrhagic lesions associated with intense pruritus. The majority of bites were located on limbs, especially on uncovered body parts, which is in accordance with literature data [14]. Systemic reactions in the form of fever, weakness and generalized lymphadenitis may also appear and cause "black fly fever" [15], which was not detected in our patients, so hospitalization was not necessary and all patients completely recovered after several weeks. Apart from the previously mentioned conditions, Simulium erythrocephalum can be a vector in transmission of a disease known as 'river blindness' (onchocerciasis), tularemia and myxomatosis [1, 15, 22, 25].

Throughout the last few decades, there have been several reports on massive Simulium erythrocephalum attacks on humans in Serbia, mostly in the region near the Danube, but also along the Tisa river where around 2.000 clinical cases of bites were registered in humans [14, 26, 27].

On the territory of the city of Novi Sad, in spring of 1999, there was a massive appearance of Simulium erythrocephalum related to high water level of the Danube [24], but no epidemiological data are available on the exact number of patients. A team of experts from the Faculty of Agriculture, University of Novi Sad, pointed to a higher risk of black fly bites in the circumstances when the Danube water level is above 450 cm, in the vicinity of the river, rural and semi-rural regions within 5 km from the water [24, 28].

Apart from its extreme anthropophilic character and high impact on human health, Simulium erythrocephalum also has a role in veterinary medicine. Its bites in animals may cause toxic allergic reactions resulting in frequent animal mortality and significant livestock losses [24, 29]. Simulium erythrocephalum may also transmit a parasite from the genus of Leucocytozoon that infests birds [23].

Conclusion

Preventive eradication of the Simulium erythrocephalum in the larval stage along the Danube is almost unfeasible because of the water speed, breadth of the river, bridges, barriers for air-treatment, harmful insecticides, and the volume of water that flows in the unit of time. The available preventive measures may include removal of underwater plants and stones in the Danube and all its tributaries, use of repellents, avoiding dark-colored clothing and application of antiperspirants and fragrances.

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FROM AN ARTESIAN WELL TO THE PROCUREMENT OF EQUIPMENT AND FURNISHING OF THE CITY ARTESIAN SPA IN NOVI SAD

OD ARTEŠKOG BUNARA DO OPREMANJA I NABAVKE NAMEŠTAJA ZA GRADSKO ARTEŠKO KUPATILO U NOVOM SADU

Rastislava KRASNIK[1, 2], Jelena ZVEKIĆ SVORCAN[1, 3], Viktor STRIKOVIĆ[4], Sara SAMARDŽIĆ[5], Branislav BOBIĆ[1, 3] and Aleksandra MIKOV[1, 2]

Summary

Introduction. In 1897, the city government of the Free Royal City of Novi Sad proposed exploration for drinking water for adequate water supply in the city. **Iodine Spa Novi Sad.** An artesian well showed to have healing properties, which was a prerequisite for the development of the future spa that grew into the famous Iodine Spa Novi Sad. The Art Nouveau building of the Novi Sad City Spa was completed in 1910. In 1909, the city authorities issued a public notice to procure the necessary equipment and furnishing. In the turbulent years that followed, the City Iodine Spa has changed its name, exterior and interior appearance, as well as its equipment and furnishings, but kept its profile as an institution that fits the needs of every individual who may seek and find professional help under its auspices.

Key words: Water Wells; Baths; Public Facilities; History of Medicine; History, 19th Century; History, 20th Century; Balneology; Hospital Design and Construction; Interior Design and Furnishings

Sažetak

Uvod. Godine 1897. gradska vlast slobodnog kraljevskog grada Novog Sada je zbog potreba za adekvatnim vodosnabdevanjem započela sa bušenjem i nalaženjem izvora pijaće vode. **Jodna banja Novi Sad.** Utvrđena je lekovitost pronađene vode, što je bio neophodan uslov za razvoj budućeg kupališta koje će prerasti u čuvenu novosadsku Jodnu banju. Secesijska zgrada novosadskog varoškog kupatila završena je 1910. godine. Grad je 1909. godine raspisao oglas za nabavku potrebnog nameštaja i opreme. U burnim turbulentnim godinama koje su usledile, gradsko jodno lekovito kupatilo menjalo je svoje ime, spoljašnji i unutrašnji izgled, opremu, nameštaj, ali je sačuvalo pečat ustanove po meri svakog čoveka, koji pod njenim okriljem i krovom traži i nalazi stručnu pomoć.

Ključne reči: arterški bunari; kupatila; javni objekti; istorija medicine; istorija, 19. vek; istorija, 20. vek; balneologija; projektovanje i izgradnja bolnice; dizajn enterijera i nameštaja

Introduction

At the end of the 19th century, the Free Royal city of Novi Sad was facing extensive development and influx of population and there was a need to improve the water supply for the growing urban population. Therefore, in order to find an additional source of drinking water in the area of the former Calvary, on December 22, 1897, well drilling started, to the total depth of 193.42 meters, which ended on March 18, 1898 [1] or, according to other sources, in April of the same year [2, 3]. That was when 24.6 °C water gushed from the well, with an output of 300 liters per minute [1, 2], or 306 liters from a pipe that was 4.85 meters high [4]. In order to protect the territory around the newly found well and confirm the healing properties of the water, intensive correspondence of the City Council was required, to obtain expert opinions and approval from a number of experts in different fields and officials of the Ministries of Agriculture, Interior Affairs and others that were based in Budapest. The mayor sent a letter to the University Institute of Public Health in Budapest on October 16, 1898, in order to get the necessary answers from several university professors of internal medicine and hygiene (Dr. W. Vincze, Dr. K. Karoly, Dr. Fodor Jozsef, and others) regarding the water quality from the artesian well near Calvary [4]. In response, dated November 8, 1898, University Professor F. Jozsef referred to the legislation of that time, where "under paragraph 100 XLV of the Public Health Law of 1876, a medicinal

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spa is a mineral spa for which in its own right, by the composition of minerals, can be said with certainty that its use is effective against major diseases" [4]. The response stated that the water was cold, slightly alkaline, hydrochloric, and mostly similar to the water of Palić Lake. According to experts, this artesian water could be effective in treating similar diseases as the water of Palić Lake, but the question was whether it would have the same effects in the city of Novi Sad, having in mind that the effect of lake water was combined with nature and fresh air, that is, the favorable influence of microclimatic factors in treatment [4]. Particularly interesting was the opinion of experts from Budapest: "If this artesian water fell into the hands of a capable manager, Novi Sad would in a short time become a famous spa center. A particular advantage of this well may also be the fact that it is located in a booming city with many residents who would benefit from regular bathing in iodine water, not to mention those who would visit the spa for personal hygiene" [4]. In the same report, based on the chemical analysis of the water from 195 meters below the surface under pressure, it was concluded that the water was pure, suitable for drinking and household purposes, without the risk of pollution [1, 4].

Iodine Spa Novi Sad

The analysis of water samples taken from several different depths was performed in 1898 by Albert Grittner, a chemist in Budapest, and again in 1914 by Dr. Wilmos Hanko. The water was found to be similar to that of Lipik, in terms of iodine and table salt content, and it was acceptable for spa use. Further tests were done in Belgrade in 1930 (analysis by Dr. A. M. Leko) and in 1948 (performed at the Federal Institute of Hygiene) [1–4]. Possibilities of application of the natural gas, present along with medicinal water, were also analyzed [5]. Chemical analysis confirmed that the water was good for drinking and contained many other mineral constituents in addition to iodine [1–5]. The idea and initiative of the Novi Sad physician Wilhelm Vilt, about the need to build an iodine bath, was supported. The project and the construction itself were entrusted to the famous Hungarian architect Imre Francsek from Budapest [2–4, 6, 7]. On December 9, 1909, the City Council unanimously approved to raise the artesian bath to the rank of a medicinal bath (spa), since the conditions presently required were met [8]. By the decision of the Hungarian Royal Ministry of the Interior Affairs, on April 26, 1911, the Novi Sad Iodine Bath was established [2, 3].

In 1909, the city authorities issued a notice to procure the necessary furniture and equipment [9]. The budget (in Austro-Hungarian crowns) for the equipment of the artesian spa of the Free Royal city of Novi Sad was planned and clearly defined. The extensive list, with the type and quality of furnishing and equipment, and resources identified, indicates that the furnishing of this facility was approached very seriously and with great responsiblity **(Figure 1)** [9, 10].

Figure 1. The list of equipment needed for furnishing the city artesian bath
Slika 1. *Spisak potrebne opreme za opremanje varoškog arteškog kupatila*

The doctor's office: a light maple desk with a fabric cover (estimated price 115.00 crowns), chairs with backrest made of the same pliable wood, sofa with two plush armchairs, a table, carpet, 10 meters of carpet path, tin spittoon with water, as well as one wall coat hanger. The lobby of the doctor's office: five chairs made of light pliable wood, a small table, a wall coat hanger and a spittoon [9, 10]. Choosing the above inventory indicates the need to provide future customers with great comfort and a sense of luxury. The cashier's desk: one lacquered pine table, two chairs, a wall-mounted coat hanger and a spittoon. Four luxury baths: four fabric-upholstered sofas with spring cushions and the same number of carpets, mirrors with matching side tables, including marble plates, wall coat hangers, light pliable wood chairs, first-class Japanese mats and a spittoon [9, 10]. It may be assumed that these luxurious bathrooms were the meeting place for the most prominent people of Novi Sad of that time. That is why a lot of attention was also paid to the time between healing baths by staying in rooms that were in the ranks of the famous European spas. 14 first class bathrooms: 14 upholstered ottomans with spring pillows, console half tables of light wood with a mirror, wall-mounted coat hangers, chairs, spittoons and second-class Japanese mats. Common first class bathroom (with 2 baths), an ottoman, a wall-mounted console with a mirror, 2 wall-mounted coat hangers, chairs, Japanese mats and spittoons [9, 10]. The inventory intended for the lobby was selected with great care and attention was paid to the smallest details. The lobby furnishings: one octagonal spring-cushion plush sofa, 4 spittoons, 2 umbrella/parasol bowls with metal stands, 8 chairs and 4 small tables. The upper and lower corridors, the lobby, all the stairs, the locker room corridors, the corridor and the stairs leading to the steam room, the drying and rest rooms had about 290 red carpet paths. The upper corridor and waiting room: 6 leather armchairs, 8 spittoons, one leather set (sofa) with two leather arm-

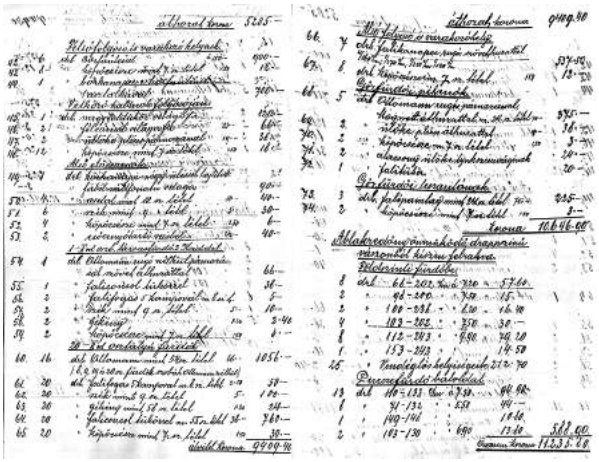

Figure 2. The list of equipment needed for furnishing the city artesian bath (continued)
Slika 2. *Spisak potrebne opreme za opremanje varoškog arteškog kupatila (nastavak)*

chairs and a small table. The hallway with locker rooms: a large lightwood wall mirror, two light wood console half tables, 2 seats with plush upholstery and 12 spittoons. The lower lobby: one circular seating set with four light wood seats and a twisted reed, 4 small tables, 6 chairs, 4 spittoons and 2 metal umbrella/parasol bowls **(Figure 2)** [9, 10].

The second-class bathroom with two bathtubs: a spring-free ottoman with, fabric coated pillows, wall-mounted console with a mirror, 2 wall-mounted coat hangers, chairs, mats and spittoons. Thirty second-class bathrooms: 16 ottomans (notes 8, 9, 10 and 20: baths without ottomans), 20 wall-mounted coat hangers, and as many chairs, mats, wall-mounted console tables with a mirror and spittoons. The lower corridor and waiting room: 7 wall fabric-coated seats with springs and 8 spittoons [9, 10]. The spa baths were also planned to provide services in the field of body and pedicure care, tailored to the users, within the steam baths. For the steam bath resting rooms, the City Council has proposed the acquisition of 5 ottomans with spring cushions, one wall mirror, 2 plush-covered seats, 2 spittoons, and 2 low seats for pedicure. The drying room after the steam bath: 3 ottomans and 2 spittoons. Beige canvas window shutters were also planned. The total budget for the equipment and furnishings was estimated to be 11.235.80 crowns [9, 10]. The invitation for procurement of the necessary equipment was officially announced and publicly released on July 12, 1909, while anonymous bids (like public procurement today) that arrived at the City Council of the Royal City of Novi Sad for the furnishing of bathrooms were considered on July 31, 1909 [9, 10].

The Art Nouveau building of Jodna Banja was completed and furnished in 1910. The exploitation of mineral drinking water began in 1911, when bottled water production started [2, 3]. With the decision of the city authorities from 1907 to build a new city hospital in Novi Sad, the foundations of future prosperous development of the health system in Novi Sad were laid [11, 12]. The increased needs for drinking water have been solved by new excavations. A new well was drilled in 1924, at a depth of 223.30 meters and initially yielded about 250 liters per minute, which subsequently also decreased. The third well was drilled in 1953, and after that several new wells as well [1]. Due to the growing interest of the population in spa facilities, the City Council decided to build a hotel next to the bath building. The hotel, designed by Djordje Tabaković, was completed in 1931. At that time, the bath building had 43 cabins, with first and second class bathtubs, three swimming pools, as well as other therapy units (electro, photo, cryo, helio, fango and physical therapy) [2, 13, 14]. The Spa water, alkaline-muriatic, hypothermic (24 degrees Celsius), has quickly gained loyal fans. There were numerous indications for its use: chronic, rheumatic and infectious inflammations of the musculature, bone and muscle diseases, fracture consequences, nervous system diseases, neuralgia, sciatica, lumbago, women's diseases, rickets and "hardening veins". It has also been recommended in chronic stomach diseases and intestinal catarrh, bile diseases, chronic constipation, urinary, as well as kidney and bladder diseases [2, 3, 13, 14].

In the turbulent years that followed, the city's iodine healing bath changed its name, external and internal appearance, equipment, furniture, but kept the mark of the institution tailored to the needs of every person who have been seeking and find professional help under its auspices.

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IN MEMORIAM
IN MEMORIAM

Prof. dr LJILJANA SOMER
(1947-2020)

Početkom januara 2020, posle duge i teške bolesti prerano je preminula prof. dr Ljiljana Somer, dugogodišnji šef Katedre za histologiju i embriologiju Medicinskog fakulteta u Novom Sadu. Poznata stara pedagoška maksima „Nije znanje znanje znati, no je znanje –znanje dati“, mogli bismo reći da je bila moto obrazovnog rada prof. dr Ljiljane Somer. Tiha, vredna, usredsređena, nenametljive vedrine, s osmehom na licu pri svakom susretu; vrstan pedagog – nastavnik mnogim generacijama studenata – to je bila Ljiljana Somer.

Rođena je 13. aprila 1947. godine u Subotici. Završila je Medicinski fakultet u Novom Sadu 1973, izabrana za asistenta 1974, magistrirala na Prirodno-matematičkom fakultetu u Novom Sadu 1980, jer Medicinski tada još nije imao magistarske studije i doktorirala na matičnom fakultetu 1984. Usavršavala se u Francuskoj i Nemačkoj. Govorila je francuski, nemački i engleski. Profesionalna orijentacija bila joj je eksperimentalna endokrinologija, eksperimentalna ortopedija, kliničko-patološka gastroenterologija. Izabrana je za docenta 1986, vanrednog 1991. i redovnog profesora 1996. godine.

Histologija kao naučna i nastavna disciplina zahteva pre svega predane ljude, pa tek onda tehnička sredstva i laboratorije. Upravo u svemu ovome nam je primer profesorka Somer i, kao dugogodišnji šef Katedre za histologiju i embriologiju, kao profesor i kao organizator rada i prijema asistenata na Katedru. Naša katedra imala je vrlo teških trenutaka, kada su prerano preminuli većina njenih članova. U jednom momentu, Katedra je imala samo jednog člana, profesorku Somer. Ona je krenula od početka i kao dugogodišnji šef, od 1997. godine, usmerila rad Katedre u sasvim novom pravcu, od prevashodno eksperimentalne za histologiju, pre svega za medicinare, i kao osnovu za učenje patologije i klinike.

Međutim, i u ranijem eksperimentalnom radu prof. Somer je još kako imala uspeha, bila je na usavršavanju u Francuskoj i Nemačkoj, nije joj bilo teško da u vreme sankcija humani materijal iz obdukcione sale nosi u Grac na elektronsku mikroskopiju, jer je mikroskop Medicinskog fakulteta bio pokvaren od devedesetih. Posebno su vredni njeni radovi iz eksperimenalne endokrinologije i alkoholizma. Njen rad o kastracionim ćelijama hipofize pacova u hroničnom etilizmu ili rad o histologiji meniskusa objavljeni su u vrlo uglednim stranim časopisima, a takvih radova imala je profesorka Somer više.

Danas Katedra ima devet članova, a sedam je ona izabrala i primila. Mi svi smo njeni direktni učenici, a ona nam je bila uvek primer kako se treba stalno usavršavati i stalno težiti ka boljem. Ona je mogla lagodno živeti samo kao profesor histologije i embriologije, čemu se i posvetila skoro svim svojim snagama, ali pored toga, ona je već kao vanredni profesor uspela da dobije specijalizaciju iz patologije, završi je 1993. i iz te oblasti je napravila još jednu karijeru, u vreme kad je i patologija bila neprofitna grana medicine. Biopsijska patologija koja je glavni posao današnjih patologa, dakle medicinska dijagnostika, bila je prof. Somer veliki izazov kome se uspešno odazvala i godinama radila na Odeljenju gastropatologije. Uvek smo mogli da se konsultujemo s njom, uvek je bila zainteresovana za dalje učenje, a naročito je negovala učenje histologije za patologe.

U ovakvim trenucima sećamo se samo dobrog, ali loših osobina profesorke Somer nije ni bilo. Sa svima je umela da bude u prijateljskim odnosima, a

da ostane dosledna, odlučna i pravdoljubiva. Ove crte nasledila je i od svog oca sudije, a i dede po majci, predratnog sudije u Subotici Milana Minovića, koji je kao sin srpskog rodoljuba poginulog u Skoplju 1905, učitelja i sveštenika Konstantina Minovića, dobio stipendiju kraljevske vlade da studira prava u Subotici. Takođe je bila duboko posvećena svojoj deci, Tijani, Daliboru i Emilu, koje smo preko nje svi poznavali i radovali se njihovim uspesima.

Bila je profesor, mentor, autor udžbenika, monografija (međunarodnog i nacionalnog značaja), autor brojnih radova objavljenih u internacionalnim i nacionalnim časopisima, naučnim skupovima; bila je rukovodilac i saradnik značajnih projekata. Velika energija jedne sitne žene – divnog čoveka i izuzetnog stručnjaka i pedagoga. Katedra za histologiju i embriologiju Medicinskog fakulteta u Novom Sadu, kojoj je posvetila čitav svoj vek, ostaje joj s dubokim pijetetom večno zahvalna.

Dušan Lalošević,
Dragica Pantić

UPUTSTVO ZA AUTORE

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Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

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Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

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Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

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Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

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Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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∗ Jedan ili više autora

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Elektronski materijal

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∗ Monografija u elektronskom formatu

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.

∗ Kompjuterska datoteka

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

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

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A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review 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.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

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 self-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 practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

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Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– 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).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. 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.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

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), materials 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.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

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

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.

Materials 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 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. 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 organization 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.

∗ *A computer file*

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

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, 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.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

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

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

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

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– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

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