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Radovi koji se objavljuju u časopisu „Medicinska istraživanja“ pripadaju oblastima: bazičnih biomedicinskih istraživanja, kliničkih istraživanja i preventivne medicine. U cilju stručne recenzije prispelih radova, Uređivački odbor časopisa je podeljen u tri sekcije: bazične, kliničke i preventivne nauke, a njihovim radom koordiniraju Urednici sekcija. Sekcija za statističku analizu podataka će biti uključena u evaluaciju statističkih apsekata prispelih radova.

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GLAVNI I ODGOVORNI UREDNIK
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ORIGINAL ARTICLE

Differences in clinical and laboratory characteristics in patients infected with COVID-19 during different epidemic waves

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Summary

Introduction/Aim: So far, the COVID-19 pandemic has seen four major epidemic waves that have affected more than 753 million people. Epidemiological studies have confirmed variability of clinical presentation of SARS-CoV-2 infection in these epidemic waves. During this period, virus mutations have contributed to greater challenges regarding treatment and prevention. The aim of the study is to determine the differences in clinical presentation, laboratory parameters, as well as the treatment outcome of patients suffering from COVID-19 during four different epidemic waves caused by different genotypic and phenotypic variants of SARS-CoV-2.

Material and Methods: We conducted retrospective study in which data were collected from hospitalized patients at the University Clinical Centre of Serbia Clinic for Infectious and Tropical Diseases in the period between March 1, 2020 and December 1, 2021. Statistical analyses, socio-epidemiological, clinical, radiographic and laboratory characteristics of patients through different epidemic waves of COVID-19 were compared.

Results: The study included 523 patients. Elevated body temperature was the first and the most common symptom of COVID-19 infection in all 4 epidemic waves, whereas cough and malaise were most common symptoms in the fourth wave. Cough was second most common symptom in third wave ($p<0.05$), following elevated body temperature, whereas malaise was second most common in the second wave. Diarrhea and nausea were significantly more common in the fourth wave compared to the previous waves ($p=0.04$ and $p=0.02$).

Conclusion: Highest values of inflammatory biomarkers were found in the second and the fourth wave. The fourth wave was characterized by the largest number of hospitalized patients, and it represented the peak of the pandemic. Treatment options varied through the waves, and corticosteroid use was most common during the fourth epidemic wave in hospital conditions.

Keywords: COVID-19, pandemic, SARS-CoV-2, clinical presentation

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INTRODUCTION

Since the beginning of the pandemic caused by a new strain of the corona virus, *SARS-CoV-2*, more than 753 million people have tested positive for the disease caused by this virus (COVID-19), with 6.8 million people having died worldwide (1). Ori et al. (2) concluded that the omicron variant was less virulent than the delta variant, which was highly infectious and first identified in 2020 (3), having lower hospitalization rate and a lower rate of severe forms of disease. Clinical presentation of COVID-19 can be mild, moderate, severe, and very severe depending on several factors such as genetic predisposition, comorbidities, duration of infection, and the immune system (4). Epidemiological data have shown that the characteristics of the clinical presentation of *SARS-CoV-2* varied during different epidemic waves. The most common symptoms were fever, dry cough, shortness of breath, malaise, loss of sense of smell and taste, myalgia, and weakness, caused by an attack on the alveolar epithelial cells in the lungs (4,5). Consequently, variations in clinical presentation in different epidemic waves were accompanied by changes in laboratory parameters and radiographic findings. Rehan et al. (6) concluded that significant availability of antigen tests aided in rapid diagnosis and subsequent isolation, while severe cases and mortality rates were lower than in previous epidemic waves. The most likely reasons for these variations in clinical presentation, disease severity, laboratory and radiographic findings are the vaccine against *SARS-CoV-2* that was launched after the third wave (7,8), weakened viral virulence, a certain collective and personal immunity achieved through a contact with the virus during the first epidemic waves. Besides, it is important to mention the availability of new antiviral therapy that came into use later on during the pandemic, as well as the application and introduction of corticosteroid and biological therapy into global and national protocols for the treatment of COVID-19 infection (9).

Literature data indicate that patients hospitalized due to complications, with a peak in the third wave (10), had a high mortality rate (26%) and that about 17% of patients admitted to hospital treatment required respiratory support and intensive care. The hospital course of treatment was often additionally complicated by hospital infections such as enterocolitis caused by *Clostridium difficile* bacteria, urinary tract infections, bacterial infections of the respiratory tract, but also by non-infectious conditions such as pulmonary embolism, cardiac arrhythmia and renal insufficiency (11).

The aim of the study is to determine differences in the clinical presentation, radiographic findings, laboratory parameters, applied therapy and the outcome of treatment in patients suffering from COVID-19 during four different epidemic waves caused by different genotypic and phenotypic variants of the *SARS-CoV-2* virus in the period between March 1st 2020 and December 1st 2021.

MATERIAL AND METHODS

Study group

A retrospective study was conducted at the Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia, the Department of Clinical Pharmacotherapy. Data were collected from patients who were hospitalized in this department due *to SARS-CoV-2* infection in the period between March 1st 2020 and December 1st 2021. Patients who met the following criteria were included in the study: I) positive PCR or Ag test for *SARS-CoV-2* before admission and II) age ≥18 years. The following data were collected from the medical history: (I) socio-epidemiological data, (II) laboratory analyses on admission and during hospital stay, (III) radiological analyses on admission and during hospital stay, (IV) data on clinical presentation on admission and during hospital stay, (V) therapy before admission and during hospital stay, (VI) data on the course and complication of the disease, and (VII) treatment outcome.

All patients were divided into 4 groups depending on the period of hospitalization:

I group (alpha strain SARS-CoV2) – the first epidemic wave from March 2020 to May 2020 (34 patients);

II group (alpha strain SARS-CoV2) – the second epidemic wave from July 2020 to August 2020 (61 patients);

III group (delta strain SARS-CoV2) – the third epidemic wave from September 2020 to February 2021 (180 patients);

IV group (omikron strain SARS-CoV2) – the fourth epidemic wave from August 2021 to December 2021 (248 patients).

Patients were divided into groups according to the waves defined by the World Health Organization (WHO). Patient data collection and retrospective study were performed in accordance with the Helsinki Declaration on the Protection of Patients' Rights.

Statistical analysis

Statistical analysis was used to compare the obtained laboratory, clinical and radiological variables through 4 different waves of the COVID-19 pandemic. Chi-square and Fisher's test were used to compare categorical variables, Mann-Whitney U test was used for ordinal and numerical data that deviated from normal distribution, while Student's T-test was used for numerical data with normal distribution. Some of the variables were described during different waves of COVID-19 pandemic using value prediction. Variables with normal distribution are described by the mean and standard, while variables outside the normal distribution are described by the median with maximum and minimum values. The values of certain categories are represented by percentages and numbers. Statistical Package for the Social Sciences (SPSS) version

23 was used to analyze patient data (SPSS Statistics, IBM Corp, Armonk, NY). The significance level of $p<0.05$ was established for all statistical tests.

RESULTS

Socioepidemiological data

A total of 523 patients who met the inclusion criteria were included in the study. Of the total number of patients, 59.1% (309/523) were men, with the average age of 56.7 years. More than half of the patients (62.1%) had at least one chronic disease, the most common of which was hypertension found in 43.4% of patients (227/523), obesity in 26.4% (138/523), diabetes in 13.4% (70/523) and atrial fibrillation in 7.6% (40/523) of patients. Vaccination in our country started on January 19, 2021 and comprised a quarter of patients (25.6%, 138/523), of which in the fourth wave, as many as 83.6% (112/138) were vaccinated with all three doses of vaccines **(Table 1 and Table 2)**.

Table 1. Characteristics of patients treated for COVID-19

Characteristics of patients (n=523)	**N (%)**
Gender (male)	309 (59,0)
Age	56,7 ± 16,0
Comorbidities	**325 (62,1)**
Hypertension	227 (43,4)
Obesity	138 (26,4)
Diabetes	70 (13,4)
Atrial fibrillation	40 (7,6)
Coronary disease	39 (7,5)
Solid tumors	42 (8,0)
Metastasis	11 (26,2)
COPD	31 (5,9)
Connective tissue disease	30 (5,7)
Neurological disease	23 (4,4)
Leukemia/Lymphoma	20 (3,8)
Cardiomyopathy	19 (3,6)
Paralysis	15 (2,9)
Hashimoto thyroiditis	10 (1,9)
Liver disease	10 (1,9)
Chronic kidney disease	9 (1,7)
Congestive heart failure	8 (1,5)
Dementia	6 (1,1)
HIV	4 (0,8)
Peptic ulcer	4 (0,8)
Intrahospital infections	**22 (4,0)**
Bacterial	16 (72,7)
UTI*	10 (45,4)
SMI**	3 (13,6)
Clostridium difficile	2 (9,0)
Syphilis	1 (4,5)
Viral/Fungal	6 (27,2)
Vaccinated	**134 (25,6)**
Sinopharm	102 (76,1)
Sputnik	14 (10,4)
Pfizer	10 (7,5)
Other	5 (3,7)
Astra Zeneca	3 (2,2)
Number of doses	
1 dose	7 (5,2)
2 doses	15 (11,2)
3 doses	112 (83,6)
Primary treatment during hospitalization	
Immunosuppressive	31 (6,0)
Biological	4 (0,8)
Antiviral	53 (10,1)
Corticosteroid	86 (16,4)
Symptomatic	17 (3,3)
No treatment	143 (27,3)
Antibiotics	304 (58,1)
Macrolides	139 (45,7)
Cephalosporins	127 (41,8)
Fluoroquinolones	121 (39,8)
Penicillin	16 (5,3)
Other	16 (5,3)
Metronidazole	5 (1,6)

COPD – chronic obstructive pulmonary disease; HIV – human immunodeficiency virus; *UTI – urinary tract infection; **SIM – skin and mucosa infection

Clinical presentation on admission and during hospital stay

Cough stood out as the leading symptom with an upward trend from the first to the fourth wave (R^2=0.998, p=0.001), followed by nausea (R^2=0.969, p=0.02) and diarrhea (R^2=0.917, p=0.04) which were significantly more often present in the later waves. Malaise showed a positive but not statistically significant frequency trend (R^2=0.762, p=0.13) **(Table 3)**.

Laboratory findings upon admission to hospital and during hospital stay

Observing the mean values of laboratory findings on admission, none of the parameters was statistically significant. Sodium had lower values in hospitalized patients (R^2=0.898, p=0.05) in all the waves. In addition to sodium, several other findings showed a positive trend of increasing values across waves (p=0.05), including fibrinogen (R^2=0.885, p=0.06), CRP (R^2=0.762, p=0.13), LDH (R^2=0.525, p=0.28) and CK (R^2=0.706, p=0.16), although none of them has shown to be statistically significant **(Table 4)**.

Table 2. Comorbidities identified in patients with COVID-19 in different epidemic waves

Comorbidities	Trend equalization	R^2	P
Total	y = -0,19x + 63,2	0,003	0,94
Hypertension	y = 3,25x + 33,2	0,772	0,12
Obesity	y = 6,41x + 6,6	0,548	0,26
Diabetes	y = 1,32x + 9,4	0,416	0,36
Atrial fibrillation	y = 0,15x + 7,4	0,013	0,89
Coronary disease	y = 1,99x + 0,9	0,574	0,24
Solid tumor	y = -1,06x + 9,3	0,823	0,09
COPD	y = -0,04x + 6,0	0,001	0,96
Connective tissue disease	y = 1,21x + 1,9	0,914	**0,04**
Neurological diseases	y = 1,2x + 0,5	0,535	0,27
Leukemia/Lymphoma	y = 0,02x + 3,9	0	0,99
Cardiomyopathy	y = 0,49x + 1,9	0,284	0,47
Paralysis	y = -0,68x + 4,9	0,189	0,57
Hashimoto thyroiditis	y = 0,88x – 1,0	0,637	0,20
Liver disease	y = -0,55x + 3,8	0,691	0,17
Chronic kidney disease	y = 0,38x + 0,7	0,123	0,65
Congestive heart failure	y = 0,42x + 0,2	0,341	0,42
Dementia	y = -0,68x + 3,3	0,896	0,05
HIV	y = -0,51x + 2,3	0,23	0,52
Peptic ulcer	y = 0,29x – 0,2	0,216	0,54

COPD – chronic obstructive pulmonary disease; HIV – human immunodeficiency virus; R^2– coefficient of determination; statistically significant values (p < 0,05) are bolded

Table 3. Comparison of symptoms in patients with COVID-19 in different epidemic waves

Symptoms	Equalizing trend	R^2	*p*
Temperature	y = 2,55x + 81,0	0,365	0,40
Cough	y = 11,66x + 31,9	**0,998**	**0,001**
Fatigue	y = 12,74x + 28,4	0,762	0,13
Dyspnea	y = -2,68x + 32,4	0,628	0,21
Myalgia	y = -0,54x + 24,6	0,006	0,93
Diarrhea	y = 3,96x + 6,6	**0,917**	**0,04**
Nausea	y = 6,0x – 1,9	**0,969**	**0,02**
Anosmia	y = 3,09x + 4,6	0,482	0,31
Headache	y = 1,51x + 8,5	0,269	0,48
Loss of taste	y = 2,69x + 4,3	0,288	0,46
Throat pain	y = -0,39x + 10,6	0,039	0,80
Chest pain	y = 3,95x – 4,1	0,485	0,30
Runny nose	y = 0,9x + 4,3	0,6	0,23
Vomiting	y = 2,33x – 0,8	0,724	0,15
Altered consciousness	y = 0,34x + 1,7	0,15	0,61
Coughing of blood	y = 0,44x + 0,7	0,175	0,58
Vertigo	y = 0,13x + 1,3	0,013	0,89
Skin changes	y = 0,2x + 1,1	0,036	0,81

R2 – coefficient of determination; statistically significant values (p < 0.05) are bolded

Table 4. Trend analysis of laboratory test values in patients with COVID-19

Laboratory values	Trend equalization	R^2	*P*
Hgb	y = 4,95x + 173	0,540	0,30
PLT	y = -2,15x + 146	0,495	0,27
CRP	y = 15,945x – 6,6	0,762	0,13
IL-6	y = 9,82x – 1,125	0,834	0,09
Urea	y = -0,15x + 6,1	0,600	0,23
Creatinine	y = 1,5x + 84,5	0,495	0,30
AST	y = 0,15x + 32,75	0,012	0,89
ALT	y = 43,75	0,000	1,00
GGT	y = 0,55x + 38,5	0,032	0,82
ALP	y = -1,35x + 67,25	0,377	0,39
LDH	y = 9,8x + 208,5	0,525	0,28
CK	y = 10,7x + 69,5	0,706	0,16
Fe	y = -0,93x + 7,75	0,788	0,11
Na	y = -2,55x + 146,25	0,898	**0,05**
K	y = 0,045x + 3,925	0,600	0,23
D-dimer	y = 0,036x + 0,53	0,158	0,60
Fibrinogen	y = 0,58x + 2,15	0,885	0,06

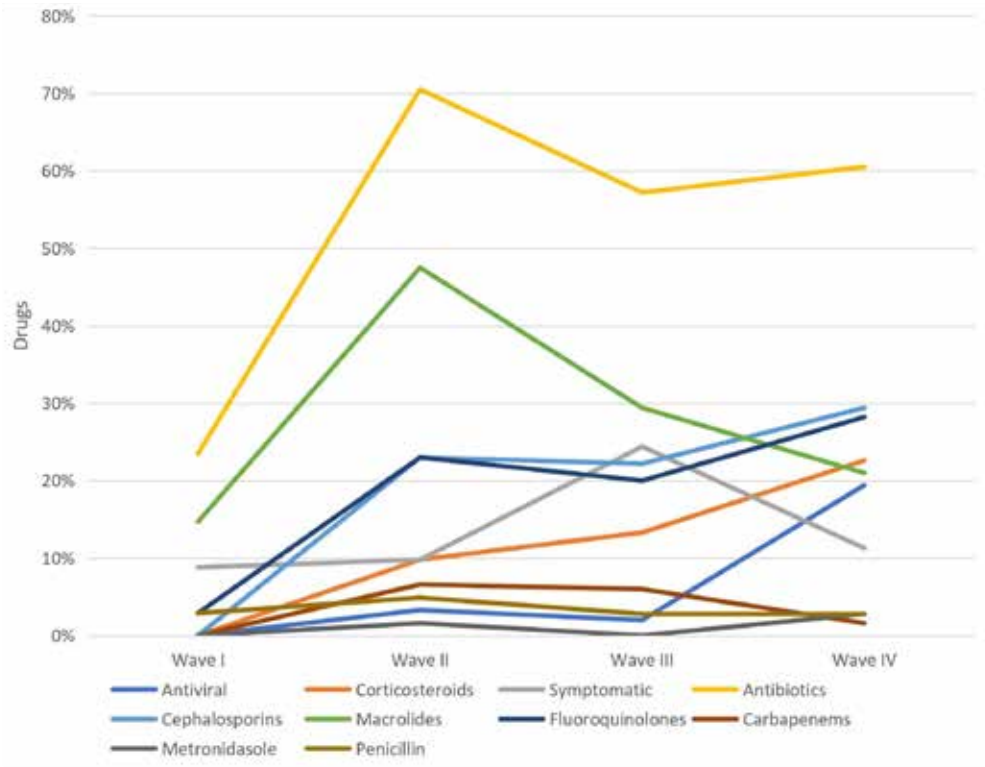
Hgb- hemoglobin; PLT- platelets; CRP- C-reactive protein; IL-6- interleukin-6; AST- aspartate aminotransferase; ALT- alanine aminotransferase; GGT- gamma glutamyl transferase; ALP- alkaline phosphatase; LDH- lactate dehydrogenase; CK- creatin kinase; Fe- iron; Na- sodium; K- potassium. R^2- coefficient of determination; statistically significant values (p < 0.05) are bolded

Radiological findings on admission and during hospital stay

The majority of patients (82.6%; 432/523) had a pathological finding on radiography of the lungs, of which 29.2% were described as spotty shadows, while 20.2% were described as undoubtable pneumonia (inhomogeneous diffuse shadows). As for the patients who underwent lung imaging with computerized tomography (CT) scan, the findings in 33.3% of cases indicated diffuse shadows, while in 18.7% the CT findings were described as diffuse consolidations. A significantly higher CT score of changes in the lungs, i.e., a more severe form of pneumonia, was registered in obese patients compared to patients with normal weight (p=0.008). Other characteristics did not prove to be statistically significant predictors of severe pneumonia (gender, comorbidities, hypertension, coronary heart disease, COPD).

Therapy before admission and during hospital stay

More than half of the admitted patients (58.1%, 304/523) used antibiotics before admission to hospital. A statistically significant, positive trend across waves was obtained for the use of corticosteroids in the treatment of patients with COVID-19 (R^2=0.972, p=0.01). The trend of antibiotic use before admission to hospital decreased signifi-



Graph 1. Distribution of drug use in four different waves of COVID-1

cantly through the waves, because of the introduction of antivirals, but was not statistically significant (R^2=0.382, p=0.38) despite a significant positive trend, both overall and especially in the two antibiotic groups administered during hospital stay – cephalosporins (R^2=0.772, p=0.12) and fluoroquinolones (R^2=0.738, p=0.14). During hospitalization, patients were treated with anticoagulant therapy in 90.4% of cases. During hospitalization, patients aged ≥66 years were more often treated with antibiotic therapy compared to patients aged 18-30 years, who were more often treated with antibiotic therapy before hospitalization **(Graph 1)**.

Complications of the disease

Complications of COVID-19 pneumonia were observed in 81/523 (15.5%) patients, the most common of which was respiratory failure in 27/523 (5.2%) patients. Intrahospital infections were recorded in 17/523 (3.2%) patients, of which urinary tract infections were most common (11/523, 64.7%). Other complications were rare, including pulmonary embolism (9/523, 1.7%), hyperglycemia (6/523, 1.1%), and new-onset cardiac arrhythmia (5/523, 1%). Complication trend analysis showed a statistically significant positive trend for the overall complication rate from the first to the fourth wave (R^2=0.915, p=0.04), especially for respiratory failure (R^2=0.944, p=0.03). No other complication showed a consistent positive or negative trend in occurrence **(Graph 2, Table 5)**.

Table 5. Comparison of complications of COVID-19 in patients hospitalized in different epidemic waves

Complications	Trend equation	R^2	*p*
Total	y = 4,05x + 0,35	0,915	**0,04**
DVT	y = 0,11x - 3E-18	0,067	0,74
PE	y = 0,69x – 0,6	0,328	0,43
HAI	y= - 0,91x + 5,75	0,358	0,40
Respiratory insufficiency	y = 2,25x – 2,0	0,944	**0,03**
Pericarditis	y = 0,87x – 0,35	0,279	0,47
Hyperglycemia	y = 0,72x – 1,2	0,600	0,23

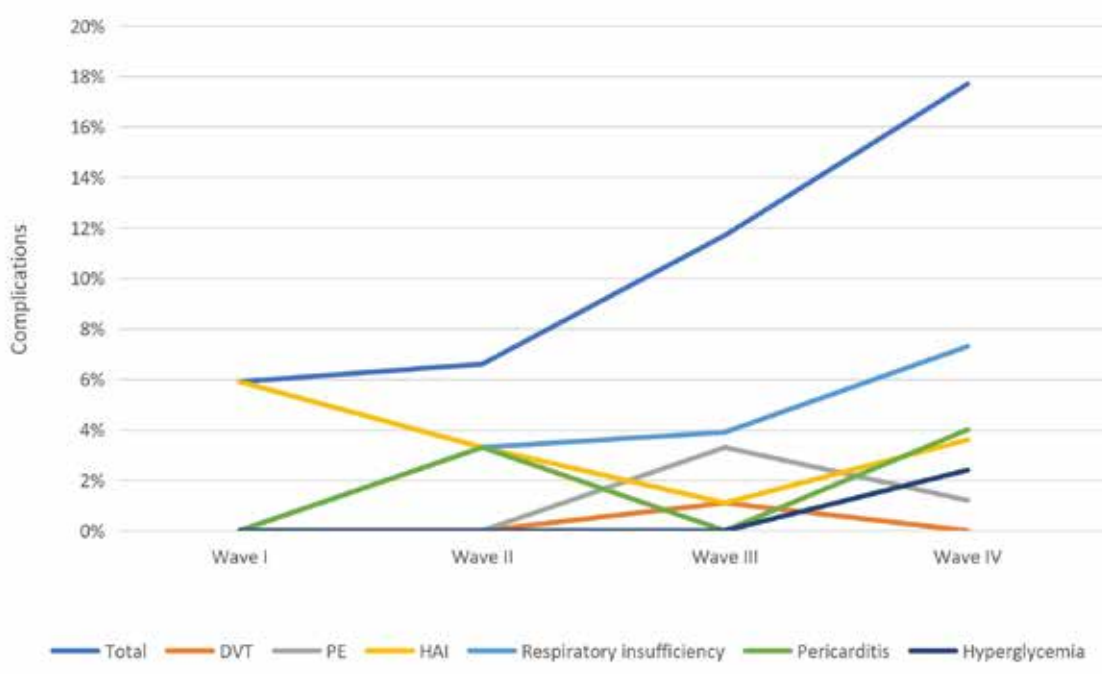
R^2 – degree of certainty; statistically significant values ($p < 0.05$) are marked in bold; DVT- deep vein thrombosis; PE- pulmonary embolism; HAI- hospitalization associated infection

Treatment outcome

As many as 91% (476/523) of patients were discharged from hospital cured, while 3.8% (20/523) of patients were transferred to the intensive care unit due to the development of disease complications. Only 3 deaths were recorded (0.6%).

Comparison of different comorbidities in COVID-19 epidemic waves

The distribution of the most common comorbidities was similar in all waves (R^2=0.003, p=0.94), with connective tissue diseases being the only chronic condition that showed a statistically significant positive trend in occurrence (R^2=0.914, p=0.04) and hypertension, which showed a positive but not statistically significant trend



DVT- deep veins thrombosis; PE- pulmonary embolism; HAI-hospitalization associated infections

Graph 2. Distribution of complications of COVID-19 in hospitalized patients in four different waves

(R^2=0.772, p=0.12). Other comorbidities, including obesity (R^2=0.548, p=0.26) and diabetes (R^2=0.416, p=0.36) did not show a statistically significant trend of occurrence from the first to the fourth wave of the pandemic.

Comparison of symptoms and clinical variables across different epidemic waves of the SARS-CoV-2

Cough had the most significant positive trend of occurrence from the first to the fourth wave of the pandemic, with the peak frequency in the fourth wave (p=0.001). An increase in the symptoms of fatigue and weakness was most significant from the first to the second wave, as well as from the third to the fourth wave, but statistical significance was not proven. Diarrhea (p=0.04) and nausea (p=0.02) had a significant upward trend during the pandemic, with a marked gradual increase in occurrence and peak in the fourth epidemic wave. Elevated body temperature, as the most common symptom, did not show oscillations in occurrence during waves (p=0.4).

Comparison of laboratory parameters in different epidemic waves of SARS-CoV-2

Laboratory parameters such as hemoglobin (Hgb), sodium (Na+), D-dimer and iron (Fe) did not change across epidemic waves. Sodium level was elevated in the laboratory results in all waves; also, a significant downward trend was present from the first to the fourth wave, but no statistical significance was shown.

Comparison of outcomes across different epidemic waves of SARS-CoV-2

At the end of all four waves, 3 deaths were recorded. The duration of hospital stay as well as the stay in intensive care units (ICU) were shorter in the last wave due to the less severe clinical presentation of patients in that period, compared to previous waves, but without statistical significance. In case of patients who were transferred from the clinical department to the ICU, the further course of the disease was not monitored, but only the outcome of the disease was recorded, therefore no information is available on the number of days spent in the ICU **(Table 6)**.

DISCUSSION

SARS-CoV-2 pandemic that began in March 2020 affected health and economy of the entire world population (12). Clinical, laboratory and radiographic picture of COVID-19 changed through epidemic waves.

In our study, we found that the most common comorbidity in patients with COVID-19 was hypertension, followed by obesity and COPD. According to literature, apart from obesity, frequent comorbidities were atrial fibrillation, coronary heart disease and solid tumors (12). In other studies, liver insufficiency was highlighted as an important comorbidity, apart from cardiovascular diseases, which were the most common comorbidity (12). In our study, liver failure was a rare comorbidity. In the group of vaccinated patients in our study, the largest number of patients with COVID-19 infection were ≥66 years of age, who were treated for at least one chronic disease.

Table 6. Complications and outcomes of treatment of COVID-19 in patients hospitalized in different epidemic waves of the *SARS-CoV2*

Patient characteristics (n=523)	**N (%)**
Complications	81 (15,5)
Respiratory insufficiency	27 (5,2)
Intrahospital infections	17 (3,2)
Urinary tract infections	11 (64,7)
Clostridium difficile infection	4 (23,5)
Pulmonary embolism	9 (1,7)
Hyperglycemia	6 (1,1)
Cardiac arrhythmia	5 (1,0)
Altered conscious	4 (0,8)
Pericarditis	2 (0,4)
Neutropenia	2 (0,4)
Deep venous thrombosis	2 (0,4)
Liver insufficiency	1 (0,2)
Acute pancreatitis	1 (0,2)
Acute myocardial infarction	1 (0,2)
Outcome	
Recovered	476 (91,0)
Transferred to ICU	20 (3,8)
Transferred to other hospital	19 (3,6)
Died	3 (0,6)
Dismissed at personal request	5 (1,0)

ICU- intensive care unit

In the first wave, mostly patients who belonged to younger population were hospitalized, with oxygen saturation <90% upon admission, and these were slightly more often female patients (13). In a study conducted by Alfonso et al. (14) the most common symptoms were fever, cough and dyspnea. In our study, the most common symptom was cough, while diarrhea and nausea were in the second and third place, respectively.

Anticoagulant therapy was administered to hospitalized patients in 90.4% of cases in our study. When comparing the waves, anticoagulant therapy was less commonly administrated in the first wave, while it was part of routine treatment in the second, third, and fourth wave. The reason for this was the positive outcome of patients treated with corticosteroids and anticoagulant therapy in later waves, whereas in the first wave, there was no experience or published data on this topic, so doctors rarely chose to treat patients with anticoagulant therapy. Same was the case with corticosteroid therapy, which was less commonly administered in the first wave, while it became part of routine treatment for COVID-19 in the later waves, based on the previous experiences of positive outcomes. At the same time, this was the reason for our group's low mortality rate, as appropriate corticosteroid and anticoagulant treatment was administered on time.

The results of laboratory results did not change significantly across the waves. The parameters that were most often above the referential values were CRP, fibrinogen, D-dimer, Na+, hemoglobin, platelets. Results of other studies are in accordance with our results, as elevated values of CRP, LDH, and accelerated erythrocyte sedimentation were highlighted in these studies as well (14).

Chest radiography represents an important diagnostic role in patients with suspected *SARS-CoV-2* infection, especially in settings where RT-PCR for *SARS-CoV-2* testing is not available or test results are delayed, as well as in patients with respiratory complaints or auscultatory verified pneumonia in which the RT-PCR test has been initially negative (15).

The most common COVID-19 complications are pneumonia (96%) and pulmonary thromboembolism (52%), as confirmed in our study, where 90.5% of patients were diagnosed with COVID-19 pneumonia. Only three lethal outcomes were observed in our study, all of which were patients transferred to the ICU unit who developed acute respiratory distress syndrome due to the COVID-19 cytokine storm and pneumonia, followed by multiorgan failure. None of our patients developed invasive fungal infections as a consequence of corticosteroid therapy.

During the COVID-19 pandemic, there has been a global increase in inappropriate use of antibiotics in the treatment of this viral infection (11). Antibiotics have been the most frequently used drugs during the pandemic. Our study showed that the younger population (33-45 years) used antibiotics more often in the treatment of COVID-19 compared to people ≥66 years. Despotovic et al. (11) showed that cephalosporins were the most frequently used antibiotics during the entire pandemic, which was also shown in our study in all waves. Along with cephalosporins, fluoroquinolones were the most commonly used drugs during all four epidemic waves of COVID-19.

Statistically, the most frequently used drugs in hospitalized patients during the *SARS-CoV-2* pandemic were corticosteroids, most likely due to their positive results in the treatment of complicated forms of COVID-19. Parrella and Marra (16) reported that the clinical use of corticosteroids in the treatment of patients with moderate to severe COVID-19 infection not only reduced the length of treatment and improved clinical outcome, but also significantly reduced mortality.

Complications of COVID-19 were recorded in 15.5% of patients and related to respiratory failure and intrahospital urinary infection. Prolonged duration of treatment in hospital conditions and the use of urinary catheter as a convenient location for urinary infections are the reason for most of these infections. Beatriz et al. (17) stated that the most common causes of urinary tract infections were *E. coli, E. faecalis and E. faecium*. In addition to urinary infections, literature data indicate that the leading co-infections that burdened healthcare in hospital conditions more than expected were central venous catheter infections and methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia (18).

CONCLUSION

The highest values of inflammatory biomarkers were recorded in the second and the fourth wave. The fourth wave recorded the largest number of hospitalized patients and represented the peak of the pandemic. The choice of therapy changed across the waves, and significantly more frequent use of corticosteroids and antiviral therapy was shown in the fourth wave.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

Since this is a retrospective observational study, the approval of the Ethics Committee of KCS was not required.

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RAZLIKE U KLINIČKIM I LABORATORIJSKIM KARAKTERISTIKAMA KOD PACIJENATA OBOLELIH OD KOVIDA 19 TOKOM RAZLIČITIH TALASA PANDEMIJE

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Sažetak

Uvod/Cilj rada: Od početka pandemije novim korona virusom (*SARS-CoV-2*), registrovana su četiri epidemijska talasa tokom kojih je obolelo više od 753 miliona ljudi. Karakteristike kliničke slike koju daje *SARS-CoV-2* varirale su tokom ova četiri talasa. Mutacije virusa su doprinele većem izazovu kada su u pitanju tok bolesti, lečenje i prevencija. Cilj studije je da se utvrde razlike u kliničkoj slici, laboratorijskim parametrima, kao i ishodu lečenja pacijenata obolelih od *COVID-19* tokom četiri različita talasa pandemije izazvanih različitim genotipskim i fenotipskim varijantama virusa *SARS-CoV-2*.

Materijal i metode: Sprovedena je retrospektivna studija tokom koje su prikupljeni i analizirani podaci hospitalizovanih pacijenata u Klinici za Infektivne i tropske bolesti UKCS u periodu od 1. marta 2020. do 1. decembra 2021. godine koji su lečeni zbog *SARS-CoV-2* infekcije. Statističkim analizama su poređene socioepidemiološke, kliničke, radiografske i laboratorijske karakteristike pacijenata kroz različite talase pandemije kovida 19.

Rezultati: U studiju su uključena 523 pacijenta. Povišena telesna temperatura je bila prvi i najčešći simptom *SARS-CoV-2* infekcije tokom sva četiri talasa pandemije, dok su kašalj i malaksalost bili najzastupljeniji simptomi u četvrtom talasu. Drugi najčešći simptom posle povišene telesne temperature u trećem talasu bio je kašalj ($p<0,05$), a malaksalost u drugom talasu. Dijareja i mučnina su bili statistički značajno češći u četvrtom talasu u poređenju sa prethodnim talasima ($p=0,04$ i $p=0,02$).

Zaključak: U drugom i četvrtom talasu su primećene najviše vrednosti biomarkera zapaljenja. U četvrtom talasu je zabeležen najveći broj hospitalizovanih pacijenata. Izbor terapije se menjao tokom talasa, te je u četvrtom talasu primećeno značajno češće korišćenje kortikosteroida u bolničkim uslovima.

Ključne reči: kovid 19, pandemija, *SARS-CoV-2*, klinička slika

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ORIGINAL ARTICLE

Congenital central hypoventilation syndrome – heterogeneous clinical presentation, ventilatory modalities and outcome

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Summary

Introduction/aim: Central congenital hypoventilation syndrome (CCHS) is a rare genetic disorder characterized by autonomic dysregulation and alveolar hypoventilation with ventilatory support being the cornerstone of long-term survival. The aim was to present different ventilatory strategies in CCHS.

Material and methods: The study included retrospectively analyzed medical records of five patients diagnosed with CCHS in a national pediatric center. Alveolar hypoventilation was evidenced by noninvasive continuous transcutaneous capnometry and central sleep-disordered breathing documented by polygraphy. Clinical evaluation included cardiac evaluation, rectal biopsies, and urinalysis of catecholamine levels. Life-threatening cardiac arrhythmias were indications for pacemaker implantation. Genetic analyses of alanine residues in paired-like homeobox 2B gene (*PHOX2B*) confirmed the diagnosis.

Results: A range of pathogenic changes in the *PHOX2B* gene resulted in varying clinical outcomes. 3/4 (75%) of patients with an early onset were ventilated continuously through a tracheostomy tube, while one patient was successfully treated with noninvasive ventilation (NIV) as the preferred option. Additionally, NIV was applied in one child with early-onset disease after decannulation. Finally, NIV was also feasible in a case with late-onset disease presented by the time of four years with symptoms of pulmonary hypertension. There were no serious side effects of ventilation, and one patient died due to cardiac arrhythmias.

Conclusion: Invasive mechanical ventilation remains the treatment of choice in most children with early-onset disease. However, the indications for NIV have been widened from overnight ventilation in the late-onset course to selected cases with early-onset disease. The timely switch from IMV to NIV has been popularized in recent years worldwide.

Key words: central congenital hypoventilation, invasive ventilation, noninvasive ventilation, decannulation

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INTRODUCTION

Central congenital hypoventilation syndrome (CCHS) is a rare incurable genetic disorder characterized by heterogeneous clinical patterns, respiratory and autonomic dysregulation being the main clinical concern (1). It has been estimated that there are about 1300 cases of CCHS worldwide with an estimated incidence of 1/148.000-1/200.000 live births (1). Most frequently (90% cases), it is caused by polyalanine repeat expansion mutation (PARM) of paired-like homeobox 2B (PHOX2B) gene located on chromosome 4 and expressed in the cells of the neural crest where it has an important role in cellular migration and differentiation. Former and latter processes are essential for the development of all respiratory and vasomotor centers and autonomic nervous system (ANS) functions (2). Thus, the heterogeneous clinical expression of CCHS is explained simply by autonomic functions of ANS in which PHOX2B takes participation: breathing, heart rate, blood pressure, body temperature, hormonal regulation, digestive function, and pupillary reactivity (3). Very few children (up to 10%) have a non-PARM genotype (NPARM) resulting from a missense, nonsense, frameshift, or stop codon mutation. CCHS is inherited in an autosomal dominant manner and mainly occurs via de novo mutations, while a small proportion of cases show germline mosaicism with/without somatic mosaicism (4).

Typically, diurnal hypoventilation with diminished respiratory drive and cyanosis in response to hypercarbia is manifested during infancy (5,6). Rarely, a proportion of children tend to have milder phenotypes with only nocturnal hypoventilation (5,6). However, both forms are often coupled with symptoms of autonomic dysregulation: cardiac (cardiac arrhythmias), intestinal (congenital megacolon, i.e. Hirschsprung disease), ocular symptoms (mostly pupilar abnormalities), and tumors of neural crest origin (neuroblastoma and ganglioneuroma) (7–10). Genotype-phenotype association shows a positive correlation between both the length of polyalanine string and non-polyalanine repeat expansion alterations and severity of the disease (11,12).

Though ANS disorders and tumors impose significant healthcare challenges with a potential to increase mortality rates, treatment of respiratory insufficiency still remains the cornerstone of long-term survival (13,14). While chronic invasive mechanical ventilation (IMV) represents a traditional treatment modality, the importance of noninvasive ventilation (NIV) has been popularized and highlighted in recent years in selected patients, e.g., after decannulation or in those with late-onset disease (14-16). With better overall medical care directed at each aspect of the disease, the field of indications for NIV has become wider in the past decade (14–16).

We intended to present the experiences of the leading national pediatric respiratory center treating patients diagnosed with CCHS with special emphasis on long-term ventilatory support. The important goal was to underline the criteria for a timely switch from invasive chronic mechanical ventilation to NIV.

MATERIAL AND METHODS

The study includes retrospectively analyzed medical records of five patients diagnosed with CCHS from 2008 to 2022 in the Department of Pulmonology of the Institute for Mother and Child Health Care of Serbia, the national center for home mechanical ventilation in children.

Criteria for diagnosis

Referrals for genetic testing were made for patients in whom sleep study results had revealed central pattern and chronic alveolar hypoventilation and who showed blunted response to hypercarbia and hypoxemia – either as an absence of respiratory drive or multiple failures to wean from mechanical ventilation without specific explanation. Additionally, the possibility of hypoventilation was considered in patients with idiopathic pulmonary hypertension, sleep disordered breathing, and signs of autonomic dysfunction. Diagnosis of CCHS was based on the number of sequence repeats in the *PHOX2B* gene.

Genetic testing

Having extracted DNA from peripheral blood, PCR analysis of the exon 3 of PHOX2B gene coding for the CCHS-associated polyalanine repeat sequence expansion was performed using primers flanking specific regions. The number of alanine repeats was quantified via gel electrophoresis of the PCR product and compared with standard alleles typically containing 20 repeats. CCHS-confirmative findings were those containing 25-33 alanine repeats. Genetic counseling for family members was recommended in each confirmed case.

Respiratory evaluation

Hypercarbia was detected by spot arterial blood gas analyses (ABG) and/or noninvasive continuous transcutaneous capnometry ($PtcCO_2$) results. More than 2% of the total sleep time (TST) with PtcCO2 > 50 mmHg was indicative of nocturnal hypoventilation (17,18). Sleep study (polygraphy) was performed simultaneously in all patients, when possible, except in patients chronically ventilated in ICU. Respiratory events were scored by two experienced physicians according to the American Academy of Sleep Medicine (AASM) (19). Flexible bronchoscopy was performed in each patient to evaluate possible structural anomalies of the airways.

Modes of ventilatory support

The onset of ARF and daily requirements for respiratory support further defined treatment strategies in subjects diagnosed with CCHS. Patients who had experienced ARF in infancy with a permanent (>16 hours a day) need for ventilatory support were considered to have an early-onset disease. They were tracheostomized, and the treatment of choice was chronic invasive mechanical ventilation with pressure-control mode. A specific protocol for decannulation was used, as described elsewhere (20). Patients diagnosed with CCHS that presented only with nocturnal hypoventilation and preserved bulbar function without neurodevelopmental delay were primarily ventilated noninvasively via nasal or full-face masks.

Prior to discharge, parents/caregivers were trained to provide satisfactory, all-day-long medical care to the children including tracheal suctioning with cough assist device usage, tube feeding when needed, and training related to basic ventilatory settings and alarms. Once discharged, patients were mechanically ventilated at home.

Efficacy of the respiratory support in each patient was evaluated regularly in three-month intervals using ABG, continuous transcutaneous capnometry, and in-built software data from the memory cards of the ventilators.

Evaluation of the autonomic nervous system

Both echocardiography and heart rhythm disturbances evaluation were part of routine workup. Life-threatening arrhythmias were indicators for pacemaker implantation. Regular cardiac reevaluation was mandatory at least twice a year as a part of follow-up.

Regular ophthalmologic evaluation was also provided from initialadmission onward, at least annually. Each patient was screened for neural crest tumors with vanilmandelic (VMA) and homovanilic (HVA) blood and urinalysis.

Unlike the aforementioned, congenital megacolon screening was not routinely included. It was conducted only when necessary – in selected patients with symptoms of intestinal obstruction. Those with mild intermittent symptoms of intestinal obstruction underwent transanal rectal biopsy, while abdominal X-ray and urgent surgery were opted for in patients with a severe acute presentation. Postcolectomy pathohistological verification of the absence of intramural ganglion cells was mandatory.

Thorough neurologic and metabolic workup was undertaken in each case to diagnose disorders that included hypoventilation as a secondary phenomenon.

RESULTS

The study included five patients diagnosed with CCHS all of whom were females aged from birth to four years. Clinical presentation, including the onset and severity of the disease correlated with genotype: those with the early-onset disease had 27 alanine residues in exon 3 of the *PHOX2B* gene, while one child with the late-onset disease had 25 alanine repeats. The main clinical and genetic features are given in **Table 1**.

A range of pathogenic changes in the *PHOX2B* gene resulted in varying degrees of cellular dysfunction, thereby influencing the phenotype of an individual patient with CCHS. Most of the patients with genotype 20/27 required continuous ventilatory support and had clinical manifestations of autonomic dysfunction including Hirschsprung disease - as seen in three out of four analyzed patients with 20/27 PARM *PHOX2B* genetic alteration (Table 1 and Table 2). In those three cases, long-term mechanical ventilation was applied througha tracheostomy tube. The mean age at tracheostomy tube insertion was three months. None of the patients had structural airway abnormalities on flexible bronchoscopy.

A milder disease course and an absence of Hirschsprung disease were confirmed in the analyzed patient harboring the 20/25 PARM *PHOX2B* genetic alteration **(Table 1 and Table 2)** and presented with late-onset disease and symptoms of pulmonary hypertension. NIV turned out to be feasible in this child. Furthermore, NIV was also used as a definite treatment option in one case with 20/27 genotype with early-onset disease after a successful extubation in the first postnatal week, as well as in one child after decannulation at the age of six.

Table 1. The main clinical and genetic features in patients with CCHS.

Patient No.	*Mutation*	*Onset*	*Initial presentation*	*Hirschsprung disease*	*Cardiac arrhythmias*	*Outcome*
I	PARM (20/27)	Early (2^{nd} month)	Acute respiratory failure	Yes	Yes (pacemaker inserted)	Alive – home ventilation
II	PARM (20/27)	Early (1^{st} month)	Acute respiratory failure	No	No	Alive – home ventilation
III	PARM (20/25)	Late (4^{th} year)	Pulmonary hypertension	No	No	Alive – home ventilation
IV	PARM (20/27)	Early (1^{st} month)	Acute respiratory failure	Yes	No	Alive – home ventilation
V	PARM (20/27)	Early (1^{st} month)	Acute respiratory failure	Yes	Yes (fatal arrhythmias)	Dead

Table 2. Modalities of ventilatory dependence and treatment in patients with CCHS.

	Hypoventilation pattern	*Initial intubation*	*Successful extubation*	*Definitive route of ventilation*	*Mode of ventilation*	*Complications of ventilation*	*Adherence to ventilation*
I	Nocturnal	Infancy	No	T-tube NIV - after decannulation	PCV AVAPS	Midfacial hypoplasia (mild)	Good
II	Nocturnal	Infancy	Yes	NIV	AVAPS	Midfacial hypoplasia (mild)	Good
III	Nocturnal	-	-	NIV	PSV	Midfacial hypoplasia (mild)	Initially poor
IV	Diurnal	Infancy	No	T-tube	PC-SIMV	No	Good
V	Diurnal	Infancy	No	T-tube	PC-SIMV	No	Good

PCV - pressure control ventilation; AVAPS - average volume-assured pressure support; PSV - pressure support ventilation; SIMV - synchronous intermittent mandatory ventilation;

Ventilatory settings were adjusted to achieve and maintain PaCO2 between 35–45 mmHg and SpO2 >94%. The initial ventilatory parameters included either Bilevel positive airway pressure or average volume assured pressure support (AVAPS) **(Table 2)**. Backup rates were slightly below physiologic values for the given age. Settings would be adjusted periodically through regular clinical assessments. Ventilator internal memory card manual analyses showed maintenance of sleep-disordered breathing in each patient in the first six years of life: constantly high AHI values (>5/h) were consistent with low percentage of patient-triggered breaths overnight, i.e. no more than 20% of total breaths were triggered by patients themselves during night.

Except for initial problems with adherence to NIV in a child with the late-onset disease which was resolved with time by proper accommodation, ventilation was implemented regularly. All patients on NIV had some degree of midfacial hypoplasia despite using various mask types – nasal, full-face, and custom-made when available. Facial skeleton abnormalities were not severe and had no impact on dental development.

While non-pulmonary complications were encountered in three out of four cases with 20/27 genotype, cardiac involvement was directly related to survival **(Table 1)**. Unfortunately, ventricular arrhythmias were fatal for one child by the age of five months, even before the scheduled pacemaker insertion.

DISCUSSION

This study synthesizes a single tertiary pediatric center experience with children diagnosed with CCHS and represents ventilatory possibilities as a mandatory part of its treatment. It particularly underlines the feasibility of noninvasive ventilation at different stages of the disease.

Home mechanical ventilation has changed the long-term perspective of infants diagnosed with CCHS (13). Consequently, non-pulmonary complications nowadays have become an increasing cause of CCHS-associated mortality. Over time, technical improvements in ventilatory equipment and better overall health care have skewed a focus of respiratory support towards the improvement of quality of life and minimizing side effects of mechanical ventilation (13). Despite being the first option in early-onset disease with unequivocal efficacy, IMV imposed serious concerns about speech and psychosocial development (21). Additional possible complications associated with tracheostomy tube (infections, bleeding, frequent admissions for tracheostomy tube replacement) coupled with a better understanding of the nature of the disease led to the necessity of introducing NIV and broadening indications for it (15,20).

While NIV was the first-line option in CCHS for late-onset disease with nocturnal hypoventilation, there were many concernsregarding the use of NIV either after decannulation or in early-onset disease as initial treatment (20). However, awareness that there was partial maturation of chemoreceptors with time as well as diminished blunted response to both hypercarbia and hypoxia while awake has strengthened the possibility of NIV usage (22). Despite undisputed advancements in ventilatory protocols regarding CCHS, NIV can be demanding. A triangle of patients, caregivers/families, and proper equipment as main determinants is essential for a successful NIV trial. The absence of at least one of these determinants may lead to NIV failure (18). Patient-related factors are of special importance – adequate neurocognitive development and normal bulbar function firmly support the use of NIV (23). Additionally, preserved airway patency documented by bronchoscopy remains inevitable for the NIV course (24). The age of the child remains an important factor of ventilatory modality, particularly when it comes to decannulation. Namely, after the failure of an early transition from invasive ventilation to NIV had been reported, some authors suggested the period from six years to adolescence as the prime time for decannulation and introduction of NIV (15,20,25,26). Still, meticulous patient recruitment can render NIV possible after removing the tracheostomy tube, even at an early age(27).

Brain tissue in children has a relatively higher metabolic rate and basal blood flow compared to adults, with

particular vulnerability to hypoxemic episodes (28,29). Besides, decreased cerebral tissue oxygenation caused by respiratory events precedes systemic desaturation (30). Although a specific contribution of cerebral oxygenation drops in the non-rapid eye movement stage (NREM) has not been studied yet, its potential negative impact on neurocognitive outcomes should not be neglected. Thus, decannulation represents a critical part of treatment and should be meticulously considered and carried out following the international protocols (20).

Despite NIV efficacy in practically all forms of disease severity, many infants have still been ventilated invasively via tracheostomy tube for multiple reasons apart from disease severity. Challenges imposed by the appropriate mask choice, adherence to ventilation, motivation of caregivers/families and long-term sequellas of NIV such as med-facial hypoplasia, used to play a significant role in tailoring the therapy concept (31–33). Meanwhile, improvements in ventilatory equipment associated with better overall health care of patients to CCHS have helped to cope with these challenges: different mask types adjusted to perfectly fit a child's face in combination with ventilatory software advancement have enabled early commencement of NIV (34). Moreover, mid-facial hypoplasia due to impaired growth of facial bones as a consequence of mask pressure mainly seeks observation instead of intervention (35,36). Additionally, the statement that PHOX2B is expressed in the rhombencephalic area important for facial development, suggested that mid-facial hypoplasia could be an integral CCHS feature instead of a mask pressure complication. Examples of invasively ventilated children with characteristic facial expressions of mid-facial hypoplasia support this remark (37). Surely, regular orthodontic follow-ups are recommended in each case (35,38). Moreover, combining different interface solutions (nasal, full-face, and custom-made masks) with family motivation is important concerning specificities of the early-childhood circadian rhythm – since the number of sleeping hours is the highest at this stage of life, problems with adherence to NIV are most frequent here.

Finally, a gradual shift from invasive ventilation to NIV – particularly with the extension of NIV to severe genotype/phenotype cases – has been conducted recently worldwide (39). Nevertheless, the necessity of long-term overnight ventilation may seemingly remain life-long, just as analyses of the percentage of patient-triggered breaths during sleep while using NIV confirmed in this study on a small-scale sample.

With improved respiratory care of the patients with CCHS, non-pulmonary complications appear to become an important factor in quality of life and overall survival. However, heterogeneous representation of cardiac arrhythmias and Hirschsprung disease was observed in patients with the same genotype in this paper. This phenotypic variability may be the result of the impact of modifier genes, or the result of incomplete penetrability of the gene due to which the observed clinical presentation in a patient may be mild or severe (40).

This study has some limitations. The small number of patients included in the study interferes with adequate statistical analysis. Technical limitations emanated from inability to conduct full polysomnography, so a potential analysis of sleep stages associated with respiratory events was not feasible. Since *PHOX2B* genetic alterations are inherited in an autosomal dominant pattern with incomplete penetrance, it is important to analyze parents of the affected child for the *PHOX2B* pathogenic variations, since early identification of parents harboring *PHOX2B* alterations can facilitate prompt evaluation and interventions to improve long-term outcomes and enable prenatal testing (40). Lack of data related to genetic testing of some parents represents one of the limitations of this study.

CONCLUSION

Congenital central hypoventilation syndrome remains a complex condition with long-term ventilatory support as an essential therapeutic tool. Apart from invasive ventilation via tracheostomy tube as an option of choice in most children with early-onset disease, noninvasive ventilation appears to be feasible in various clinical scenarios: except for overnight ventilation in the late-onset course and after decannulation, the indication area has been widened to meticulously selected cases with the early-onset disease.

AUTHOR CONTRIBUTIONS

Each author contributed to the conception and design of the work; MB and AS contributed to the acquisition and analysis of data; MB prepared the draft of the manuscript; each author was in charge of the interpretation of data; the final version of the paper was reviewed and approved by all the authors.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICAL APPROVAL

The study protocol was approved by the local Ethical committee (decision number 5/48). The research was conducted according to the Declaration of Helsinki.

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SINDROM KONGENITALNE CENTRALNE HIPOVENTILACIJE - HETEROGENOST KLINIČKE PREZENTACIJE, MODALITETI VENTILATORNE POTPORE I ISHODI

Mihail Basa[1], Jelena Višekruna[1], Bojana Gojsina Parezanović[1], Tijana Grba[1], Marina Andjelković[3], Aleksandar Sovtić[1,2]

Sažetak

Uvod/cilj: Sindrom centralne kongenitalne hipoventilacije (CCHS) predstavlja redak genetski uslovljen poremećaj karakterisan autonomnom disregulacijom i respiratornom insuficijencijom zbog koje je neophodno sprovođenje dugotrajne mehaničke ventilacije kao osnove preživljavanja. Cilj rada je predstavljanje modaliteta ventilatorne potpore u lečenju CCHS-a.

Materijal i metode: Retrospektivnom analizom medicinske dokumentacije obuhvaćeno je pet pacijenata sa dijagnozom CCHS-a lečenih u tercijarnoj pedijatrijskoj ustanovi. Alveolarna hipoventilacija dokumentovana je kontinuiranom neinvazivnom transkutanom kapnometrijom**,** a centralni poremećaj disanja u spavanju potvrđen je poligrafski. Dijagnostička obrada obuhvatala je i kardiološku evaluaciju, rektalnu biopsiju i analizu kateholamina u urinu. Teški poremećaji srčanog ritma predstavljali su indikaciju za implantaciju pejsmejkera. Dijagnoza bolesti potvrđena je genetskom analizom broja alaninskih rezidua u *PHOX2B* genu.

Rezultati: Heterogenost mutacija *PHOX2B* gena rezultovala je razlikama u kliničkom ispoljavanju bolesti. Mehanička ventilacija preko traheostome primenjivana je kod 3/4 (75%) dece sa ranim početkom bolesti u neonatalnom dobu, dok je kod jednog deteta iz date grupe uspešno sprovođena neinvazivna ventilacija (NIV). Takođe, NIV je bila opcija izbora u periodu posle dekanilmana kod jednog deteta sa ranim početkom bolesti. Konačno, NIV je sprovođena u slučaju deteta sa kasnim početkom bolesti prezentovanom simptomima plućne hipertenzije u uzrastu od četiri godine. Ventilacija je kod sve dece sprovođena redovno, a uzrok smrti u jednom slučaju bio je poremećaj srčanog rada.

Zaključak: Iako invazivna mehanička ventilacija ostaje prva terapijska opcija kod dece sa ranim oblikom CCHS-a, vremenom je došlo do proširenja indikacija za primenu NIV-a od kasnog ka odabranim slučajevima sa ranim početkom bolesti. Značaj dekanilmana i prelazak na NIV postaje sve veći u svetskim centrima.

Ključne reči: centralna kongenitalna hipoventilacija, invazivna ventilacija, neinvazivna ventilacija, dekanulacija

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ORIGINAL ARTICLE

Causes and outcomes of respiratory distress in late preterm infants - tertiary neonatal intensive care unit experience

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Summary

Introduction/Aim: Respiratory distress (RD) is the most common cause of morbidity in preterm infants. The current study was undertaken to determine causes of RD in late preterm infants, analyze characteristics of RD regarding gestational age, compare different clinical forms of RD and determine the factors influencing the unfavorable outcome of late preterm infants with RD.

Methods: The study included infants born between 34 0/7 and 36 6/7 weeks of gestation with RD hospitalized at the Institute for Mother and Child Health Care of Serbia „Dr Vukan Cupic". Demographic and clinical characteristics, clinical signs and course of the disease, the occurrence of complications, as well as quantification of disease severity were analyzed. The outcome was assessed through mortality, duration of mechanical ventilation, and length of hospital stay. Patients were ranked and compared according to gestation and type of RD. Descriptive and analytic statistical methods were used for analyzing the results.

Results: The study comprised a total of 65 late preterm newborns with RD, the majority of whom were male (n=44, 67.7%). Respiratory distress syndrome (RDS) (46.2%) and transient tachypnea of the newborn (TTN) (40%) were the most common causes of RD. TTN was most prevalent in neonates in the 36th gestational week, whether RDS was most present before 35th gestational week. The average length of hospital stay was 15 days and mortality in the group was 1.5%.

Conclusion: The most prevalent causes of RD in late preterm infants are RDS and TTN. Late preterm infants represent a vulnerable group of newborns, with a heightened risk of associated morbidity and mortality.

Key words: respiratory distress, late preterm, transient tachypnea of the newborn, respiratory distress syndrome

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INTRODUCTION

Late preterm newborns are born between 34 0/7 and 36 6/7 gestational weeks. They represent 70% of preterm born infants, with a frequency of around 8% of all newborns (1). Physical appearance of late preterm newborns is similar to term infants. However, due to insufficient physiological maturity and usually delayed postnatal adaptation, late preterm newborns are susceptible to specific diseases that can significantly affect the overall neonatal morbidity and mortality. Notably, approximately 50% of late preterm newborns require treatment in neonatal intensive care units (NICUs), and they experience high rates of readmissions, and three times higher mortality compared to full-term newborns (2).

The incidence of respiratory distress (RD) is significantly higher in late preterm than in term newborns and is inversely proportional to gestational age (2, 3). At 34 weeks of gestation, the lung volume is only 50% of the lung volume in a full-term newborn, and alveolar walls are around one-third thicker, contributing to reduced lung compliance (4). The most common causes of RD in late preterm infants include respiratory distress syndrome (RDS), transitory tachypnea of the newborn (TTN), pneumonia, idiopathic persistent pulmonary hypertension, aspiration syndrome, air-leak syndrome.

This research aims to identify the causes of RD in late preterm infants, analyze characteristics of RD in relation to gestational age, compare various clinical forms of RD, and determinate the factors that influence the adverse outcomes of late preterm infants with RD.

MATERIALS AND METHODS

This study is an observational and descriptive retrospective analysis conducted on late preterm newborns with RD who were admitted to the NICU of the Institute for Mother and Child Health Care of Serbia „Dr Vukan Cupic", over a two-year period. The Institute for Mother and Child Health Care of Serbia „Dr Vukan Cupic" is a pediatric hospital providing tertiary-level care in the NICU, but it does not contain a maternity department. The study group included newborns delivered between 34 0/7 and 36 6/7 gestational weeks with the diagnosis of RD. Gestational age was determined according to the mother's last menstrual period. The American College of Obstetricians and Gynecologists' (ACOG) definition of late preterm birth was used (5). The diagnosis of RD was made according to clinical, laboratory, and radiographical findings.

The following demographic and clinical characteristics were analyzed: gender, age, type of delivery, birth weight (BW), Apgar score (AS), small for gestational age (SGA), risk factors for preterm birth, and RD. Additionally, clinical symptoms and signs of RD were observed during hospital stay: type of respiratory pathology, need for supplemental oxygen, intubation and surfactant administration, treatment complications (air leak syndrome, persistent pulmonary hypertension), and co-morbidities. X-ray of the lungs and heart and echocardiography were used to estimate the presence of pulmonary hypertension. Treatment outcome was assessed through survival rate, duration of mechanical ventilation and the length of hospital stay. Patients were compared according to gestational age, and the type of RD.

Disease severity assessment

Disease severity was assessed according to the internationally used scores Score for Neonatal Acute Physiology II (SNAP II) and Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE II) (6).

SNAP II score assesses six parameters: mean arterial pressure, body temperature, the ratio of arterial oxygen partial pressure (PaO2) to fractional inspired oxygen (FiO2) (PaO2/FiO2 ratio), serum pH, multiple seizures, and urine output. Normal values are evaluated with zero points, while deviations are evaluated with a minimum of 5 and a maximum of 28 points. SNAPPE II score consists of three additional perinatal variables recognized as important predictors of newborn survival: BW, AS, and SGA. Normal values are evaluated with zero points, whereas alterations are evaluated with 10 to 18 points.

Statistical analysis

Statistical analysis was done using the SPSS Statistics for Windows version 21.0 software program (IBM Corp., Armonk, NY, USA). Descriptive data are presented in the form of measures of central tendency (arithmetic mean, median), measures of variability (standard deviation, percentiles), and relative numbers (structure indicators). Student's t-test, Mann-Whitney U test, One Way ANOVA and Kruskal Wallis test were performed to determine differences between numerical data. Pearson's chi-squared test and Fisher's exact test were used to determine the significance between nominal variables. The association between risk factors and RDS and TTN was evaluated by univariate logistic regression. Differences at $p<0.05$ were considered to be statistically significant.

RESULTS

The study included 65 late preterm newborns with RD, and the majority of them were male (n=44, 67.7%). In order to assess their characteristics, type of RD and therapy strategy, newborns were divided into three groups according to gestational week: 34 – 34+6 (group I), 35 – 35+6 (group II) and 36 – 36+6 (group III) **(Table 1)**. BW was significantly different between groups, with low-

Table 1. Characteristics of the study participants according to the gestational week.

	All N=65 N (%)	Group I 34–34+6 GW N=16 N (%)	Group II 35–35+6 GW N=14 N (%)	Group III 36–36+6 GW N=35 N (%)	p
BW	2815.8 ±399.5	2533.7 ±379.0	2770.0 ±209.8	2963.1 ±399.4	**0.001**
BW<10 percentile	2 (3.1)	1 (6.3)	0 (0)	1 (2.9)	0.609
BW>90 percentile	7 (10.8)	1 (6.3)	0(0)	6 (17.1)	0.413
Twins	6 (9.2)	2 (12.5)	1 (7.1)	3 (8.6)	0.863
Cesarean section	31 (47.7)	8 (50)	7 (50)	16 (45.7)	0.942
Maternal morbidity	24 (36.9)	8 (50)	4 (28.6)	12 (34.3)	0.428
PPROM	3 (4.6)	2 (12.5)	1 (7.1)	0 (0)	0.125
Antenatal corticosteroids	6 (9.2)	2 (12.5)	4 (28.6)	0 (0)	**0.007**
AS 1 minute	8 (7-9)	8 (6-8)	8.5 (8-9)	9 (7-9)	**0.040**
AS 5 minute	9 (8-10)	8 (6-9)	9 (8-9.5)	9 (8-10)	**0.054**
AS 5 ≤ 7	14 (21.5)	4 (25.0)	3 (21.4)	7 (20.0)	0.922
Resuscitation in delivery room	7 (10.8)	2 (12.5)	2 (14.3)	3 (8.6)	0.816

GW – gestational week; BW – birth weight; PPROM – preterm premature rupture of the membranes; AS – Apgar score

Table 2. Types of the RD among study participants according to the gestational week.

	All N=65 N (%)	Group I 34–34+6 GW N=16 N (%)	Group II 35–35+6 GW N=14 N (%)	Group III 36–36+6 GW N=35 N (%)	p
RDS	30 (46.2)	13 (81.3)	8 (57.1)	9 (25.7)	**0.005**
TTN	26 (40)	2 (12.5)	5 (35.7)	19 (54.3)	
Others	9 (13.8)	1 (6.3)	1 (7.1)	7 (20.0)	

GW – gestational week; RDS – respiratory distress syndrome; TTN – transient tachypnea of the Newborn

est BW in the group born between 34 and 34+6 weeks (p=0.001). The use of antenatal corticosteroids was most frequently recorded in group II (p=0.007). AS in 1st and 5th minute were lowest in group I (p=0.040 and p=0.054, respectively) **(Table 1)**.

Causes of RD among all included participants were RDS (n=30, 46.2%), TTN (n=26, 40%), amniotic fluid aspiration (n=2, 3.1%), persistent pulmonary hypertension of the newborn (PPHN) (n=1, 1.5%), meconium aspiration syndrome (MAS) (n=2, 3,1%), and pneumothorax (n=4, 6,1%). Most common type of RD in the group born between 34 and 34+6 weeks was RD, and among infants born between 36 and 36+6 weeks it was TTN (p=0.005) **(Table 2)**. Univariant logistic regression showed 8,15-fold higher risk for RDS in infants born before 35 weeks. Newborns with BW below 2500 g had a 3,47-fold increased risk for developing RDS.

As for the therapy used, only surfactant administration was significantly different between study groups, commonly used in the group born between 34 and 34+6 gestational weeks (p=0.005). Pneumothorax occurrence was statistically most frequent in group II (p=0.013). PPHN frequency was similar across groups (p=0.258) **(Table 3)**.

Table 3. Therapeutic procedures and their duration across groups according to the gestational week.

	All N=65 N (%)	Group I 34–34+6 GW N=16 N (%)	Group II 35–35+6 GW N=14 N (%)	Group III 36–36+6 GW N=35 N (%)	p
Surfactant	12 (18.5)	17 (43.8)	3 (21.4)	2 (5.7)	**0.005**
MV	37 (56.9)	9 (56.3)	11 (78.6)	17 (48.2)	0.159
MV duration	7 (3-8)	8 (2-12)	7 (4-8)	5 (2-7)	0.144
O2 duration	3 (2-5)	4 (2-6)	3.5 (2.5-5)	3 (2-5)	0.603
Pneumothorax	11 (16.9)	2 (12.5)	6 (42.9)	3 (8.6)	**0.013**
PPHN	13 (20)	1 (6.3)	4 (28.6)	8 (22.9)	0.258

GW – gestational week; MV – mechanical ventilation; PPHN - persistent pulmonary hypertension of the newbor

Table 4. Therapeutic procedures and their duration according to the type of RD

	RDS N=30		TTN N=29		Others N=9		P
	N	%	N	%	N	%	
Surfactant	11	36.7	0	0	1	11.1	**0.002**
MV	23	76.7	7	26.9	7	77.8	**<0.001**
MV duration	8	2-12	7	4-8	5	2-7	0.144
NIV	14	46.7	3	11.5	3	33.3	**0.017**
O2	28	93.2	25	96.2	9	100	0.685
O2 duration	4	2-6	3.5	2.5-5	3	2-5	0.603
iNO	3	10	0	0	1	11.1	0.24

RDS – respiratory distress syndrome; TTN - transient tachypnea of the newborn; MV – mechanical ventilation; NIV – noninvasive ventilation; O2 – oxygen; iNO – inhalational nitric oxide

Furthermore, the investigation of therapy modalities and their duration among different types of RD showed a statistically significant difference among groups for surfactant administration, with its most often use among newborns with RDS (p=0.002). The need for invasive mechanical ventilation and noninvasive ventilation support was significantly less frequent in newborns with TTN in comparison to other types of RD (p<0.001 and p=0.017, respectively) **(Table 4)**.

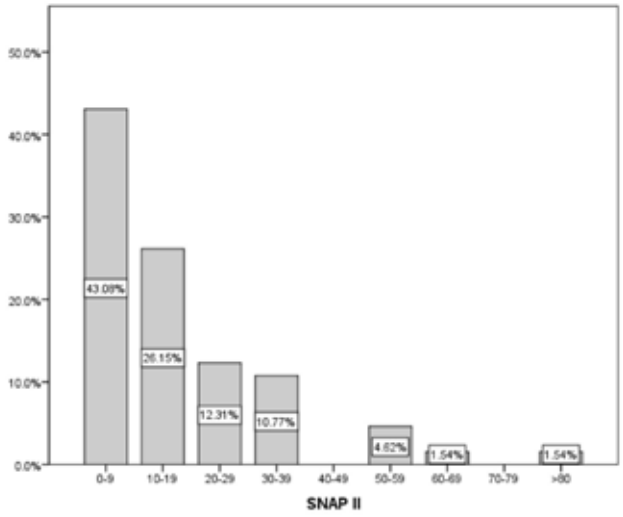

Figure 1. Distribution of investigated near-term newborns according to the SNAP II score.

Our results showed that 28 of our study patients (43,08%) had SNAP II score between 0 and 9, while 5 of them (7,7%) had a score with high mortality risk **(Figure 1)**. According to the SNAPPE II existing tables for calculating the mortality risk for the category of newborns with body weight > 1500g, 89,2% had risk below 5,2% (score between 0 and 39), while 10,8% had mortality risk between 15,4 and 66,7% (score 40 and higher) **(Figure 2)**.

Group I had the longest hospital stay, which was statistically significant (p=0.015). Of the late preterm infants, only one patient born between 35 and 35+6 gestational weeks experienced the fatal outcome. In terms of the SNAP II score, Group II had the highest score, although the differences between the groups were not statistically significant (p=0.055). The SNAPPE II scores did not show significant differences between the groups (p=0.121) **(Table 5)**.

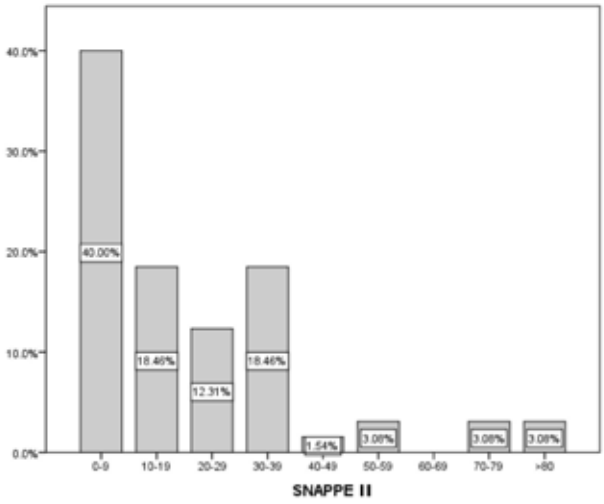

Figure 2. Distribution of investigated near-term newborns according to the SNAPPE II score

Regarding the type of RD, patients with RDS had the longest hospital stay , which was statistically significant (p=0.015). Additionally, SNAP II score was significantly higher in the group diagnosed with TTN compared to other types of RD (p=0.055) **(Table 6)**.

DISCUSSION

Prematurity represents a leading cause of morbidity and mortality in the neonatal period worldwide. The incidence of premature births shows an increasing trend despite the efforts made during past years (7). The rate of preterm birth increased between 1981. and 2003. by 31% (from 9,1% to 12,3%). It is largely influenced by an increased number of births in near-term gestation, which rose by 40% in the mentioned period (8). Data from the year 2010 showed that among live singleton births, late preterm births ranged from 3.0% to 6.0% and constituted between 65% and 75% of preterm births in high-income

Table 5. Length of hospital stay, fatal outcome, SNAP II and SNAPPE II scores in RD patients according to the gestational week.

	All N=65 N (%)	Group I 34–34+6 GW N=16 N (%)	Group II 35–35+6 GW N=14 N (%)	Group III 36–36+6 GW N=35 N (%)	p
Length of hospital stay	15 (10-22)	21.5 (13-35)	17 (11-24)	12 (9-18)	0.015
Fatal outcome	1(1.5)	0(0)	1(1.5)	0(0)	NA
SNAP II	12 (5-24)	16 (8.5-26)	19.5 (5-32)	5 (5-16)	0.055
SNAPPE II	16 (5-30)	20 (8.5-35.5)	23 (5-34)	10 (5-26)	0.121

GW – gestational week; SNAP II - Score for Neonatal Acute Physiology II; SNAPPE II - Score for Neonatal Acute Physiology Perinatal Extension II

countries (9, 10). In Serbia, the frequency of preterm newborns increased from 5.7% to 6.7% between 2009 and 2014, along with an increase in near-term births from 3,9% to 4,7 % (17% increase) (11).

In our study, the majority of the included newborns were male (67.7%), which is in correlation with the findings of a higher morbidity and mortality rate among late preterm males in comparison to females (12). BW was significantly different between the groups according to gestational age, with the lowest BW in the group born between 34 and 34+6 weeks. BW below 10 percentiles was found in only 2 out of 65 patients. Low BW as a consequence of intrauterine growth restriction (IUGR) might represent an indication for delivery between 34 and 36+6 weeks (13). Gilbert et al. described lower RDS incidence in infants with IUGR born before 30 gestational weeks, while in late preterm gestation this incidence was significantly higher (14). Our results showed most frequent use of antenatal corticosteroids in the group born between 34 and 34+6 weeks. Only 9.2% of all the mothers got this therapy. Such low incidence of mothers prenatally treated with corticosteroids might reflect its insufficient protective effect for severe RDS development. Antenatal administration of corticosteroids in mothers with expected preterm birth has been reported to increase the survival rate, decrease the risk of RDS and the use of mechanical ventilation, necrotizing enterocolitis, and intraventricular hemorrhage (15). Prenatal corticosteroid therapy is recommended in all pregnant women with the risk of birth before 34 weeks (16). Furthermore, corticosteroid administration between 34 and 36 weeks lowers the risk of respiratory morbidity, but not the risk of mortality (15). AS lower than 7 in the 5th minute was found in 21,5% of included newborns, while 10,8% showed the need for resuscitation. AS in the 1st and the 5th minute were lowest in the group born between 34 and 34+6 gestational weeks. Low AS increases the frequency of respiratory disorders in late preterm newborns. The incidence of perinatal asphyxia characterized as AS lower than 7 in the 5th minute is 3-fold higher in late preterm neonates than in term neonates (17).

Most frequent causes of RD in near-term infants included in this study were RDS and TTN. Other less common causes were also recorded, such as amniotic fluid aspiration, PPHN, MAS, and pneumothorax. The most common type of RD in the group born between 34 and 34+6 weeks was RDS, and in the group born between 35 and 35+6 weeks was TTN. According to the results of univariate logistic regression, newborns below 35 gestational weeks had an 8,15-fold increased risk of RDS occurrence. Additionally, near-term infants with low BW (below 2500 g) had a 3,47 times higher risk of developing RDS. In a study conducted by Rubaltelli et al. (18), the incidence of RD in late preterm infants was reported to be approximately 30%, while in term newborns the incidence was only 1%. Another study demonstrated that RDS was the most common type of RD in newborns between 34 and 34+6 weeks, with the occurrence rate of around 10% (17). A study from 2018 showed that approximately 9% of late preterm infants developed RDS or TTN (19). This frequency decreases with gestation, showing the rate of 0.3% in term newborns at 40 weeks (17). One large observational study of 19000 preterm and 166000 term newborns showed a decrease in the TTN incidence with an increasing gestational age, resulting in

Table 6. Length of hospital stay, fatal outcome, SNAP II and SNAPPE II scores in different types of RD.

	RDS N=30		TTN N=29		Others N=9		p
	N	%	N	%	N	%	
Fatal outcome	1	3.3	0	0	0	0	NA
Length of hospital stay	21.5	13-35	17	11-24	12	9-18	**0.015**
SNAP II	16	8.5-26	19.5	5-32	5	5-16	**0.055**
SNAPPE II	20	8.5-35.5	23	5-34	10	5-26	0.121

RDS – respiratory distress syndrome; TTN – transient tachypnea of the newborn; SNAP II - Score for Neonatal Acute Physiology II; SNAPPE II - Score for Neonatal Acute Physiology Perinatal Extension II

its frequency of 6,4% in neonates born at 34 weeks, 2,5% in those born at 36 weeks, and only 0,3% in term infants (17). This could be due to impairment of hormonal stimuli that accompany a normal term delivery, and which is responsible for establishing successful pulmonary transition by activating biochemical processes responsible for absorption of lung fluid from the alveoli.

In our research, we observed that among the complications of RD treatment, PPHN occurred in 16.9% of cases, while pneumothorax was present in 20% of cases.

Patients included in this study had severe types of RD and needed hospitalization in tertiary center, which accounts for a high incidence of complications. More than half of the study patients had a need for mechanical ventilation, with a mean duration of 7 days, 30% were on noninvasive ventilation, 18,5% of newborns received surfactant, and 6.2% were treated with iNO. Other studies showed inconsistent results regarding therapy use in near-term newborns with RD (20). One investigation showed an incidence of ventilatory support of 6.6% in 34 weeks and 3% in 36 weeks, and surfactant administration depended of gestational age (2,2 – 7,4%) (17). Oppositely, Shaikh et al. (21) described the need for MV in 78,5% of infants born at 34 weeks, while it decreased to 15% at 36 weeks.

The average duration of hospital stay in our study was 15 days. Comorbidities of our patients, such as jaundice, feeding difficulties, infections, could have been the reason for a longer hospital stay. The frequency of readmission is significantly higher in late preterm than in term infants, showing an incidence of 6,3% in newborns between 35 and 36 gestational weeks and 2,4% after 40 weeks (22). An earlier study showed that mean hospital length stay for infants in NICU with RD was 12 days for those born at 34 weeks, 8 days for 35 weeks and 6 days for neonates born at 36 weeks (17).

Only one patient had the lethal outcome, which resulted in mortality rate of 1,5% for our study group. Mortality is found to be 4,6-fold higher in near-term than in term neonates (23). The analysis of late preterm infants with RD found the mortality rate of 0,8% at 34 weeks, 0,4% at 35 weeks, and only 0,1% at 40 gestational weeks (17).

We observed disease severity through the calculation of SNAP II and SNAPPE II scores. More than 40% of our study patients had SNAP II score between 0 and 9, and less than 8% had a score with high mortality risk. According to the SNAPPE II existing tables for calculating the mortality risk for the category of newborns with body weight > 1500g, 89,2% of our patients had a risk below 5,2% (score between 0 and 39), while 10,8% had mortality risk between 15,4 and 66,7% (score 40 and higher).

Our study results showed the highest frequency of pneumothorax in the group born between 35 and 35+6 gestational weeks. Regardless RD type, one research found pneumothorax to be one of the most frequent complications of both the disease and its treatment (24). Oppositely, some earlier investigations failed to detect an increased incidence of pneumothorax in newborns aged 34 to 36+6 weeks (17).

CONCLUSIONS

Based on the results presented in this study, we can draw certain conclusions.

The most common types of RD in late preterm infants are RDS and TTN. Among the infants included in our study, the mortality rate was 1,5%. However, mortality risk in infants with high SNAPPE scores exceeded 15%.

RDS is most frequently observed in infants born before 35 weeks of gestation and with low BW. Late preterm infants with RDS often require surfactant administration and experience longer hospital stays compared to other types of RD.

TTN typically requires less frequent use of invasive and noninvasive mechanical ventilation. It also carries a lower risk of complications and comorbidities.

Late preterm newborns with the highest incidence of TTN are born between 36 and 36+6 weeks of gestation. Furthermore, their likelihood of experiencing complications and comorbidities decreases as their gestational age increases.

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UZROCI I ISHODI LEČENJA RESPIRATORNOG DISTRESA KOD NOVOROĐENČADI KASNE PRETERMINSKE GESTACIJE - ISKUSTVO TERCIJARNOG NEONATALNOG CENTRA

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Sažetak

Uvod/Cilj: Respiratorni distres (RD) je najčešći uzročnik morbiditeta kod preterminske novorođenčadi. Cilj ove studije je utvrđivanje najčešćih uzroka RD-a kod kasne preterminske novorođenčadi, analiza karakteristika RD-a u odnosu na nedelju gestacije i utvrđivanje faktora koji utiču na nepovoljan ishod kasne preterminske novorođenčadi sa RD-om.

Metode: Ovom studijom su obuhvaćena novorođenčad gestacijske starosti 34 0/7 do 36 6/7 nedelja sa RD-om, hospitalizovana u Institutu za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić". Analizirane su demografske i kliničke karakteristike, klinički znaci i tok bolesti, pojava komplikacija, kao i kvantifikovanje težine bolesti pomoću neonatalnih skorova. Ishod lečenja je praćen kroz preživljavanje, trajanje mehaničke ventilacije i dužinu hospitalizacije. Pacijenti su poređeni prema gestaciji, kao i prema vrsti oboljenja. Za obradu podataka korišćene su metode deskripitivne i analitičke statistike.

Rezultati: Studijom je obuhvaćeno 65 ispitanika, od kojih je većina muškog pola. Najčešći uzroci RD-a bili su respiratorni distres sindrom (RDS) (46,2%) i tranzitorna tahipneja novorođenčeta (TTN) (40%). TTN je bila najzastupljenija kod novorođenčadi u 36. nedelji gestacije, a RDS ispod 35. nedelje gestacije. Novorođančad su prosečno bila hospitalizovana 15 dana, a mortalitet je iznosio 1,5%.

Zaključak: Najčešći uzroci RD-a kod kasne preterminske novorođenčadi su RDS i TTN. Ovo je osetljiva kategorija novorođenčadi sa visokim rizikom za teške komplikacije, udruženi morbiditet i smrtni ishod.

Ključne reči: respiratorni distres, predterminsko novorođenče, tranzitorna tahipneja, respiratorni distres sindrom

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ORIGINAL ARTICLE

The association of myasthenia gravis and immune-mediated myopathies

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Summary

Introduction/Aim: Myasthenia gravis (MG) is a chronic autoimmune disease of the neuromuscular junction, characterized by muscle weakness and fatigability. Idiopathic inflammatory myopathies (IIM) are an immune-mediated group of diseases characterized by progressive painful proximal weakness of the extremities. The coexistence of these two diseases is extremely rare and so far, only about fifty cases have been reported worldwide. The aim of this study was to analyze the frequency of coexistence of IIM and patients with *de novo* MG.

Material and Methods: The study is retrospective in nature and was conducted at the "Neurology Clinic", University Clinical Center of Serbia. It included 97 patients diagnosed with myasthenia gravis between January 1, 2014 and December 31, 2018.

Results: The average age of the MG patients was 54.1±18.9 years. At the time of diagnosis, 19 (19.6%) participants had at least one of the anamnestic data observed as potential indicators for the existence of immune-mediated myopathy. Finally, one patient clinically presented with generalized seropositive (anti-AchR positive) myasthenia gravis associated with the diagnosis of antisynthetase syndrome. In addition, the main characteristics of patients with combined occurrence of *de novo* MG and antisynthetase syndrome are presented.

Conclusion: Although the simultaneous occurrence of MG and IIM is a very rare phenomenon, we need to think about the possibility of combined occurrence of these two autoimmune diseases, with the aim of early recognition and adequate treatment, and thus a better prognosis of both diseases.

Keywords: Myasthenia gravis, inflammatory myopathy, antisynthetase syndrome, coexistence

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INTRODUCTION

Myasthenia gravis (MG) belongs to the group of autoimmune diseases in which antibodies directed against different postsynaptic membrane antigens lead to neuromuscular transmission impairment (1). About 80% of MG patients have antibodies directed against the nicotinic acetylcholine receptor (AchR), while 40% of initially seronegative patients have antibodies directed against muscle-specific tyrosine kinase (MuSK) (2). This rare disease is clinically characterized by variable weakness and excessive fatiguability of various skeletal muscles, especially after repeated or prolonged muscle activity, but also by an improvement in strength after rest or after the administration of anticholinesterase medication (3). In 15% of patients, the disease presents solely with eye symptoms, while in more than two-thirds of the patients, generalized weakness of facial muscles, bulbar musculature, limb muscles, and sometimes also respiratory musculature weakness are observed (4). On the other hand, idiopathic inflammatory myopathies (IIM) belong to the group of acquired, immune-mediated muscle diseases that are clinically manifested by progressive and often painful muscle weakness, predominantly of the proximal musculature of the extremities (5). A significantly elevated serum muscle enzyme creatine kinase (CK) value is a typical laboratory finding in these patients (6). Other systemic manifestations often occur in these patients, such as interstitial lung disease, arthritis, arthralgia, Raynaud's phenomenon, and various skin changes (7). Myositis-specific and myositis-associated autoantibodies can be detected in the serum taken from such patients (7).

Although both disorders are considered part of the "autoimmune spectrum of neuromuscular diseases", they are clinically, electrophysiologically, and pathophysiologically different entities. Thus, their co-occurrence is extremely rare and so far, only around 50 cases have been described worldwide, mostly in the form of case reports and small case series (7–9). However, none of the described cases belonged to this part of Europe.

Thus, the aim of our study was to analyze the frequency of signs and symptoms of IIM in a large cohort of MG patients, as well as the specificity of the clinical characteristics of these patients.

MATERIAL AND METHODS

Subjects

The study included 97 patients who were diagnosed with myasthenia gravis in the period between January 1, 2014 and December 31, 2018 at the Clinic for Neurology, University Clinical Centre of Serbia (UCCS). The diagnosis of myasthenia gravis was established in all patients based on the typical clinical presentation (in the form of weakness and pathological fatigue of various skeletal muscles), positive pharmacological test (positive neostigmine test), and/or decremental response to repetitive nerve stimulation (RNS) and/or positive findings of specific antibodies (AchR or MuSK) (8). The presence of signs and symptoms of idiopathic inflammatory myopathy in MG patients (those with elevated serum levels of creatine kinase (CK) and/or lactate dehydrogenase (LDH) and/or aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT)), was assessed according to EULAR /ACR criteria (European League Against Rheumatism/American College of Rheumatology) (10). The diagnosis of IIM was further confirmed using the Web calculator for IIM of the Department of Biostatistics, Karolinska Institute, Stockholm, Sweden (9). Also, the presence of current electrophysiological criteria for the IIM diagnosis was analyzed in all MG patients (10). All patients signed informed consent to participate in the study and the study was approved by the Ethical Board of the Neurology Clinic, University Clinical Centre of Serbia and performed in compliance with the Declaration of Helsinki. In order to strengthen the certainty of the established diagnosis, data were collected at two time-points (at the moment of diagnosis and during the follow-up visit six months later). Patients who did not have a follow-up outpatient examination after six months, as well as patients who were already treated with corticosteroid and other immunosuppressive therapy (azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil, and rituximab) at the time of MG diagnosis (non-drug naïve), were excluded from this study.

Methods

Sociodemographic and diagnostic data were collected from patients and their medical records, both at the time of initial testing and retesting. The existence of provoking or precipitating factors (stress, infection, pregnancy, surgery, and malignancy), and the presence and treatment of idiopathic hyperlipidaemia (statins and/or fibrates) were taken into consideration. Other therapeutic modalities and significant comorbidities (including other autoimmune diseases) were also noted. Disease severity was evaluated in accordance with the *Myasthenia Gravis Foundation of America* (MGFA) clinical classification at both time points. MGFA clinical classification divides MG presentations into different classes by clinical features with increasing disease severity. There are 5 main classes and several subclasses. The MGFA classifies MG forms as pure ocular (class I), mild generalized (class II), moderate generalized (class III), severe generalized (class IV), and MG requiring intubation/myasthenic crisis (class V) (11). The term "improvement" or "deterioration" of the MGFA score was defined as a change greater than or equal to one degree according to the MGFA classification. For patients in whom the change in MGFA score did not meet the criteria for change, the condition

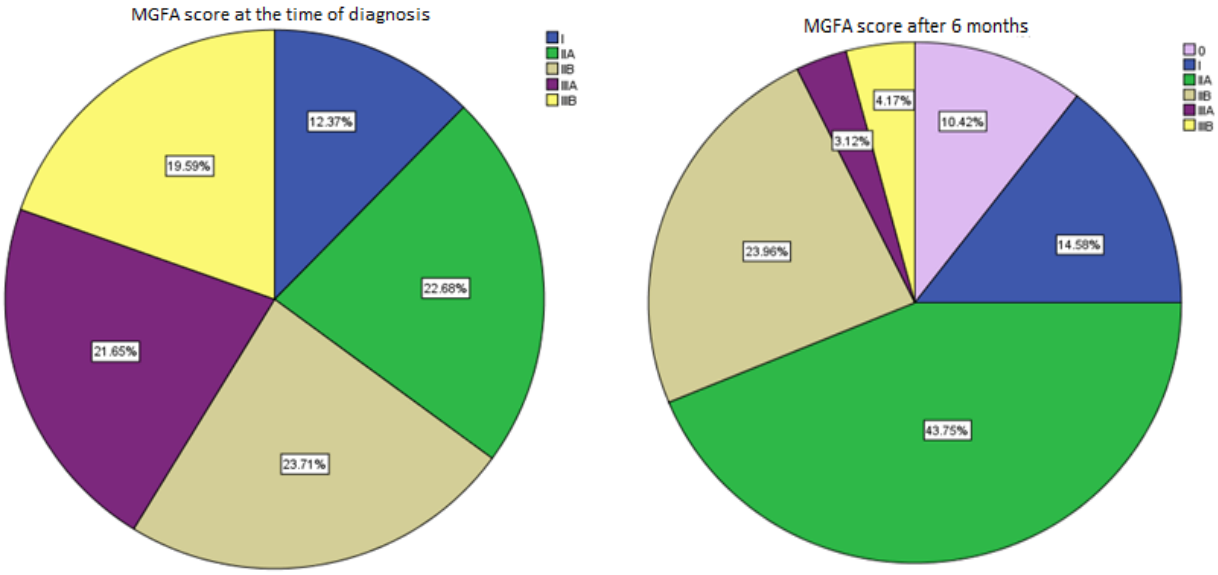


Figure 1. The frequency distribution of patients according to MGFA score at the moment of diagnosis and after 6 months (n= 97)

was considered "unchanged". Patients were thoroughly neurologically examined using quantitative scores to assess muscle weakness and fatigue: *The Quantitative Myasthenia Gravis Score* (QMGS), and the *Medical Research Council-Sum Scale* (MRC-SS) (12, 13).

The following laboratory parameters were analyzed in all subjects: complete blood count and biochemical parameters, including the values of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), thyroid-stimulating hormone (TSH), free thyroxine fraction (fT4), anti-thyroglobulin antibodies (anti-Tg), thyroid peroxidase antibodies (anti-TPO), antinuclear antibodies (ANA) and antineutrophil cytoplasmic antibodies (ANCA). Patients were also tested for the presence of myositis-specific antibodies (Jo-1 (histidyl-transfer RNA synthetase), SRP (signal recognition particle), Synthetase, Ku, Mi-2 (NuRD subunit), and Ro 52 (anti-SSA 52 - anti–Sjögren's-syndrome-related antigen A) antibodies), as well as anti-AchR and anti-MuSK antibodies specific for MG. In addition, as part of the diagnostic protocol, RNS tests, electromyographic (EMG) evaluation, and computed tomography of the chest (chest CT examination) were performed in all patients.

Statistical analysis

Nominal and numeric data were tested using descriptive statistics methods. The assumption of data normality was tested with the Shapiro-Wilks test. The IBM SPSS program (The Statistical Package for the Social Sciences, version: SPSS v22) was used for statistical analyses.

RESULTS

A total number of 97 patients with confirmed diagnosis of *de novo* MG were included in our study. The average age of our patients was 54.11±18.98 years, of which 51 (52.6%) were females. The main socioepidemiological features of our MG patients are presented in **Table 1**.

Table 1. Main socioepidemiological characteristics of our patients with MG (n=97)

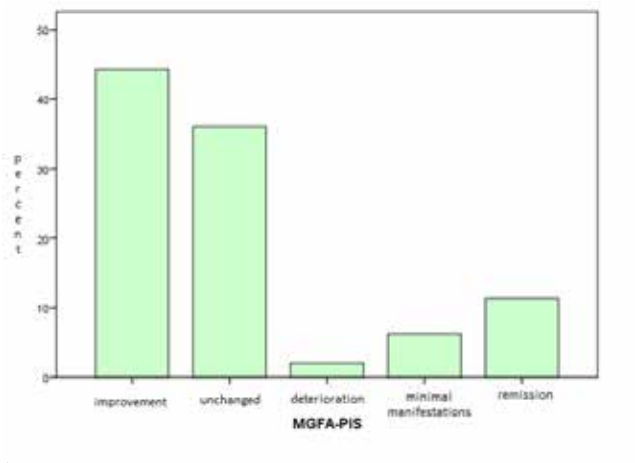
Patient characteristics	
Males (n (%))	46 (47.4%)
Females (n (%))	51 (42.6%)
Age of onset (year, mean ± SD)	54.11±18.98
Disease duration > 1 year (n (%))	54 (56.7%)

The ocular form of MG was noted in 11.3% of patients, while the remaining part of the cohort had the generalized form of the disease. The stages of MG according to the MGFA classification are shown in **Figure 1**.

In 40 (41.2%) patients, there was no change in the MGFA score during the diagnostic follow-up. The distribution of disease outcomes assessed according to MGFA Post-intervention Status (MGFA-PIS) is shown in **Figure 2**.

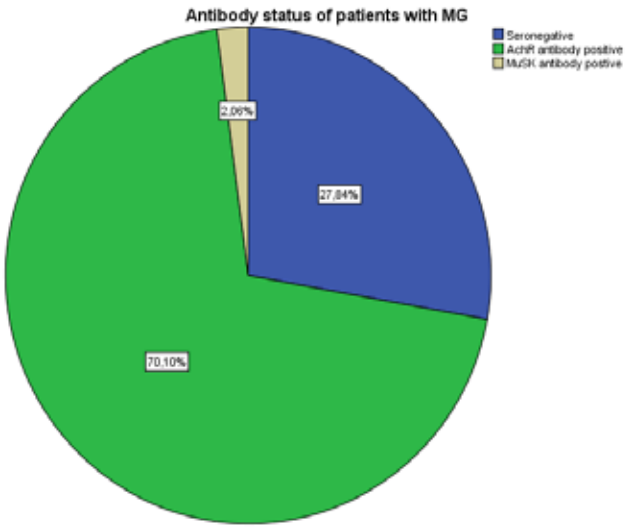
No patient had a primary manifestation of myasthenia gravis in the form of a myasthenic crisis. **Table 2** and **Table 3** show the clinical presentation and neurological status of patients with myasthenia gravis.

As part of the diagnostic workup, the presence of AchR antibodies was observed in 68 patients (70.1%), while MuSK antibodies were detected in two patients (2.1%). Tests for the presence of other rare antibodies (seronegative MG, SN-MG) in MG were not performed. The antibody status of our patients with MG is shown in **Figure 3**.



*MGFA-PIS- MGFA Post-intervention Status

Figure 2. The frequency distribution of patients according to the change in MGFA score according to MGFA-PIS (n= 97)



AchR - acetylcholine receptor; MuSK - muscle-specific tyrosine kinase

Figure 3. Antibody status of our patients with myasthenia gravis (MG).

Table 4 shows the main laboratory and electrophysiological data of our MG patients.

Of all analyzed MG patients, at the time of diagnosis, 19 (19.6%) patients had at least one anamnestic data which was considered a potential indicator for the existence of immune-mediated myopathy (pain in muscles and joints, joint swelling, different skin changes, fever, and other autoimmune diseases). One patient was found to have significantly elevated serum creatine kinase value (value typically observed in IIM) at the time of diagnosis of MG. After entering the available data into the IIM calculator, the patient was classified as a case of "probable idiopathic inflammatory myopathy" with an estimated probability of 62-99% (min-max).

In the remaining 96 patients in whom the diagnosis of myasthenia gravis was confirmed, the existence of any subtype of idiopathic inflammatory myopathies was not noted.

All patients were treated with adequate symptomatic and/or immunosuppressive therapy. Therapeutic modalities used to treat our patients with MG are shown in **Table 5**. Most of our patients (97%) were treated with acetylcholinesterase inhibitors, 92.8% with corticosteroid therapy, 45.4% with azathioprine, and 8.2% with cyclosporine A. The second-line therapy was also applied in a smaller percentage of patients – therapeutic plasma

Table 2. Main clinical characteristics of MG patients (n= 97)

History	**First examination** (n (%))	**Examination after 6 months** (n (%))
Drooping eyelids*	77 (79.4%)	54 (55.7%)
Double image*	26 (26.8%)	5 (5.2%)
Difficulty speaking*	51 (52.6%)	18 (18.6%)
Difficulty chewing*	57 (58.8%)	33 (34%)
Difficulty swallowing*	39 (40.2%)	20 (20.6%)
Neck weakness*	44 (45.4%)	18 (18.6%)
Heavy breathing*	2 (2.1%)	1 (1%)
Upper extremity pain	7 (7.2%)	7 (7.2%)
Arm weakness	49 (50.5%)	34 (35.1%)
Proximal arm weakness	45 (46.4%)	29 (29.9%)
Distal arm weakness	32 (33%)	19 (19.6%)
Lower extremity pain	9 (9.3%)	12 (12.2%)
Leg weakness	41 (42.3%)	32 (33%)
Proximal leg weakness	39 (40.2%)	30 (30.9%)
Distal leg weakness	28 (28.9%)	14 (14.4%)
Significant fatigue*	10 (10.3%)	10 (10.3%)
Autoimmune diseases	8 (8.2%)	8 (8.2%)
Other comorbidities	51 (52.6%)	46 (47.4%)
Hyperlipidemia	8 (8.2%)	3 (3.1%)

*Symptoms mainly characteristic of myasthenia gravis;

Table 3. Main neurological findings of our patients with MG (n= 97)

Clinical findings	First examination (n (%))	Examination after six months (n (%))
Ptosis	77 (79.4%)	53 (54.6%)
Double vision	26 (26.8%)	5 (5.2%)
Masticatory muscles weakness	57 (58.8%)	34 (35.1%)
Masticatory muscles fatigue	57 (58.8%)	34 (35.1%)
Mimic muscles weakness	67 (69.1%)	48 (49.5%)
Mimic muscles fatigue	67 (69.1%)	47 (48.5%)
Soft palate weakness	45 (46.4%)	19 (19.6%)
Soft palate fatigue	44 (45.4%)	19 (19.6%)
Tongue weakness	38 (39.2%)	14 (14.4%)
Tongue fatigue	38 (39.2%)	14 (14.4%)
Neck anteflexion weakness	36 (37.1%)	18 (18.6%)
Neck anteflexion fatigue	35 (36.1%)	18 (18.6%)
Neck retroflexion weakness	35 (36.1%)	11 (11.3%)
Neck retroflexion fatigue	35 (36.1%)	11 (11.3%)
Arm weakness	49 (50.5%)	35 (36.1%)
Proximal arm weakness	45 (46.4%)	29 (29.9%)
Distal arm weakness	32 (33%)	18 (18.6%)
Arm fatigue	50 (51.5%)	39 (40.2%)
Proximal arm weakness	47 (48.5%)	33 (34%)
Distal arm weakness	32 (33%)	21 (21.6%)
Leg weakness	41 (42.3%)	32 (33%)
Proximal leg weakness	39 (40.2%)	30 (30.9%)
Distal leg weakness	28 (28.9%)	14 (14.4%)
Leg fatigue	41 (42.3%)	34 (35.1%)
Proximal leg fatigue	39 (40.2%)	33 (34%)
Distal leg fatigue	28 (28.9%)	17 (17.5%)

exchange (PLEx) was applied in 18 (18.6%) patients and intravenous immunoglobulins (IVIg) in two (2.1%) patients. As mentioned above, no patient included in this study was treated with immunosuppressive or immunomodulatory therapy before the onset of MG.

A brief presentation of the case

A 71-year-old patient, previously treated solely for the diagnosis of essential arterial hypertension, clinically presented with generalized seropositive (anti-AchR positive) myasthenia gravis. The spectrum of neuromuscular complaints in our patient was comprised of predominantly proximal muscle weakness of the upper and lower extremities and a slight difficulty swallowing, which was accompanied by bilateral semi-ptosis, with clear fatigability (MGFA IIIA). Laboratory analyses showed the presence of elevated creatine kinase (8,071 U/l), serum potassium levels (5.5 mmol/l) and LDH (590 U/L), thrombocytopenia (68 10^9/L), and increased erythrocyte sedimentation (24 mm/h). After RNS conduction, the patient fulfilled the electrophysiological criteria for the presence of MG (consistent decrement of 13%). As part of the

Table 4. Main laboratory and electrophysiological parameters of MG patients at the time of diagnosis and the follow-up examination (n= 97)

Laboratory findings	First examination (n (%))	Examination after 6 months (n (%))
Decreased RBC	6 (6.2%)	-
Elevated WBC	23 (23.7%)	-
Decreased WBC	1 (1%)	-
Decreased PLT	3 (3.1%)	-
Decreased HGB	4 (4.1%)	-
Elevated ESR	26 (26.8%)	-
Elevated CRP	14 (14.4%)	-
Elevated fibrinogen	1 (1%)	-
Elevated K^+	1 (1%)	-
Elevated CK	3 (3.1%)	-
Elevated LDH	9 (9.1%)	-
Elevated AST	2 (2.1%)	0 (0%)
Elevated ALT	10 (10.3%)	2 (2.1%)
Elevated GGT	3 (3.1%)	4 (4.1%)
Elevated fT4	5 (5.2%)	0 (0%)
Decreased fT4	17 (17.5%)	0 (0%)
Elevated TSH	5 (5.2%)	2 (2.1%)
Elevated Anti-TPO antibodies	7 (7.2%)	2 (2.1%)
Elevated anti-Tg antibodies	7 (7.2%)	1 (1%)
Elevated ANA	9 (9.3%)	4 (4.1%)
Elevated ANCA	2 (2.1%)	1 (1%)
Positive myositis profile	1 (1%)	1 (1%)
Positive Anti-AchR antibodies	68 (70.1%)	-
Positive Anti-MuSK antibodies	2 (2.1%)	-
RNS test	62 (63.9%)	-

AchR- acetylcholine receptor; ALT- alanine transaminase; AST- aspartate transferase; ANA- antinuclear antibodies; ANCA- antineutrophil cytoplasmic antibodies; CK- creatine kinase; CRP- c-reactive protein; ESR- erythrocyte sedimentation rate; fT4- free thyroxine; GGT- gamma-glutamyl transferase; HGB- haemoglobin; K^+-kalium; LDH- lactate dehydrogenase; MuSK- muscle-specific tyrosine kinase; PLT- platelet count; RBC – red blood cell count; RNS- repetitive nerve stimulation; TSH- thyroid stimulating hormone; TPO- thyroid peroxidase; Tg- thyroglobulin; WBC – white blood cell count.

performed immunoserological analyses, the presence of anti-Jo-1 antibodies was observed, and the patient was diagnosed with the co-occurrence of myasthenia gravis and antisynthetase syndrome (symmetrical proximal and painful weakness of the arms and legs, elevated serum CK and LDH values, elevated erythrocyte sedimentation rate (ESR), positive myositis panel). The patient was treated with anticholinesterase and corticosteroid therapy according to therapeutic protocols for both MG and antisynthetase syndrome, respectively. The control neurological examination verified the improvement of

Table 5. Therapeutic modalities used to treat our patients with MG

Symptomatic and immunosuppressive therapy	Percent of treated cases (%)
First-Line treatment options	
Acetylcholinesterase inhibitors	97%
Steroids	92.8%
Azathioprine	45.4%
Cyclosporine A	8.2%
Second-Line treatment options	
Therapeutic plasma exchange	18.6%
Intravenous immunoglobulins	2.1%

the neurological findings (MGFA IIA), with persistence of mild but not painful weakness and fatigue of the mimic muscles, as well as the normalization of the laboratory biochemical parameters.

DISCUSSION

Similar clinical presentations of both diseases are frequently the reason for a diagnostic delay, which is important from the therapeutic point of view, bearing in mind the frequent need for more aggressive immunosuppressive therapy in these patients (7). In the broadest sense, MG and IIM can be considered part of the autoimmune neuromuscular disorder spectrum. The clinical presentation of both entities can be similar, with a range of possible overlapping symptoms and signs, which represents a significant diagnostic challenge. Studies have shown that patients with IIM, unlike patients with MG, usually do not have ocular symptoms, such as diplopia, ptosis, and/or ophthalmoparesis. However, bulbar involvement is often found in both diseases (8). Suspicion of the possible association of IIM with MG can also be raised when there is non-fatigable weakness or a continuous increase in CK before starting immunosuppressive therapy (7). Moreover, the possibility that prescribing immunosuppressive or immunomodulatory therapy in patients diagnosed with one of these two diseases could "mask" or modify the clinical manifestation of the other disease should not be ignored. The co-occurrence of these two chronic disorders has been found only in a small number of patients so far (14). Namely, the incidence of myasthenia gravis ranges from 1.7 to 30 per million individuals per year, while the average incidence of inflammatory myopathy is 5 per million individuals (15,16). Therefore, it is not surprising that the concomitant appearance of myasthenia gravis and inflammatory myopathies is extremely rare. In the most extensive publications to date, Garibaldi et al. and Huang et al. describe no more than 50 cases of coexistence of these entities (7, 8). In the largest observed Italian cohort of 441 MG patients, 2.9% of patients with MG and IIM were detected, of which 10 patients were diagnosed with both entities simultaneously. Although in a smaller cohort of patients, the frequency of *de novo* coexistence was confirmed in 1.03% of patients in our study, which does not differ significantly from the data in the available literature. Furthermore, our cohort of MG patients did not differ from the aforementioned cohorts of MG patients in terms of their clinical and sociodemographic characteristics (7).

When the frequency of MG and individual forms of inflammatory myopathies were analyzed, it was observed that individual cases of overlap of MG with polymyositis (now anti-synthase syndrome), inclusion body myositis (IBM), autoimmune necrotizing myopathy, and dermatomyositis have been described so far (8, 17–19). Compared to the findings in our patient with a positive titer of anti-Jo-1 antibodies, only three case reports so far have reported a case of an antisynthetase syndrome associated with MG, of which only one had positive anti-Jo-1 antibodies. In the remaining two patients, the presence of anti-PL7 (anti-threonyl-tRNA synthetase) and anti-Ej (anti-glycyl-tRNA synthetase) antibodies was detected (20, 21). According to the available literature at the time of writing this paper, our results represent the first data on the coexistence of IIM and MG in the area of Southeastern Europe.

Our patient met all currently valid criteria for the diagnosis of MG. The predominance of pain and muscle weakness, and uncharacteristically slow response to therapy, were the reasons for further examination, which was also the most common reason for re-examining the diagnosis in the previous literature. Inflammatory myopathy was suspected based on significantly elevated serum CK values and then confirmed by a combination of clinical presentation and positive antibody findings. The finding of anti-Jo-1 antibodies in patients with idiopathic inflammatory myopathies is rare and ranges from 1-20% of all patients (22). On the other hand, CK elevation is considered the main laboratory indicator of myocyte damage, along with an increase in LDH and potassium, but it can be associated with other pathological conditions and is still a completely asymptomatic laboratory-isolated entity. In our patient, there were no associated symptoms and signs of other manifestations otherwise described in the literature (23, 24) which could be explained by the short duration of the disease in this case.

Certain authors report an association of the coexistence of IIM and MG with an increased frequency of malignancy, which was also not the case with our patient (25). Uchio et al. showed that the prevalence of thymoma in patients with IIM and MG was as high as 70%, compared to 10% in patients with MG (9). Some authors propose an explanation according to which the occurrence of MG with IIM is not a coincidence, but the association with thymoma may indicate the presence of complex pathogenetic mechanisms between these two autoimmune disorders. However, using chest CT examination we excluded any abnormalities of the thymus and signs of intestinal lung disease in our patient.

Spanish authors have described an MG patient with

similar sociodemographic and treatment characteristics compared to the data of our patient, but his anti-synthetase syndrome was complicated with the finding of alveolitis and other pulmonary manifestations (26). After the diagnosis of IIM, the patient was treated with rituximab, while in our patient, the treatment with classic immunosuppressants proved to be sufficient to suppress the inflammatory process, regulate laboratory indicators of myositis, and reduce the clinical picture.

CONCLUSION

The concomitant occurrence of MG and IIM is rarely observed. We have described the case of co-occurrence of antisynthetase syndrome and myasthenia gravis in our patient, underlining that MG patients with atypical clinical and diagnostic features should be screened for the presence of IIM. Thus, neurologists should think about the possibility of the combined occurrence of these two rare but treatable diseases, with the aim of early recognition and more adequate treatment, and therefore a better prognosis for such patients.

Study limitations

The shortcomings of our study are the small number of included patients and the short follow-up period. Data on further rheumatological, pulmonological, or dermatological treatment were not available at the moment of study conduction. Also, a major shortcoming of the study is the fact that a definitive diagnosis of myositis was not established because a muscle biopsy was not performed. To obtain solid evidence that explains the reasons for the co-occurrence of inflammatory myopathies with myasthenia gravis, basic research is needed for the definitive exploration of the autoimmune basis of both conditions, as well as multicentric research, which could solve the problem of a small number of patients in most of the previous studies.

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POVEZANOST MIJASTENIJE GRAVIS I IMUNSKI-POSREDOVANIH MIOPATIJA

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Sažetak

Uvod/Cilj rada: Mijastenija gravis (MG) je hronično autoimuno oboljenje neuromišićne spojnice, koje se karakteriše slabošću dominantno proksimalne muskulature svih ekstremiteta, uz zamorljivost. Inflamatorne miopatije (IM) su imunski posredovana, heterogena grupa oboljenja koje karakteriše postojanje progresivne bolne slabosti proksimalne muskulature ekstremiteta. Iako se obe bolesti smatraju delom autoimunog spektra neuromišićnih bolesti, one su klinički, elektrofiziološki, ali i patofiziološki različiti entiteti. Koegzistencija ova dva oboljenja je izuzetno retka i do sada je širom sveta zabeleženo samo pedesetak slučajeva. Cilj ovog istraživanja bila je analiza učestalosti postojanja koegzistencije IM i pacijenata sa de novo MG.

Metode: Studija je retrospektivnog karaktera i sprovedena je na Klinici za neurologiju Univerzitetskog kliničkog centra Srbije. U studiju je bilo uključeno 97 pacijenata kod kojih je dijagnoza mijastenije gravis postavljena u periodu od 1. januara 2014. godine do 31. decembra 2018. godine.

Rezultati: Prosečna starost navedenih ispitanika je iznosila 54,1±18,9 godina. Od svih analiziranih pacijenata, u trenutku postavljanja dijagnoze njih 19 (19,6%) je imalo barem jedan od anamnestičkih podataka posmatranih kao potencijalni indikator za postojanje imunski posredovane miopatije. Finalno, kod jednog pacijenta je klinički prezentovano postojanje generalizovane seropozitivne (*anti-AchR* pozitivne) mijastenije gravis udruženo sa postojanjem dijagnoze antisintentaza sindroma. U daljem teksu su prikazane ključne karakteristike pacijenta sa udruženom pojavom MG i antisintentaza sindroma.

Zaključak: Premda je istovremena pojava MG i IM veoma redak fenomen, neophodno je imati na umu mogućnost udruženog javljanja ova dva autoimuna oboljenja, sa ciljem što ranijeg prepoznavanja i adekvatnijeg lečenja, a samim tim i bolje prognoze obe bolesti.

Ključne reči: Mijastenija gravis, inflamatorna miopatija, antisintetaza sindrom, koegzistencija

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ORIGINAL ARTICLE

Is fecal calprotectin a dependable indicator of activity in inflammatory bowel diseases?

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Summary

Introduction/Aim: Fecal calprotectin (FCP) is an S100 protein biomarker used in diagnostic and monitoring algorithms of inflammatory bowel diseases (IBD). The role of FCP is established in differentiating inflammatory from functional bowel diseases, predicting relapse of IBD, and monitoring response to IBD therapy. The therapeutic strategy "treat-to-target" includes the normalization of laboratory biomarkers including FCP to attain mucosal healing (MH) as a result of effective Crohn's disease (CD) and ulcerative colitis (UC) treatment. Our research aimed to assess the relationship of FCP values in IBD patients with endoscopic and histological scores of disease activity.

Material and methods: We performed a cross-sectional study at the Clinic for Gastroenterohepatology, University Clinical Center of Serbia, encompassing 223 diagnosed IBD patients (110 CD and 113 UC). The concentration of FCP was analyzed from the first morning stool. The endoscopic activity of IBD was evaluated using the endoscopic Mayo score for UC, Simple Endoscopic Score (SES-CD) for CD, and Rutgeerts score in case of a prior operation. The Geboes grading score was used to evaluate IBD histological activity. Due to discontinuous bowel involvement in CD, histopathological grading was limited.

Results: Our results did not identify any statistically significant relationship between FCP and histological scores in patients with Crohn's disease (FCP median 950.98, PH median 3.57; p= 0.22). While FCP values did not show a correlation with the Rutgeerts score, we did observe a notable correlation between FCP and the SES-CD. In UC patients, values of FCP strongly correlated with endoscopic and histological grading (FCP median 1162.62, PH median 3.67; p = 0.011).

Conclusion: FCP has shown to be a useful and reliable biomarker for assessing UC disease activity, while its applicability is restricted when it comes to CD.

Keywords: fecal calprotectin, ulcerative colitis, Crohn's disease

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INTRODUCTION

Fecal calprotectin (FCP) is a commonly used biomarker of inflammation in the management of IBD (1, 2). It is a cytosolic S100 protein distinguished by a heteromeric two-subunit A8/A9 complex (1). Calprotectin as an innate immune protein has antimicrobial properties affecting immunomodulation (1, 3, 4). Namely, FCP is used to distinguish inflammatory and functional bowel diseases, predict relapse in IBD patients, and monitor treatment efficacy (1, 5, 6). The concentration of FCP in stool samples reflects the presence of calprotectin, which is released during an inflammatory process by recruited immune cells that infiltrate and damage the intestinal wall. Nevertheless, the optimal cut-off values of FCP have been controversial and vary among several measurement kits (2,7).

Mucosal healing (MH) in IBD was highlighted in the treat-to-target era, with the preferable use of noninvasive biomarkers in everyday practice. Nevertheless, the evaluation of mucosal healing in Crohn's disease (CD) and ulcerative colitis (UC) is still based on colonoscopy and histopathologic examinations. Current research data indicate a strong association between the endoscopic grading of UC activity and FCP levels (7, 8). Furthermore, the FCP value has been reported as a valuable predictor of disease recurrence in UC patients and an important marker for therapy algorithms (6, 7). Published data indicate that endoscopically active CD can be identified by elevated FCP with sensitivity of up to 97%, while specificity ranges from 45% to 98% (9).

Owing to the challenging scoring of Crohn's disease histologic activity, most studies have concentrated on ulcerative colitis (10). According to previous research, FCP showed the ability to predict histological MH due to an identified notable correlation with histological activity in UC patients. The aim of our study was to investigate the utility of FCP as an activity marker in Crohn's disease and ulcerative colitis.

MATERIAL AND METHODS

A cross-sectional research conducted at the Clinic for gastroenterohepatology, University Clinical Center of Serbia was in accordance with the Helsinki Declaration and approved by the Ethics Committee (decision number 1393/12).

Patients

The study enrolled a total of 223 patients diagnosed with IBD, including 110 cases of CD and 113 cases of UC**.** The IBD diagnosis was determined by conventional clinical, laboratory, endoscopic, and histological findings in accordance with the European recommendations from 2019 (11). Out of 110 CD patients, 9 were intraoperatively diagnosed.

Stool samples

Stool samples of the patient's first-morning stool were analyzed in the laboratory of the Clinic for Gastroenterohepatology. The concentration of FCP was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). As per the manufacturer's specifications, FCP is less than 50 mg/kg in general population. The cut-off value for elevated FC in our study was 200mg/kg.

Endoscopic and histological assessment of IBD activity

All patients encompassed in our research underwent colonoscopy with terminal ileoscopy using Olympus endoscopes, specifically the CF-H180AL video colonoscope with High-Definition Television (HDTV). Successive intestinal biopsies were collected and separately analyzed by a pathologist.

Endoscopic evaluation was performed using the Simple Endoscopic Score for Crohn's disease (SES-CD) and the endoscopic Mayo score for UC (13). The score values were determined by experienced endoscopists. SES-CD graded the extent of ulceration, inflammation, and stenosis in five defined sections of the bowel with values less than 2 indicating remission, mild disease suggested by the range 3 to 6, moderate nature ranging from 7 to 15, and scores ≥15 representing severe endoscopic findings. The postoperative risk of CD relapse was established using the Rutgeerts score categorizing remission or disease recurrence (14). Endoscopic findings after ileocolic anastomosis were described as remission in cases of lesion absence or less than 5 aphthous lesions (score 0-1), while recurrence was denoted with more than 5 aphthous or larger lesions, diffuse ileitis, or presence of ulcerations and stenosis (score 2-4) (14). Endoscopic Mayo score in UC cases assessed the colon for erythema, vascular mucosa, vulnerability, lesions, and spontaneous bleeding with values from 0 to 3 (13).

The histological activity of UC was evaluated using Geboes scores ranging from 0 to 5.4 with elevated values suggesting a more significant presence of chronic inflammation. Although target biopsies of endoscopically active CD were analyzed, grading by the Geboes system was limited by the discontinuous nature of the disease.

Statistics

Data analyses were conducted using SPSS software version 23 (Chicago, IL). The Gaussian distribution of variables was assessed using the Shapiro-Wilk test. Summary statistics were applied encompassing measures such

as mean, median, and standard deviation. Spearman's corelation test was applied to investigate the association between analyzed variables, with p-values < 0.05 considered statistically significant.

RESULTS

Features of the IBD patients included in our research are outlined in **Table 1**.

Table 1. Characteristics of Study Participants

Subjects' parameters	CD (n = 110)	UC (n = 113)
Age, y	37 ± 11	44 ± 14
Females, n (%)	53 (48.2)	55 (48.7)
Smoking, n (%)	44 (40.0)	39 (34.5)
IBD family history, n (%)	16 (14.5)	14 (12.4)
NSAID use, n (%)	25 (22.7)	30 (26.5)
FCP, mg/kg (median)	1200.0	2000.0
Localization of disease		
[a]L1/[b]E1 (%)	32 (29.1)	18 (15.9)
[a]L2/[b]E2 (%)	28 (25.4)	35 (30.9)
[a]L3/[b]E3 (%)	50 (45.5)	60 (53.2)
Phenotype of disease	60 (54.5)	45 (39.8)
[c]B1/[d]S1 (%)	26 (23.6)	28 (24.8)
[c]B2/[d]S2 (%)	24 (21.9)	40 (35.4)
[c]B3/[d]S3 (%)		

Localization of CD according to Montreal classification, L1— ileum, L2—colon, L3—ileocolonicic; [b]Extent of UC according to Montreal classification, E1—ulcerative proctitis, E2—left side UC, E3—extensive UC; [c]Behavior of CD according to Montreal classification, B1— nonstricturing, nonpenetrating disease, B2—stricturing, B3–penetrating.; [d]Severity of UC according to Montreal classification, S1—mild, S2—moderate, S3—severe. CD, Crohn's disease; UC, ulcerative colitis; NSAID, non-steroid anti-inflammatory drugs

There was no notable statistical correlation observed between the degree of histological activity in CD and levels of fecal calprotectin **(Figure 1)**. During our analysis of the CD group of patients, we found a significant positive correlation between the degree of endoscopic activity assessed by SES-CD and FCP values (p = 4.9e-) **(Figure 2)**. Nevertheless, our results did not find a correlation of statistical importance regarding endoscopic activity in operated CD patients and FCP (p = 0.7) **(Figure 3)**

In patients with UC, a significant correlation displayed in **Figure 4** was detected between FCP and mucosal histological activity (p = 0.011). Moreover, results regarding the UC group of patients indicated a strong correlation between fecal calprotectin and endoscopic activity (p = 0.00013) **(Figure 5)**, highlighting the importance of FCP as an activity marker in UC.

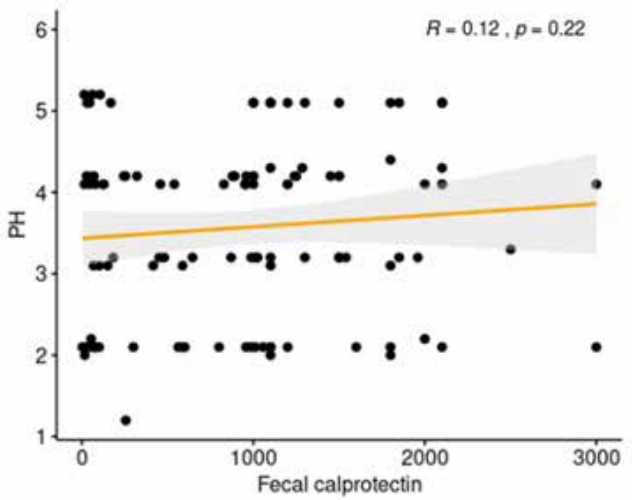

Figure 1. Correlation of FCP levels and histological activity in CD

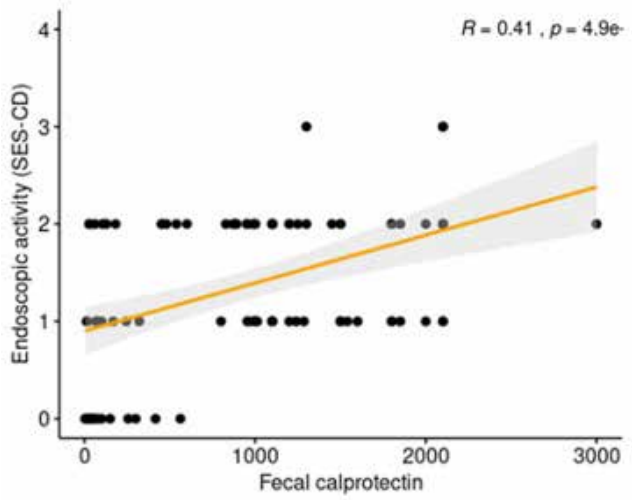

Figure 2. Correlation of FCP levels and endoscopic activity (SES-CD score) in CD

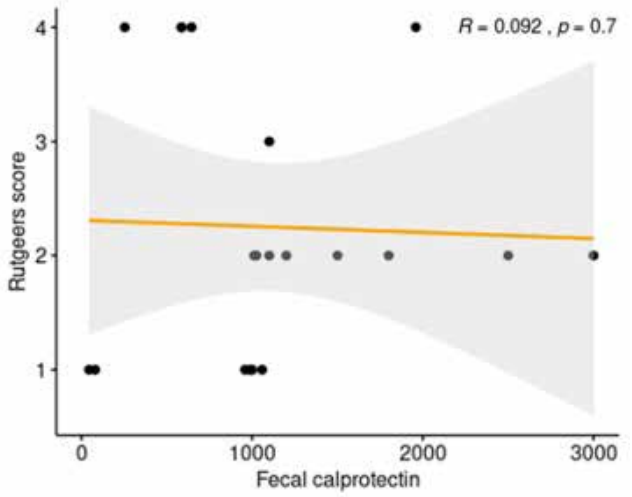

Figure 3. Correlation of FCP and Rutgeerts values in operated CD patients

DISCUSSION

Calprotectin is an antimicrobial protein with elevated values detected in a variety of immunological and immunopathological conditions (10). A significant over-

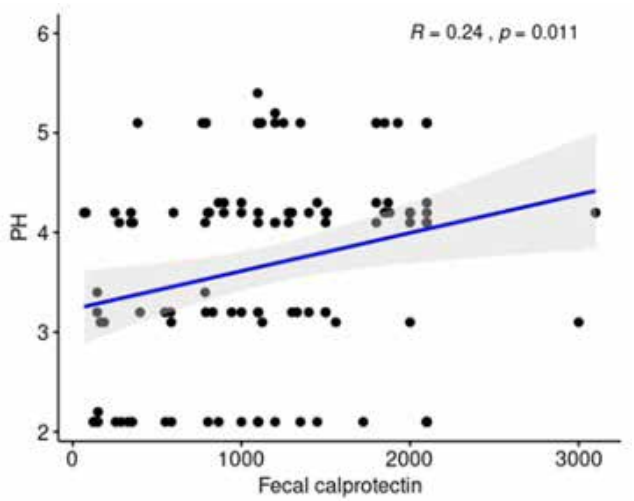

Figure 4. Correlation of FCP values and histological activity in the UC

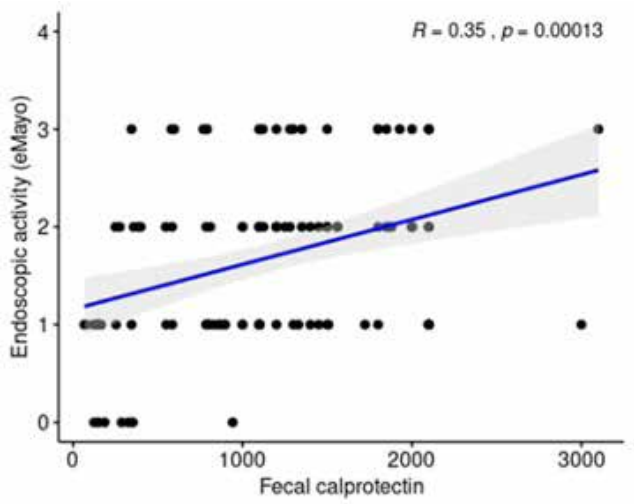

Figure 5. Correlation of FC values and endoscopic activity (eMayo score) in the UC

lap between symptoms of functional and inflammatory disorders has increased the colonoscopy rate. Hence, non-invasive diagnostic markers are needed in everyday clinical practice.

As early as 1997, Roseth et al. highlighted the utility of FCP in IBD diagnosis with elevated values registered in UC compared to controls (15). Nevertheless, consistent challenges occurred across different research studies regarding variability in the sensitivity and specificity of the FCP threshold. Published data such as studies by Jensen MD et al. and Diamanti et al. indicated that the interpretation of fecal calprotectin levels can be demanding, underscoring the complex nature of diagnosing gastrointestinal disorders, especially IBD (16, 17). Possible reasons for the diverse cut-off values of FCP in IBD might be in different clinical settings and demographic and clinical characteristics of IBD patient cohorts. Our study's threshold value of FCP was 200 mg/kg in IBD patients.

Examining the status of MH in individuals with IBD is based on endoscopic and histologic activities. So far, several scoring systems have been developed based on endoscopic and histopathologic findings. Furthermore, recent studies reported a correlation between these scores and FCP levels (10, 15, 18).

According to our results, we found a notable correlation between FCP and the score of endoscopic activity in the UC study group. Based on our findings, we have used a suitable threshold for FCP in assessing endoscopically active UC individuals. Furthermore, a strong correlation was observed linking FCP and mucosal histological activity in UC as evaluated by the Geboes grading system. According to the outcomes derived from our study, FCP represents an important and valuable biomarker in the management of UC patients. These results align with previous studies that have also demonstrated the importance of FCP in diagnosing and following disease activity in ulcerative colitis (10, 15, 18). Moreover, Hart L. et al. and Mak WY et al. reported different FCP thresholds for distinguishing endoscopically "silent" ulcerative colitis (18, 19). Additionally, Schoepfer AM et al. found that FCP had greater efficacy as an indicator of endoscopic activity in ulcerative colitis in comparison with C-reactive protein and clinical presentation of the disease (20)

The management of CD and postoperative courses has a crucial role in optimizing long-term outcomes with regular endoscopic evaluation for monitoring disease activity. Nevertheless, the field of endoscopy faces significant challenges in standardizing scoring systems for consistent and reliable evaluation (21, 22, 23). The results of our study did not observe the link between levels of FCP and endoscopic activity in operated CD patients assessed with a scoring system developed by Rutgeerts. However, interpreting these results might be constrained due to the small number of operated CD patients included in our study. Nevertheless, SES-CD showed a notable correlation with FCP in our CD study group. In line with our results, Sipponen et al. showed a strong link between CD endoscopic activity using CD Endoscopic Index of Severity and FCP, while suggesting FCP values of 200 μg/g as a significant threshold for predicting endoscopic relapse (21). In alignment with previous findings, Moein S. et al. found FCP to be an important and applicable biomarker in predicting endoscopic activity both in UC and CD patients (22).

The IBD histologic activity has also been associated with FCP, but the majority of studies have been focused on UC (23) So far, conducted studies have a relatively low number of UC patients. Nevertheless, our study included 223 IBD patients. Although we reported a correlation between FCP and histologic activity in UC using the Geboes scoring system, CD histologic activity showed no correlation with the values of FCP. However, recently published research data reported that FCP can be used as a reliable predictor of histological remission in IBD (10, 24).

CONCLUSIONS

FCP represents a valuable non-invasive biomarker in the routine clinical practice of IBD patients. The disease activity in patients with UC is strongly linked with levels of FCP. However, its application in CD is currently limited.

Future studies on IBD management should be focused on investigating the monitoring ability of FCP in different personalized therapy algorithms and its prognostic significance in the disease course such as the need for hospitalization or surgery.

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DA LI JE FEKALNI KALPROTEKTIN POUZDAN MARKER KOD INFLAMATORNIH BOLESTI CREVA?

Sanja Dragašević[1,2], Milica Stojković Lalošević[1,2], Andreja Nikolić[1], Nikola Panić[2,3], Vladimir Milivojević[1,2]

Sažetak

Uvod/Cilj: Fekalni kalprotektin (FCP) je S100 protein koji se koristi kao biomarker u algoritmima dijagnostike i praćenja inflamatornih bolesti creva (IBC). FCP ima ulogu u diferencijaciji inflamatornih od funkcionalnih bolesti creva, predikciji relapsa IBC i praćenju odgovora na IBC terapiju. Terapijska strategija "*treat-to taget*" za IBC podrazumeva i normalizaciju laboratorijskih biomarkera uključujući FCP u cilju postizanja mukoznog zaceljenja kao rezultata efikasnog lečenja Kronove bolesti (KB) i ulceroznog kolitisa (UK). Cilj rada je ispitivanje korelacije vrednosti FCP sa endoskopskom i histološkom aktivnosti bolesti kod pacijenata sa IBC.

Metodologija: U studiju preseka koja je sprovedena na Klinici za gastroenterohepatologiju Univerzitetskog Kliničkog Centra Srbije uključeno je 223 IBC pacijenta (110 KB, 113 UK). Nivo vrednosti FCP detektovane su iz uzorka prve jutarnje stolice. Endoskopska aktivnost određena je primenom endoskopskog Mayo skora za UK i Pojednostavljenog Endoskopskog skora za KB (SES-CD) . Rutgeerts skor je korišćen kod prethodno operisanih KB pacijenata. Histološka aktivnost opisana je primenom Geboes grading sistema. Primena histološkog skoring sistema u KB je limitirana diskontinuiranom lokalizacijom u crevu.

Rezultati: Nije uočena statistički značajna korelacija između FCP i histološke aktivnosti KB (FCP median 950.98; PH median 3.57, p= 0.22). Značajna korelacija detektovana je između FCP i endoskopske aktivnosti KB, dok je ista izostala sa Rutgeerts skorom. FCP je značajno korelisao sa histološkom i endoskopskom aktivnosti kod UK (FCP median 1162.62; PH median 3.67, p = 0.011).

Zaključak: FCP je praktičan, neinvazivan marker u proceni endoskopske i histološke aktivnosti kod pacijenata sa UK, dok postoji limitiranost primene kod KB.

Ključne reči: fekalni kalprotektin, ulcerozni kolitis, Kronova bolest

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ORIGINAL ARTICLE

Neurological complications of severe influenza a in children

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Summary

Introduction: Neurological complications of Influenza infection in pediatric population vary in incidence and severity. The central nervous system is most often affected by encephalitis and encephalopathy. Acute necrotizing encephalopathy (ANE) is the most serious complication. The aim of this case study was to present a variety of severe cases of neuro-influenza in pediatric patients.

Methods: The study presents five children who were hospitalized in the pediatric intensivecare unit due to neurological complications of InfluenzaA infection during the winter season of 2022/2023. The identification of the causative agent was carried out by the reverse–transcription–polymerase chain reaction or by the detection of viral antigens in the samples of nasopharyngeal swabs.

Results: Four out of five presented patients were male. The average age at admission was 6 years (min. 7 months, max. 11 years). All patients had an acute disturbance of consciousness at admission and four of them had seizures. Neurological complications of Influenza A infection presented as acute encephalitis, posterior reversible encephalopathy syndrome, acute disseminated encephalomyelitis, encephalopathy, and ANE. Neurological signs appeared approximately four days after the first symptoms. There were no lethal outcomes. Younger patients had more severe brain damage and took longer to recover. None of the children had been vaccinated against the flu.

Conclusions: Children presenting with acute neurological deterioration during influenza season should be evaluated for influenza-associated central nervous system complications even if the respiratory involvement is mild or there are no respiratory signs at all. Magnetic resonance imaging is the most important tool for early diagnosis.

Keywords: Influenza, children, encephalitis, encephalopathy

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Table 1. Demographic data, vaccinal status and clinical presentation in five patients with Influenza A.

Case number	Age	Sex	Clinical presentation at the beginning of disease	Vaccinal status	Time from the onset of the disease to the appearance of neurological signs (days)	Neurological signs
1	8y 10m	F	Fever up to 39˚C Headache Photophobia Vomiting	No Vaccine	3	Seizures Loss of consciousness Disorientation
2	10y 4m	M	Cough Fever over 39˚C Headache Vomiting Hypertension	No Vaccine	11	Seizures Loss of consciousness Visual hallucinations Agitation
3	11y 2m	M	Fever over 40˚C Vomiting Diarrhea	No Vaccine	3	Seizures Altered consciousness
4	1y 4m	M	Fever up to 40˚C Vomiting Diarrhea Somnolence	No Vaccine	2	Seizures, prolonged disorder of consciousness
5	7m	M	Fever over 38˚C	No Vaccine	2	Cyanosis Apnea Episodes of altered consciousness

y-years, m-months, F-female, M-male

INTRODUCTION

Influenza A is a viral contagious infectious disease that is primarily manifested by symptoms and signs of acute respiratory disease. It is estimated that the causative agent of the disease, influenza A virus, infects tens of millions of people every year, mostly young children. A typical clinical presentation in this population includes high fever and gastrointestinal complaints such as reduced appetite, nausea, and vomiting. However, depending on the region and severity of the symptoms, some studies have reported that 10–30% of pediatric patients with influenza A may have neurological complications, including influenza-associated encephalopathy (IAE), encephalitis, acute disseminated encephalomyelitis (ADEM), transverse myelitis, aseptic meningitis, Guillain-Barre syndrome, and cerebrovascular disease (1). Fever may cause febrile convulsions (1, 2). Acute necrotizing encephalopathy (ANE), which has mortality rate of about 30% and which affects about 70% of children, is the most severe brain complication of influenza (1, 3, 4). Similar results related to the incidence of neurological complications of the infection caused by influenza A were presented in a study in Croatia, but our study is the first report from Serbia in children (5). We present five pediatric cases of severe neurological complications caused by Influenza A. The goal of this case study was to present variations in neurological presentations and neuroimaging in pediatric patients.

METHODS

This retrospective casereport study included children with neurological manifestation of InfluenzaA virus who were admitted to the University Children's Hospital in Belgrade, during the winter season of 2022/2023, from December 2022 to April 2023. The identification of the causative agent was carried out by the molecular metho(reverse–transcription–polymerase chain reaction (RT-PCR)) or by detection of viral antigens in the samples of nasopharyngeal swabs, because molecular assay or viral culture are the "gold standard" for determining influenza A virus (6). We excluded COVID-19 infection in all patients by RT-PCR analysis of nasal swabs. The data were obtained by analyzing the existing medical documentation. Electroencephalography, magnetic resonance imaging (MRI), and computerized tomography (CT) were performed.

RESULTS

Four out of five presented patients were male. The average age at admission was 6 years and 6 months (ranging from 7 months to 11 years of age). Demographic data, vaccination status, and clinical presentation are shown in **Table 1**. Comorbidities in our patients were as follows: patient number 1 lost consciousness after a febrile illness at the age of 6; patient number 3 was diagnosed with type 1 diabetes mellitus and was well regulated; and patient number

Table 2. Neuroimaging, electroencephalography, disease course and outcome in five patients with Influenza

Patient	MR	CT	EEG	Diagnosis	Days at PICU	Day of discharge	Neurological sequels at discharge
1	Signal changes of the parietal and occipital cortex of both hemispheres	Cerebral edema	Encephalopathic	Acute encephalitis	6	12	None
2	Posterior reversible encephalopathy with supratentorial cortico-subcortical lesions of the holohemispheric type along the "watershed" zones	CT examination done in another institution described as normal	Encephalopathic	Posterior reversible encephalopathy syndrome (PRES)	4	15	None
3	Disseminated fronto-parietal supratentorial white matter lesions and lesions in the brainstem, basal ganglia and cerebellum	Indirect signs of cerebral edema, bilateral hypodensities of the thalamus	Encephalopathic	Acute disseminated encephalomyelitis (ADEM)	5	8	Decreased gross motor strength
4	Massive lesions of the white matter of both hemispheres of the cerebrum, cerebellum, thalamus and brainstem	Supra- and infratentorial lesions affecting the thalamus, dorsal pons, medial cerebellar peduncles and white matter of the cerebellar cortex	Encephalopathic	Acute necrotizing encephalopathy (ANE)	21	47	Altered state of consciousness with occasional spontaneous eye opening and preserved reaction to light and painful stimuli, uttering few meaningful words
5	Multiple bilateral cortico-subcortical changes in all cerebral lobes, signal changes in basal ganglia and thalamus	Cerebral edema	Focal epileptic activity	Encephalopathy	14	40	Generalized hypotonia, hyperreflexia, poor eye tracking

5 had macrocephaly without any neurological symptoms. Influenza A infection was confirmed in patients 1, 2, and 4 by RT-PCR and in patients 3 and 5 by rapid antigen test. All patients had flu-like symptoms with mild respiratory symptoms or with no symptoms. All of them also had acute disturbance of consciousness (ADOC) at admission, while four out of five had seizures. Neurological signs appearedapproximately 4 days (range 2-11) after the first symptoms. There were no lethal outcomes. All patients had different forms of neurological complications caused by virus influenza disease and required treatment in the pediatric intensive care unit (PICU). Diagnosis based on neuroimaging examinations, electroencephalography, and laboratory findings are shown in **Table 2**. MRI findings are shown in **Figures 1-5** for all the patients. Patients 4 and 5 were treated with plasmapheresis and intravenous immunoglobulins (IVIG) because they had severe liver dysfunction, while patient 4 was additionally treated with pulse doses of methylprednisolone. Both patients had increased levels of C-reactive protein (CRP) and significantly increased liver enzymes. Patient 2 also developed glomerulonephritis due to Influenza A infection. Laboratory examinations of all patients at admission are shown in **Table 3**.

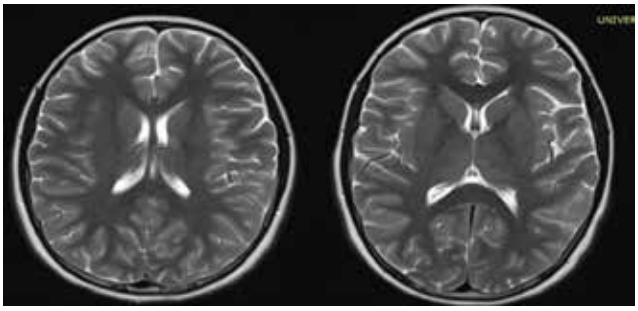

Figure 1. MRI findings of the endocranium of patient 1: signal changes of the parietal and occipital cortex of both hemisphere

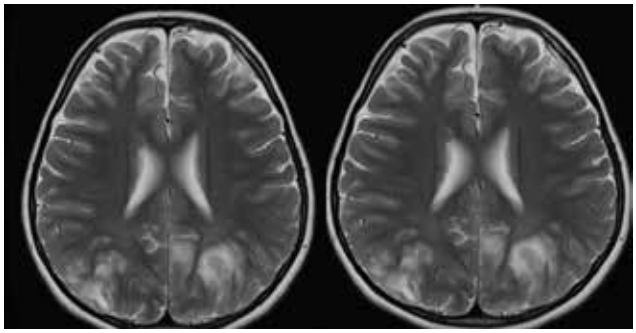

Figure 2. MRI findings of the endocranium of patient 2: posterior reversible encephalopathy with supratentorial cortico-subcortical lesions of the holohemispheric type along the "watershed" zones

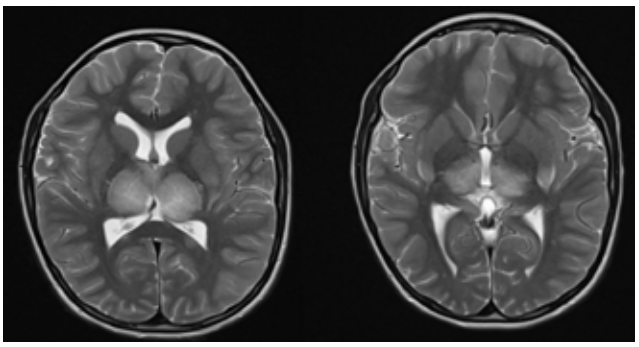

Figure 3. MRI findings of the endocranium of patient 3: Ddisseminated frontoparietal supratentorial white matter lesions and lesions in the brainstem, basal ganglia and cerebellum

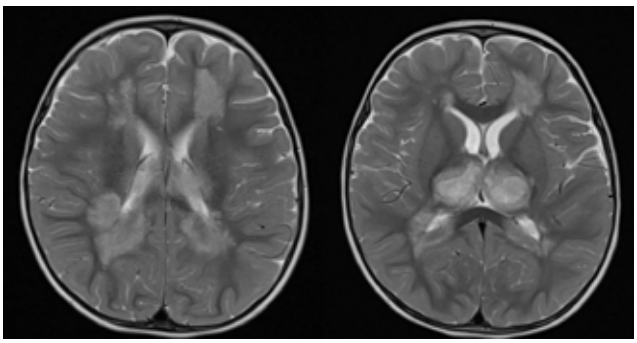

Figure 4. MRI findings of the endocranium of patient 4: massive lesions of the white matter of both hemispheres of the cerebrum and cerebellum, thalamus and brainstem

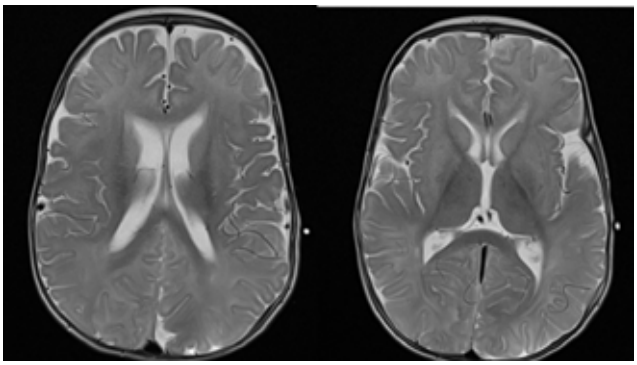

Figure 5. MRI findings of the endocranium of patient 5: multiple bilateral cortico-subcortical changes in all cerebral lobes, signal changes in basal ganglia and thalamus

DISCUSSION

All patients had different types of brain involvement which represent the diversity of neuroinfuenza, despite having relatively similar clinical presentations at admission. Although influenza viruses are known to increase the risk of secondary bacterial infections, particularly pneumonia and sepsis, our study group did not experience any of these complications (5). Acute necrotizing encephalopathy is described as the most severe form of influenza encephalopathy. It is acuteencephalopathy, with no inflammation and with symmetrical bilateral lesions of the thalami and other deep brain structures, and it presents with necrosis or hemorrhage (4).According to statistics, it affects about one-fifth of children with influenza encephalopathy, and the only documented case from our study is consistent with these figures (4). Acute necrotizing encephalopathy presents with fever, deteriorating consciousness, personality changes, seizures, focal deficits, and coma (6). Early steroid administration may be associated with a favorable prognosis, most likely as a result of the interruption of cytokine elevation and reduction of inflammation (7). The neuroinflammation-related brain edema can also be controlled by corticosteroids (8). This therapy had positive results for our patient and minimized brain sequelae.

Serious central nervous complications of the flu are more common in children under 2 years of age (2). Younger children in our study group (patients 4 and 5, aged 16 months and 7 months, respectively) presented with more severe clinical symptoms, recovered more slowly, and had neurological sequelae at discharge. Cerebral edema, signal changes in the cortical and subcortical white matter, bilateral lesions of the thalami and other deep brain structures, particularly in the brain stem, surrounding the white matter, and cerebellar medulla, are some of the neuroimaging changes of influenza-associated encephalitis and encephalopathy (2). The MRI scans of patients 1, 3, and 5 were comparable to previously published data (2). According to reports, significant MRI changes are associated with disease severity and have excellent prognostic value for outcomes (9–13). For IAE, there are no specific laboratory markers. Elevated enzyme levels are a common sign of liver dysfunction. An increased level of white blood cells and noticeably elevated liver enzymes are seen in ANE blood tests. These signs do not, however, exclusively point to the diagnosis of neuro-influenza (2). The results of our laboratory tests are not differentially symptomatic. Although acute necrotizing encephalopathy in children can result from other respiratory tract infections besides influenza A, it is most frequently caused by influenza A: rubella, measles, varicella, human herpes simplex virus, SARS-CoV-2, and

Table 3. Laboratory examination data of five children with Influenza A at admission

Laboratory examination	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
WBC count (10^9/L)	11,9	7,3	6,6	4,1	10,4
CRP (mg/L)	4,5	4	5,1	10,4	12,2
ALT (U/L)	34	39	40	78	97
AST (U/L)	35	58	80	105	241

WBC-white blood cell, PCT-procalcitonin, ALT- alanine aminotransferase, AST- aspartate aminotransferase

human herpes viruses 6 and 7. It was crucial to rule out SARS-CoV-2 infection as the cause of infection, especially since all patients in our study received care during the Covid-19 pandemic.

CONCLUSIONS

Changing consciousness and seizures are the main clinical signs of neuro-influenza. All children with neurological symptoms must be screened for CNS flu-related complications during the flu season. MRI is the most accurate tool for early diagnosis and treatment.This is a potent tool for the diagnosis of neurological damage to brain structures and serves as evidence of the brain diversity of the same virus, such as influenza A. The severity and course of the disease may be altered by the early administration of IVIG, corticosteroids, and plasmapheresis. The differential diagnosis of acute disturbance of consciousness following flu-like symptoms must take other viral infections into account.

Ethical approval

This research and publication were approved by the Ethical committee of the University Children's Hospital (approval number 018 19/02).

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NEUROLOŠKE KOMPLIKACIJE TEŠKOG OBLIKA INFLUENZE A KOD DECE

Snežana Rsovac[1,2], Vesna Selaković[1], Jasna Kalanj[1,2], Katarina Milošević[1,2]

Sažetak

Uvod: Neurološke komplikacije infekcije virusom Influenza A u pedijatrijskoj populaciji javljaju se sa različitom učestalošću i ozbiljnošću kliničke slike. Centralni nervni sistem može biti zahvaćen na različite načine, najčešće u vidu encefalitisa i encefalopatije. Najozbiljnija komplikacija je akutna nekrotizirajuća encefalopatija (ANE). Cilj ove studije slučajeva jeste prikaz raznolikosti teških oblika neuroinfluence kod pedijatrijskih pacijenata.

Metode: Studija obuhvata petoro dece koja su zbog neuroloških komplikacija infekcije Influenzom tip A hospitalizovana na odeljenju pedijatrijske intenzivne nege tokom zime 2022/2023. godine. Identifikacija uzročnika sprovedena je reakcijom lančanog umnožavanja pomoću reverzne transkriptaze (RT-PCR) ili detekcijom virusnih antigena u uzorcima nazofaringealnog brisa.

Rezultati: Četiri od pet bolesnika bilisumuškog pola. Prosečna starost na prijemu bila je 6 godina i 6 meseci (min. 7 meseci, maks. 11 godina). Svi bolesnici su na prijemu imali akutni poremećaj svesti, a četvoro je imalo konvulzije. Neurološke komplikacije Influenze A manifestovale su se kao: akutni encefalitis, sindrom posterior nereverzibilne encefalopatije, akutni diseminovani encefalomijelitis, encefalopatija i ANE. Neurološki znaci su se pojavili u proseku četiri dana nakon prvih simptoma bolesti. Bolest se u svim slučajevima završila ozdravljenjem. Mlađa deca su imala veće oštećenje mozga i duži period oprovaka od starije dece. Nijedno dete nije bilo vakcinisano vakcinom protiv gripa.

Zaključci: Kod dece sa akutnim neurološkim pogoršanjem tokom sezone gripa treba razmotriti potencijalno postojanje neuroloških komplikacija infekcije gripom, čak i u slučajevima blagih ili nepostojećih respiratornih tegoba. Magnetna rezonanca je najvažnija dijagnostička procedura za blagovremeno postavljanje dijagnoze.

Ključne reči: Influenza, deca, encefalitis, encefalopatija

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ORIGINAL ARTICLE

The influence of vitamin B6 on cardiac oxidative stress, cardiometabolic and histological markers in monocrotaline-induced heart failure in wistar albino rats

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Summary

Introduction/Aim: Heart failure (HF) induced by monocrotaline (MCT) is common in the pulmonary arterial vessels remodeling mechanisms with increased pulmonary resistance and oxidative stress markers. The purpose of this study was to validate the hypothesis that the treatment with vitamin B6 could affect HF by modulating cardiometabolic and oxidative stress biomarkers, and the structure of the rat heart.

Material and Methods: Male Wistar albino rats were divided into 3 groups: blank solution-exposed control (C physiological saline 1ml/kg 28 days ip., n=8), B6 (vitamin B6 7mg/kg/day 28 days ip., n=8), and MCT+B6 (MCT 50mg/kg once ip. plus vitamin B6 7mg/kg/day 28 days ip., n=8).

Results: The four-week vitamin B6 treatment significantly affected certain biochemical parameters. The activity of key antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX) did not change, whereas the total glutathione (GSH) was significantly decreased in the MCT+B6 group. This was followed by a slightly decreased level of the total glutathionylation observed in the MCT+B6 group. The parameters of protein oxidative damage (reactive carbonyl derivates, thiol groups and nitrotyrosine) did not significantly change in the MCT+B6 group. An increasing trend in RV and LV wall thickness was observed in the MCT+B6 compared to the C group, as well as in Ki67 and PCNA positivity.

Conclusion: The four-week treatment with vitamin B6 significantly affected certain biomarkers. The activity of SOD and nitrotyrosine content did not change, while GPX activity, total glutathione and total glutathionylation level were decreased in the MCT+B6 group. We observed an increase in RV and LV wall thickness in the MCT+B6 group compared to the C group, as well as in Ki67 and PCNA positivity.

Key words: heart failure, monocrotaline, vitamin B6, oxidative stress, rats

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INTRODUCTION

Vitamin B6 in its active form, pyridoxal 5'-phosphate (PLP) is a cofactor of a variety of enzymes which are necessary for metabolism of amino acids, lipids and carbohydrates (1), but it also has an important role as co-factor in many inflammatory pathways (2). Vitamin B6 serves as a co-factor for two main enzymes in transsulfuration metabolic pathway of homocysteine, cystathionine β-synthase and cystathionine γ-lyase (3). This pathway is important for synthesis of hydrogen sulfide (H_2S) and cysteine which is the key amino acid for the function of tripeptide glutathione (GSH) (4). GSH is an important intracellular antioxidant protecting cells form reactive oxygen species (ROS) and it also takes part in the regulation of inflammatory response by mediating in cytokine synthesis and the activation of transcription factors, like nuclear factor-kappa B (NF-κB) and hypoxia-inducible factor-1α (HIF-1α) (5). In addition, the relevance of H_2S in blood pressure modulation, glucose uptake and metabolism of cardiomyocytes has emerged recently (6). Indeed, decreased H_2S levels have been found in patients with congestive heart failure (7). It has been proposed that beneficial effects of vitamin B6 on cardiovascular system are mediated by reducing inflammation and the degree of oxidative stress (8). Low vitamin B6 levels may interrupt the metabolism of homocysteine and lead to hyperhomocysteinemia which is a risk factor for coronary artery disease (9). A study on experimental animals by Mahfouz et al. (10) showed that a treatment with vitamin B6 and vitamin C achieved antioxidant effects in rats with experimentally developed hyperhomocysteinemia and the maintained ability of their aortas to produce prostacyclin at a normal rate. Deficiency of vitamin B6 is reflected in an increase of lipid peroxidation and a reduction of antioxidant defense systems, which can be in correlation with atherogenesis (11, 12). Vitamin B6 deficiency is really rare in developed countries, however low levels of vitamin B6 have been detected in patients suffering from alcoholism, diabetes, in women using oral contraceptives, and in smokers (13).

HF as one of the leading mortality causes worldwide, is accompanied with complex metabolic changes, including oxidative stress, inflammation, fibrosis, angiogenesis, and apoptosis. Valid HF experimental method (via pulmonary arterial hypertension-PAH) is achieved by MCT application. Obstruction of pulmonary blood vessels increased the afterload of the right ventricle leading to its malfunction and failure (14). Lung toxicity of MCT is caused by its effects on nitric oxide metabolism, antiapoptotic, and proliferative factors and membrane proteins (15). MCT affects the nuclear factor-E2-related factor (Nrf2) pathway leading to an increased caspase three activation, oxidative damage and inflammation (16). In an already failing heart, increased production of ROS could lead to adverse myocardial remodeling accompanied by HF progression (17).

Taking all these effects into consideration, the aim of this study was to investigate if a treatment with vitamin B6 could modify biochemical, oxidative stress, histomorphometric and immunohistochemical parameters, as well as the damage of rat heart tissue in a model of the right ventricle failure developed by intraperitoneal monocrotaline application.

MATERIAL AND METHODS

Animal ethics report

We treated animals in compliance with the Guide for the Care and Use of Laboratory Animals (8th ed., National Academies Press) and the European Directive for the Welfare of Laboratory Animals (No. 2010/63/EU). Our research has been credited by the Ethical Council for the Welfare of Experimental Animals, Ministry of Agriculture, Forestry and Water Management, Veterinary Directorate, Republic of Serbia (No. 323-07-01339/2017-05/2).

Experimental animals

Male Wistar albino rats were used in our study, with body mass around 160 g and age 25–30 days at the beginning of the experiment. The experimental animals were placed in transparent Plexiglas cages with a wood-chip floor, in pairs per each cage. Basic physiological needs, such as food and water, were accessible ad libitum. The ambient conditions such as 12 h light–dark cycle (the light period beginning at 7:30 a.m.), temperature (21 ± 2 °C), and humidity (55% ± 5%) were constant.

Protocol of the experiment

A single intraperitoneal (ip.) application of MCT (50 mg/kg body mass) was used for inducing HF, and the induced HF animal model was verified earlier (18). The experimental period lasted for 28 days. The experimental animals were split into 3 groups: blank solution-exposed control (C physiological saline 1ml/kg 28 days ip., n=8), B6 (vitamin B6 7mg/kg/day 28 days ip., n=8), and MCT+B6 (MCT 50mg/kg once ip. plus vitamin B6 7mg/kg/day 28 days ip, n=8). The animals were euthanized after the experimental period. Heart tissue and blood samples of all animals were gathered for particular analysis (biochemical, histomorphometric and immunohistochemical).

Biochemical analysis

Competitive immunoassay using direct, chemiluminescent technology on an ADVIA Centaur XP system (Siemens Healthcare Diagnostics, Tarrytown, New York,

USA) was used for serum homocysteine (Hcy) measuring. Glucose, urea (UREA) and creatinine (CREA) levels, the lipid profile parameters (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG)), total proteins (TP), albumin (ALB), marker of liver function (aspartate aminotransferase (AST), alanine aminotransferase (ALT)), α-amylase (α-AMY), and alkaline phosphatase (ALP)) were determined by using spectrophotometry commercial kits (Siemens Healthcare Diagnostics Inc., Newark, New Jersey, USA) on an automatic analyzer (Dimension Xpand, Siemens). Friedewald 's equation was used for the estimation of LDL-C level. Commercial test based on a particle enhanced turbidimetric immunoassay technique on a Dimension Xpand analyzer (Siemens) was used for measuring the serum C-reactive protein (CRP) concentration. The levels of cardiac troponin T (hs cTnT) were determined with a highly sensitive assay using the Roche Cobas e601 automated analyzer (Roche Diagnostics, Mannheim, Germany). The automated electrochemiluminescence immunoassay on a Cobas E601 analyzer (Roche Diagnostics) was used for measuring the concentration of interleukin (IL)-6. The concentration of fibrinogen was measured by the modified Clauss assay (Siemens Healthineers Headquarters, Erlangen, Germany) and the activity of von Willebrand factor (vWF) was determined by particle enhanced assay INNOVANCE® VWF Ac using a BCS XP analyzer (Siemens Healthineers Headquarters).

Oxidative stress analysis

Uric acid (UA) serum levels were measured spectrophotometrically on an automatic biochemical analyzer (Dimension Xpand, Siemens) using commercial kits (Siemens Healthcare Diagnostics Ltd., Frimley, Camberley, UK). Cardiac tissue homogenate was used for measuring other oxidative stress parameters. The heart was washed with saline (0.9% NaCl) after isolation, and then dried on filter paper. Homogenization was carried out in 50 mmol/L RIPA buffer (radioimmunoprecipitation assay), pH 7.4, with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), and it was centrifuged at 14,000 rpm for 30 min. Up to the moment of specific analyses, the cardiac tissue was frozen at –80 °C. The activity of superoxide dismutase (SOD) was measured spectrophotometrically (19), and the activity of glutathione peroxidase (GPX) was measured by the coupled assay procedure (20). One unit of enzyme activity is presented as millimoles of NADPH oxidized per minute, assuming $6.22x10^{3}\cdot L^{-1}\cdot mol^{-1}\cdot cm^{-1}$ to be the molar extinction coefficient value of NADPH at 340 nm. The content of thiol (P-SH) groups was appointed according to the method of (21) and presented as micromoles per gram of protein. Total glutathione (GSH) was measured spectrophotometrically and presented as nanomoles per milligram of protein (22). The content of reactive carbonyl derivates (RCD) was measured according to the method of Levine et al. (23). The level of protein oxidation was supervised by determination of a classic carbonyl reagent, 2,4-dinitrophenylhydrazine. Spectrophotometric measurement of RCD content was presented as micromoles per gram of protein. The nitrotyrosine content in rat heart tissue was determined by an ELISA test. Standard curves (24) were used for the nitrotyrosine content determination and the results were presented as nanomoles per litre. Bicinchoninic Acid Protein Assay kit (BCA-1) (Sigma–Aldrich) was used for determination of protein concentration.

Determination of the total S-glutathionylation

Heart tissues were homogenized in radioimmunoprecipitation assay buffer supplemented with protease cocktail inhibitor and N-ethylmaleimide for measuring the total S-glutathionylation levels. Identical amounts of proteins (40 g) from the purified homogenate were separated by sodium dodecyl sulfate – polyacrylamide gel electrophoresis on Criterion™ TGX precast gels (4%–15%) (Bio-Rad, USA) under non-reducing conditions. The transfer of proteins is implemented to a nitrocellulose membrane. After that, immunodetection was determined by using a primary mouse monoclonal anti-glutathione antibody (Abcam, Cambridge, UK) followed by incubation with goat anti-mouse antibody (Dako, Glostrup, Denmark). Chemiluminescent detection system (kit ECL Western blotting analysis system, GE Healthcare, Amersham) was used for visualization performing. ImageLab software (Bio-Rad) was used for densitometric analysis.

Histomorphometric and immunohistochemical analysis

Heart tissue was regularly oriented and transversely cut in 3 mm sections. The treated tissues were then set up in 4% neutral buffered formaldehyde, and fixed by the immersion procedure for 24 h. In the next step, it was inserted in paraffin, cut in 5 mm serial sections until the complete wall thickness of heart appeared. After all, the tissue was stained with hematoxylin and eosin. Ten measurements per heart were made from the endocardium to epicardium of the left ventricle (LV), right ventricle (RV) free wall thickness, and interventricular septum (IVS) thickness. The average dimension of a cardiomyocytes was calculated by measuring the diameter of 100 cardiomyocytes (at the nucleus level) of the LV free wall. Olympus BX 41 microscope was used for analyzing all slides. All slides were photographed with an Olympus C-5060 wide zoom digital camera and Olympus DP-soft Image Analyzer program. All the values were presented in micrometres. All tissue specimens were immunohistochemically stained using anti-Ki67 (RM-9106-S0 Ki67 clone SP6,

dilution ratio 1:200, ThermoScientific) and anti-PCNA (RB-9055-P0 PCNA rabbit polyclonal 1:200, ThermoScientific). After dewaxing and rehydration, a heat-induced antigen retrieval procedure using Tris–EDTA at pH 9.0 for 30 min was performed on all tissue sections. After that, sections were washed with TBS and incubated with the primary antibody diluted at an adequate ratio. The commercial UltraVision/DAB staining kit (Thermo Scientific LabVision TL-060-HD) was used for treating the sections. Immunoreactions were subsequently developed by using DAB. The sections were counterstained with Mayer's hematoxylin. Negative controls were performed on myocardial tissue sections using the same methodology but with the omission of primary antibody. Olympus BX 41 microscope was used for analyzing all the slides, which were photographed with an Olympus C-5060 wide zoom digital camera and Olympus DP-soft Image Analyzer program. The results were analyzed on 100 counted cardiomyocytes by determining the number of cells expressing a percentage of Ki67 and PCNA positivity and were expressed as percentages.

Statistical analysis

Obtained data were expressed as means ± SD. Discrete variables were presented as frequencies and percentages. ANOVA with post hoc analysis as well as a non-parametric variant of the Kruskal–Wallis test and Mann–Whitney test were used to compare the values between the groups, depending on the data distribution. The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

In the experimental model of monocrotaline-induced HF, the effects of four-week vitamin B6 treatment were assessed on biochemical, oxidative stress, histomorphometric and immonohistochemical parameters. Rats were divided according to the protocol regimen into 3 groups: control group (C), vitamin B6 treatment (B6), and MCT-induced HF group on vitamin B6 treatment (MCT+B6).

Biochemical analysis

Levels of Hcy did not differ significantly between MCT+B6, B6 and the control group (7.68 ± 2.26 vs. 7.21 ± 1.05 vs. 10.19 ± 3.87 μmol/L). However, in the group of rats treated only with vitamin B6 in comparison with the control group a beneficial effect for blood glucose and HDL concentrations was noticed. On the other hand, glucose concentrations were significantly increased in MCT+B6 group compared to B6 group (7.33 ± 0.78 vs. 5.88 ± 0.31 mmol/L). The levels of total cholesterol and its HDL fraction were increased in the sera of rats that were treated with vitamin B6 after MCT-induced heart injury (CHL = 2.18 ± 0.48 mmol/L; HLD = 1.47 ± 0.29 mmol/L), compared to the control group (CHL = 1.45 ± 0.23 mmol/L; HDL = 0.64 ± 0.09 mmol/L). The levels of triglycerides did not differ significantly between groups. In B6 group, higher activity of AST, ALT and ALP were determined in comparison to the control group ($p<0.05$), but there were no changes in the activity of AST, ALT and ALP between MCT+B6 and C groups. Levels of TP and ALB had decreasing trend in the MCT+B6 group compared to the C group. The concentration of uric acid was not significantly changed comparing the groups. The activity of α-AMY in the sera of rats was lower in the C group compared to the MCT + B6 group (3097.4 ± 386.83 U/L vs. 3460.00 ± 80.41 U/L).

In all tested groups, in each sample, CRP was under the limit of detection (2 mg/L). We got similar results for the concentration of IL 6 (in all tested groups 100% of samples were under 1.5 pg/mL).

Fibrinogen values were under 1.8 g/L in 75% MCT+B6 group samples. The treatment with vitamin B6 caused a decrease in von Willebrand factor (vWF) concentration in rats with MCT-induced HF (36.87 ± 26.01 %).

Troponin T increased after vitamin B6 treatment (190.71 ± 75.36 ng/L) compared to the control group (57.00 ± 87.68 ng/L). The levels of UREA and CREA were significantly higher in the MCT+B6 group in comparison with the B6 group (22.77 ± 8.56 mmol/L vs. 12.30 ± 2.18 mmol/L, 55.71 ± 5.09 μmol/L vs. 38.10 ± 2.92 μmol/L, respectively). The results are presented in **Table 1**.

Oxidative stress analysis

The key antioxidant enzymes, SOD and GPX, the main non-enzymatic antioxidant, GSH, as well as the parameters of oxidative damage of proteins (thiol, carbonyl groups and nitrotyrosine), were evaluated in all tested groups. Following B6 treatment, the activity of SOD did not change in the MCT + B6 group compared to C group (495.00 ± 96.42 U/ml vs. 480.60 ± 53.43 U/ml) **(Fig. 1 A)**, while GPX activity **(Fig. 1 B)** was decreased in both B6 and MCT + B6 group in comparison with the C group (197.27 ± 29.90 U/ml vs. 317.60 ± 151.48 U/ml), but without reaching statistical significance. The total GSH concentrations were decreased in rats treated with B6 compared to the control group (33.23 ± 3.06 nmol/mg of proteins vs. 70.62 ± 11.44 nmol/mg of proteins) which is more potentiated in the MCT+B6 group **(Fig. 1 C)**. The intraperitoneal B6 application with MCT-induced HF very slightly decreased the content of thiol groups in comparison with C group (211.71 ± 38.96 μmol/g of proteins vs. 308.45 ± 39.24 μmol/g of proteins) **(Fig. 2 A)**. The content of RCD showed an increasing trend in comparison with the control group (26.99 ± 13.68 μmol/g of proteins vs. 18.22 ± 5.40 μmol/g of proteins) **(Fig. 2**

Table 1. Certain biochemical parameters in the serum of the experimental animals

Groups/ parametres	C	B6	MCT+B6
°Hcy (µmol/L)	10.19 ± 3.87	7.21 ± 1.05	7.68 ± 2.26
°GLUC (mmol/L)	7.17 ± 0.58	5.88 ± 0.31 #	7.33 ± 0.78^
°CHL (mmol/L)	1.45 ± 0.23	1.35 ± 0.13	2.18 ± 0.48^
°HDL (mmol/L)	0.64 ± 0.09	1.12 ± 0.11 #	1.47 ± 0.29^
°TGL (mmol/L)	0.77 ± 0.17	0.91 ± 0.21	1.18 ± 0.30
°AST (U/L)	222.80 ± 49.56	331.00 ± 36.26 #	190.29 ± 30.32^
°ALT (U/L)	69.50 ± 8.77	121.40 ± 13.33 #	61.14 ± 11.91^
°ALP (U/L)	347.80 ± 70.38	540.40 ± 119.90 #	346.00 ± 80.41^
°TP (g/L)	60.60 ± 1.96	58.20 ± 2.97	52.43 ± 2.82^
°ALB (g/L)	30.40 ± 1.58	34.30 ± 1.34 #	22.43 ± 1.62
°UA (µmol/L)	77.20 ± 14.11	62.25 ± 11.68	73.14 ± 20.05
°α-AMY (U/L)	3097.4 ± 386.83	3856.90 ± 952.10	3460.00 ± 80.41^
¥IL6 < 1.5 (pg/mL)	8 (100%)	8 (100%)	8 (100%)
¥CRP < 2 (mg/L)	8 (100%)	8 (100%)	8 (100%)
¥Fibrinogen < 1.8 (g/L)	8 (100%)	3 (37.5%)	6 (75%)
¥D Dimer < 0.5 (mg/L)	2 (25%)	6 (75 %)	7 (87.5%)
¥vWF Ac (%)	15.40 ± 0.89	130.52 ± 31.34 #	36.87 ± 26.01^
hsTnT (ng/L)	57.00 ± 87.68	12.55 ± 3.70	190.71 ± 75.36 ^
°UREA (mmol/L)	9.90 ± 0.54	12.30 ± 2.18	22.77 ± 8.56^
°CREA (µmol/L)	29.00 ± 1.76	38.10 ± 2.92	55.71 ± 5.09^

C - saline 1ml/kg/day i.p., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day
Hcy-homocysteine, GLUC-glucose, CHL-total cholesterol, HDL-high density lipoprotein, TGL-triglycerides, AST-aspartate-aminotransferase, ALT- alanine - aminotransferase, ALP-alkaline phosphatase, TP-total proteins, ALB-albumin, UA-uric acid, α-AMY- α-amylase, IL6-interleukin 6, CRP-C- reactive protein, vWF-vonWillebrand Factor, hsTnT-troponin T, UREA-urea, CREA-creatinine;
°- results are presented as x ± SD; ¥ - results are presented as n (%)
– P < 0.05 versus C group, ^ – P < 0.05 versus B6 group.

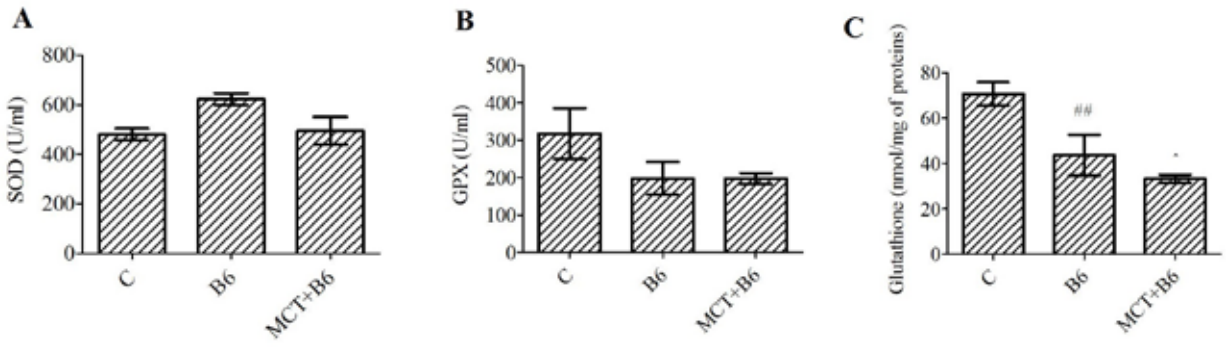
B), and the nitrotyrosine content did not change in the MCT+B6 group compared to the C group (5.13 ± 0.35 nmol/L vs. 5.23 ± 0.82 nmol/L) (Fig. 2 C).

Total S-glutathionylation measurements

The total protein glutathionylation level was assessed by Western blot in 3 sample groups (C, B6 and MCT+B6). Monoclonal anti-glutathione antibody was used to investigate the total protein glutathionylation. Figure 3A showed bands reacting with anti-glutathione antibody in 3 investigated sample groups (C, B6 and MCT+B6). The total glutathionylation level decreased in the MCT+B6 group in comparison with the C group but without statistical significance (Fig. 3 B). Densitometric analysis also showed decreased protein glutathionylation levels in the MCT+B6 group compared to the B6 group with borderline significance (p = 0.057).

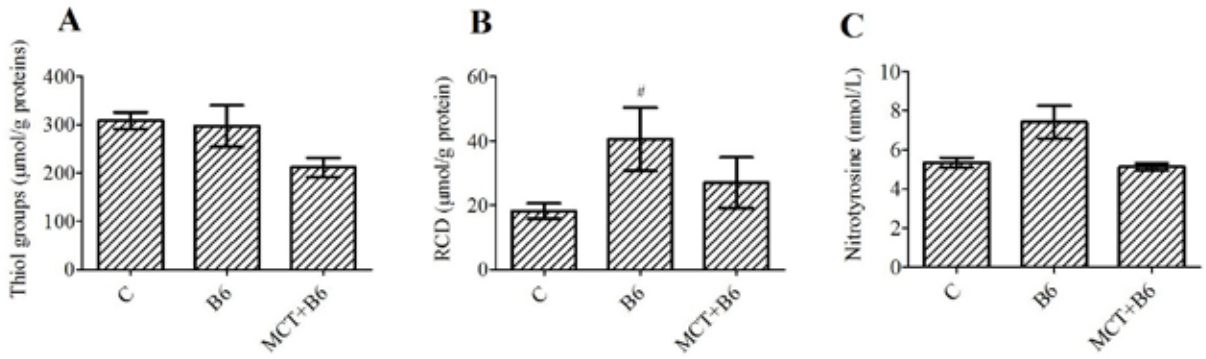
Histomorphometric and immunohistochemical analysis

The average dimension of cardiomyocytes of the LV free wall was significantly lower in the B6 group compared



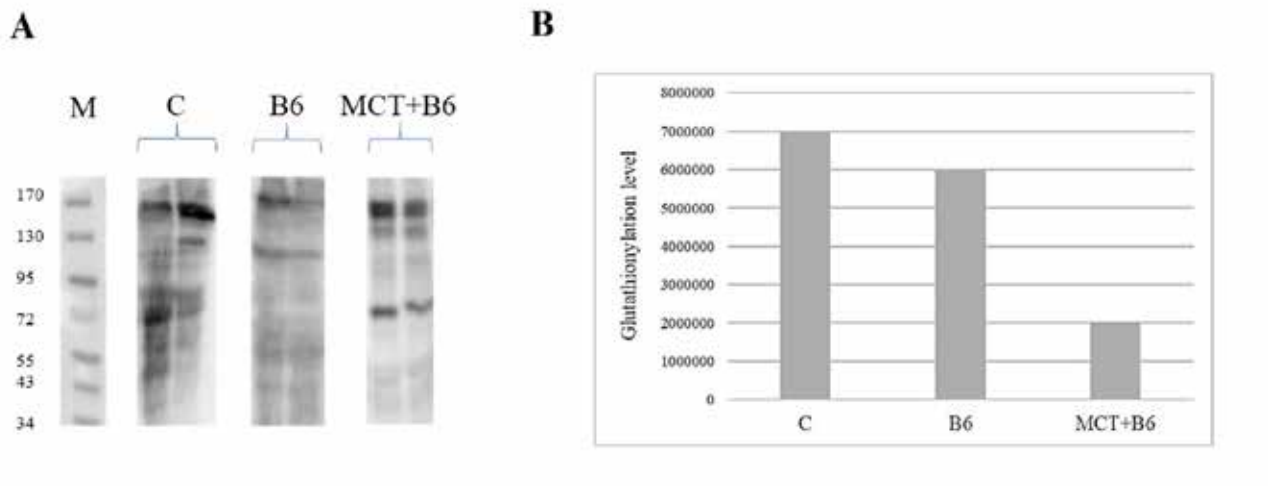
C - saline 1ml/kg/day ip., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day
– P < 0.01 versus C group; ^ – P < 0.05 versus B6 group

Figure 1. A. Superoxide dismutase (SOD) activity in rat cardiac tissue; B. Glutathione peroxidase (GPX) activity in rat cardiac tissue; C. Glutathione content in rat cardiac tissue



C - saline 1ml/kg/day ip., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day
– P < 0.01 versus C group

Figure 2. A. The amount of thiol (P-SH) group in rat cardiac tissue; B. The content of reactive carbonyl derivates (RCD) in rat cardiac tissue; C. The content of nitrotyrosine in rat cardiac tissue



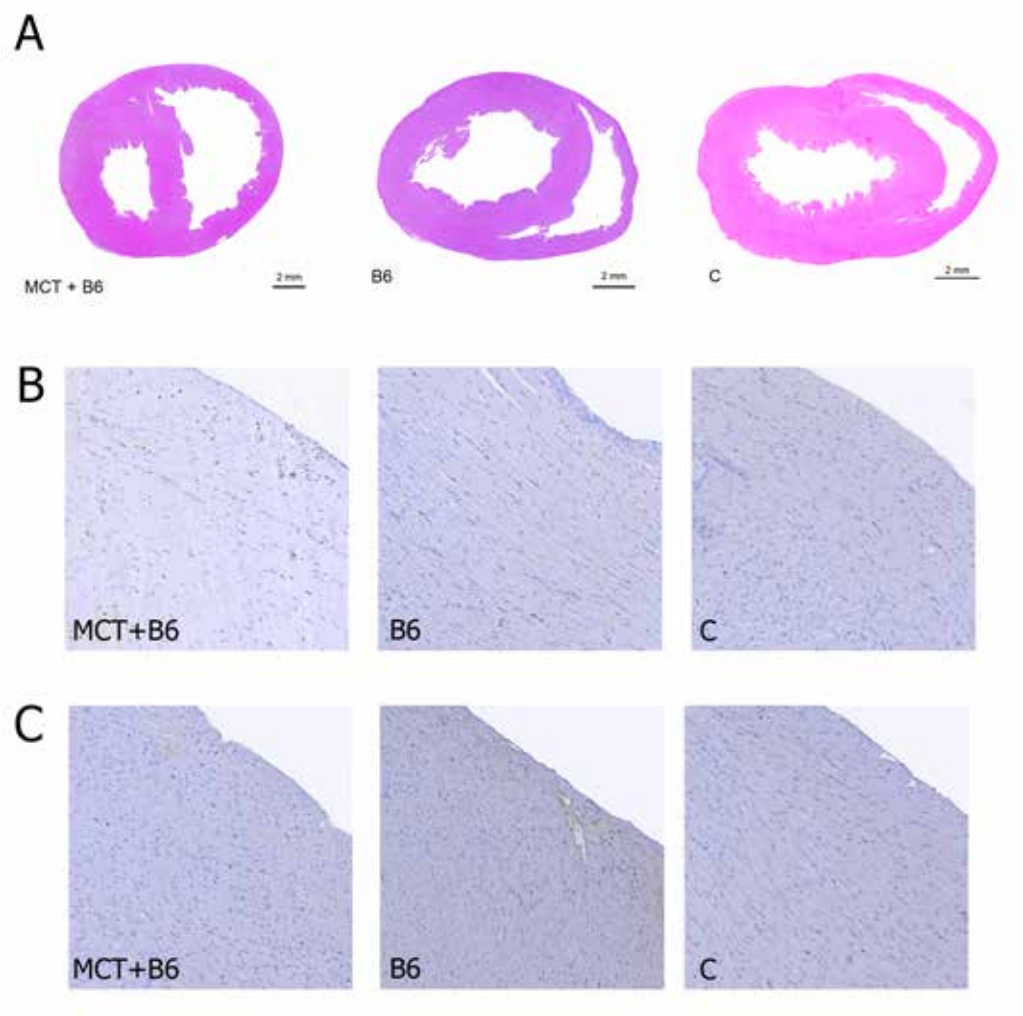
C - saline 1ml/kg/day ip., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day

Figure 3. A. The level of total protein glutathionylation by Western blot; B. Densitometric analysis of obtained blots: ImageLab software (Bio-Rad, USA)

Table 2. Histomorphometric and immunohistochemical parameters of rat heart

Parameters	C	B6	MCT+B6
LV wall thickness (μm ± SD)	2363.81 ± 346.21	1943.37 ± 96.36	2259.30 ± 382.00
RV wall thickness (μm ± SD)	835.48 ± 144.63	707.58 ± 100.63	1000.71 ± 292.25
IVS thickness (μm ± SD)	2112.61±296.05	1762.42 ± 79.28	2194.91 ± 410.28
Dimension of cardiomyocyte of the LV free wall (μm ± SD)	22.50 ± 1.90	17.62 ± 1.43 #	25.21 ± 2.83 ˆ
Ki67 (RV) (%±SD)	1.25± 1.26	1.60 ± 0.89	17.50 ± 10.60 #
Ki67 (LV) (%±SD)	1.00 ± 0.82	1.00 ± 0.71	5.00 ± 4.24 #
PCNA(RV) (%±SD)	0.20 ± 0.45	0.40 ± 0.55	6.3 ± 1.41 #
PCNA(LV) (%±SD)	0.20 ± 0.45	0.20 ± 0.45	6.6 ± 0.78 #

C - saline 1ml/kg/day ip., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day
LV – left ventricle; RV – right ventricle; IVS – interventricular septum; SD – standard deviation
– P < 0.05 versus C group, ˆ – P < 0.05 versus B6 group



C - saline 1ml/kg/day ip., B6 - vitamin B6 7mg/kg/day, MCT - 50mg/kg once ip. + vitamin B6 7mg/kg/day

Figure 4. A. Representative histological slides of rat's hearts in experimental groups; B. Representative immunohistochemical slides of rat hearts in experimental groups (Ki67 proliferation marker); C. Representative immunohistochemical slides of rat hearts in experimental groups (PCNA proliferation marker)

to the C group (17.62 ± 1.43 μm vs. 22.50 ± 1.90 μm, $p < 0.05$). There was an increasing trend in RV wall thickness in the MCT+B6 group compared to the C group (1000.71 ± 292.25 μm vs. 835.48 ± 144.63 μm). IVS thickness did not change in the MCT+B6 group compared to the control group (2194.91 ± 410.28 μm vs. 2112.61 ± 296.05 μm). The same trend was observed in LV wall thickness. Ki67 positivity in the RV wall was increased in MCT+B6 group compared to the C group (17.50 ± 10.60% vs. 1.25 ± 1.26%). A similar pattern of Ki67 positivity was recorded in the LV wall between MCT+B6 and C groups (5.00 ± 4.24% vs. 1.00 ± 0.82%). PCNA positivity in the RV wall was increased in MCT+B6 group compared to the C group (6.3 ± 1.41% vs. 0.20 ± 0.45%), and the same increase was observed in the LV wall (6.6 ± 0.78% vs. 0.20 ± 0.45%). The results are presented in **Table 2** and **Figure 4 (A, B, C)**.

DISCUSSION

In this study, we found some beneficial effects of the four-week treatment with vitamin B6 on histomorphometric and immunohistochemical parameters in monocrotaline-induced right heart failure. Indeed, an increasing trend in RV and LV wall thickness, as well as, in Ki67 and PCNA positivity in rats with heart failure treated with B6 vitamin compared to control rats was determined. Interestingly, based on a significant decrease in total glutathione concentrations, unchanged SOD and GPX activity, as well as on the increasing trend of glutathyonylation obtained in the control group treated only with vitamin B6, we hypothesized a potential involvement of vitamin B6 in redox signaling and regulation.

It has been well established that the experimental model of heart failure (HF), induced by intraperitoneal injection of the macrocyclic alkaloid monocrotaline (MCT), causes changes in various cardio-metabolic and oxidative stress parameters (REF). In our research, we reviewed retrieved information regarding potential effective impacts of vitamin B6 and determined the effects of four-week vitamin B6 treatment on cardiometabolic and oxidative stress biomarkers, but also the impact on histomorphometric and immunohistochemical changes in MCT-induced HF. Namely, the activity of key antiox-

idant enzymes, SOD and GPX, as well as, parameters of oxidative damage to proteins, such as thiol groups, reactive carbonyl derivates and nitrotyrosine did not change in the MCT+B6 group compared to the control group. However, a significant decrease was determined only for the total GSH concentrations, followed by a decreasing trend in protein glutathionylation levels in rats with heart failure treated with vitamin B6. Interestingly, in the control group treated only with vitamin B6 a significant decrease in total glutathione concentrations, and an increasing trend of protein glutathionylation in comparison with the control group, clearly implies its potential involvement in redox regulation. Namely, the important role of protein S-glutathionylation in redox regulation of different signaling pathways has emerged recently. Moreover, it has been shown that perturbations in protein glutathionylation status might be involved in the pathogenesis of atherosclerosis and cardiac hypertrophy, underlying the possible molecular mechanism of redox disbalance in ethiology of cardiovascular diseases (25). Until now, antioxidant effect of vitamin B6 was based mainly on its effect on inhibiting superoxide radical generation and reducing lipid peroxidation (26). Recently, it has been shown that a beneficial effect of vitamin B6 supplementation on the function of aortic endothelium in old rats is mediated by stimulation of H_2S biosynthesis, reduction of oxidative/nitrosative stress and lipid peroxidation along with an increase in constitutive NO synthesis (27). In contrast, vitamin B6 deficiency increases lipid peroxidation and reduces antioxidant defenses, and it also shows a connection with atherogenesis (28). It has been observed that vitamin B6 is important as a significant and detached risk factor for cardiovascular diseases (29). Some case-control studies have shown that vitamin B6 deficiency is obviously associated with higher cardiovascular risk (9), even though some clinical trials evidenced ineffectiveness of vitamin B6 supplementation on recurrence of cardiovascular events (30).

The experimentally MCT-induced HF have effect primarily on pulmonary vessels without an effect on systemic vessels and right heart hypertrophy (31). In our study, we observed an increasing trend in RV wall thickness in the MCT+B6 group compared to the C group. Moreover, Ki67 and PCNA positivity, both in the RV and LV wall, was increased in the MCT+B6 group compared to the control group. Those histomorphometric and immunohistochemical changes were accompanied with a significant increase in troponin T and impaired renal function. Although most of the investigated parameters of oxidative stress was not significantly changed in the heart failure rats in comparison with the healthy ones, a significant decrease in glutathione concentrations might contribute to disturbances in redox sensitive signaling pathways involved in the pathogenesis of RV hypertrophy. Moreover, some studies have demonstrated a higher RV susceptibility to oxidative stress damage in comparison with the LV (32). In our previous study, we found that supplementation with folic acid reduced cardiomyocyte proliferation in the RV wall even if it did not significantly reduce hypertrophy of the RV wall (33).

In our study, we observed no change in Hcy plasma level in all investigated groups. Vitamin B deficiency (primarily folate, vitamin B6 and B12) could result in hyperhomocysteinemia, via the obstruction of Hcy detoxification, leads to oxidative imbalance and overbalance in ROS production. ROS have induced the DNA base damage, DNA strand breaks, and accelerated telomere shortening (34). Some studies describing the relationship between Hcy and telomere shortening are in conflict. A certain number of these reported an inverse association between telomere length and Hcy (35), and others did not (36). The high level of plasma Hcy promotes chronic systemic inflammation through the oxidative stress induction in different types of tissue and a subsequent intracellular and extracellular damage (37, 38). That connection between Hcy and chronic inflammation, is not a one-way road. Steele et al. (39) have shown, through *in vitro* experiments, a concentration-dependent fashion. Pro-inflammatory cytokines (interleukin-1β (IL-1β) and TNF-alpha) have modified the cells' redox state and increased extracellular Hcy concentration. Furthermore, Ulvik et al. (40) have shown that systemic inflammation increased the catabolism of vitamin B6 and cellular uptake, and resulted in reduced B6 plasma concentrations. Moreover, vitamin B6 was also a strong predictor of death. In the crude model, subjects with the highest vitamin B6 concentrations had a 59% lower risk to die during follow-up. It has been shown that subjects with the highest age-corrected leukocyte telomere length (LTL) had a higher median concentration of vitamin B6 and, at the same time, a lower plasma Hcy concentration.

Current studies indicate ineffectiveness of vitamin B6 supplementation for the prevention of cardiovascular disease recurrence. Nevertheless, it remains open to determine whether vitamin B6 supplementation is effective for the primary CVD prevention. Given that vitamin B6 occurs through various physiological forms and in combination with other nutrients and other B vitamins which have a role in the complex vitamin B6 metabolism, the question arises whether supplementation with a single high-dose of vitamin B6 is effective as an adequate intake of this vitamin. More studies are needed to find the optimal, right dose and an adequate combination of vitamin B6 forms for maximizing the efficacy and minimizing detrimental effects, with respect to genetic susceptibility and environmental factors to properly estimate the role of B vitamins in the primary prevention of CVD.

CONCLUSIONS

In this study, we observed that four-week treatment with vitamin B6 had some beneficial effects on certain

cardio-metabolic biomarkers in monocrotaline-induced right heart failure. A four-week treatment with vitamin B6 significantly affected certain biomarkers like glucose, HDL cholesterol, aminotransferase, alkaline phosphatase and amylase activities, vonWillebrand factor activity, cardiac troponin T, urea and creatinine. The activity of SOD did not change, while GPX activity decreased in the MCT+B6 group. The total glutathione values and the content of thiol groups had a decreasing trend in the MCT+B6 group. The content of reactive carbonyl derivates showed an increasing trend, and the nitrotyrosine content did not change in the MCT+B6 group. The total glutathionylation level was decreased in the MCT+B6 group. We observed an increase in RV and LV wall thickness in the MCT+B6 group compared to the C group, as well as in Ki67 and PCNA positivity.

Acknowledgement

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Conflict of interest statement

The authors declare that there are is conflict of interest associated with this work.

Author Contributions

Conceptualization, A.S.R., J.J.U., T.D.; methodology, A.S.R., J.J.U., T.D., M.L.B., J.R., D.T.; software, D.T., I.N., M.D.; validation, A.S.R., T.D., S.S., M.L.B., J.R., N.M.; formal analysis, J.J.U., D.T., T.D., S.M., J.R., I.N., M.D., S.S., M.L.B., N.M., A.S.R.; research, J.J.U., D.T., T.D., J.R., I.N., M.D.; resources, A.S.R., S.S.; data curation, J.J.U., D.T., T.D., J.R., I.N., M.D.; writing-original draft preparation, J.J.U.; writing—review and editing, D.T., T.D., S.M., A.S.R.; visualization, J.J.U., D.T., T.D. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and the European Directive for welfare of laboratory animals No: 2010/63/EU. The study was approved by the Ethical Council for the Welfare of Experimental Animals, Ministry of Agriculture, Forestry and Water Management, Veterinary Directorate, Republic of Serbia (Number: 323-07-01339/2017-05/2).

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UTICAJ VITAMINA B6 NA MARKERE OKSIDATIVNOG STRESA, KARDIOMETABOLIČKE I HISTOLOŠKE MARKERE U USLOVIMA MONOKROTALINOM INDUKOVANE SRČANE SLABOSTI KOD WISTAR ALBINO PACOVA

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Sažetak

Srčana insuficijencija (HF) izazvana monokrotalinom (MCT) je dobro poznata po mehanizmima remodelovanja plućnih arterijskih krvnih sudova sa povećanom plućnom rezistencijom i povećanim markerima oksidativnog stresa. Svrha ove studije je bila da se potvrdi hipoteza da tretman vitaminom B6 može uticati na srčanu insuficijenciju modulacijom biomarkera kardiometaboličkog i oksidativnog stresa, i strukture srca pacova. Mužjaci *Wistar albino* pacova podeljeni su u 3 grupe: kontrola izložena praznom rastvoru (C fiziološki rastvor 1ml/kg 28 dana ip., n=8), B6 (vitamin B6 7mg/kg/dan 28 dana ip., n=8), i MCT+B6 (MCT 50 mg/kg jednom ip. plus vitamin B6 7 mg/kg/dan 28 dana ip., n=8). Četvoronedeljni tretman vitaminom B6 značajno je uticao na određene biohemijske parametre. Aktivnost ključnih antioksidativnih enzima, superoksid dismutaze (SOD) i glutation peroksidaze (GPx) nije se promenila, dok je ukupni glutation (GSH) značajno smanjen u grupi MCT+B6. Nakon toga je usledilo blago smanjenje nivoa ukupne glutationilacije, uočeno u grupi MCT+B6. Parametri oksidativnog oštećenja proteina (reaktivni karbonilni derivati, tiol grupe i nitrotirozin) nisu se značajno promenili u grupi MCT+B6. Uočen je trend povećanja debljine zida desne (RV) i leve (LV) komore u grupi MCT+B6 u poređenju sa kontrolnom (C) grupom, kao i povećanje Ki67 i PCNA pozitivnosti.

Ključne reči:srčana insuficijencija, monokrotalin, vitamin B6, oksidativni stres, pacov

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REVIEW ARTICLE

Perinatal complications following excisional treatment of cervical dysplasia

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Summary

Cervical cancer is one of the most common malignant tumors in women. Mass screenings have significantly decreased its incidence, while causing an increase in precancerous cervical lesions that are mainly diagnosed in women of reproductive age who still have not fulfilled their reproductive goals.

The aim of surgical treatment of these premalignant lesions is to prevent the development of cervical cancer, with minimal risks to the reproductive function. The most important perinatal complication is preterm delivery, usually coupled with preterm premature rupture of the membranes and chorioamnionitis. This results in prematurity with low birth weights, which can further result in increased neonatal morbidity and mortality. Data on the incidence of spontaneous miscarriages in treated women are non-consistent, however, it is believed that the incidence is higher in the second trimester.

Possible predictors of complications are the amount of excised tissue, the type of excision technique, age at the time of surgery, and the length of the period between treatment and conception. Re-excision of the cervix is an additional risk factor for perinatal complications. The risk of perinatal complications is the highest after cold knife conization, followed by laser conization, whereas LEETZ excision means the lowest risk – this is primarily explained by the variations in the cone size.

Having in mind that not all precancerous cervical lesions progress to cancer and that all types of excision treatments are associated with an increased incidence of perinatal complications, an adequate approach would entail primarily well-selected patients, i.e., treating only those women who are at real risk of developing cancer. The surgical treatment must be adapted to provide minimal risks for perinatal complications, maximal oncologic safety, and minimal risk of residual and/or recurring disease during a woman's lifespan.

Keywords: cervical dysplasia, excision, perinatal outcome, obstetric complications

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INTRODUCTION

Cervical cancer is one of the most common cancers in female population worldwide (1). It has been reported that the incidence and mortality rate of cervical cancer have declined in high-resource settings due to vaccination and screening programs as well as the timely treatment of precancerous cervical lesions diagnosed by screening (1). Moreover, mass screenings have resulted in a profound increase in the incidence of cervical premalignant lesions, which are now mainly diagnosed in women of reproductive age (2).

The mean age of women diagnosed with cervical intraepithelial neoplasia (CIN) grade 3 is nowadays 31-34 years in most populations (3). Timely treatment of these premalignant lesions is important for reducing the incidence of cervical cancer with minimal risks of adverse effects on the reproductive function, i.e., infertility, perinatal morbidity, and mortality (3, 4, 5). An increased risk of perinatal complications following cervical conization was demonstrated during the last century (6). Furthermore, surgical treatment of cervical premalignant lesions is associated with additional long-term morbidity in terms of the impairment of psycho-social and emotional well-being, changes in sexual life, which all additionally diminish reproductive outcomes and health-related quality of life (HRQoL) in women affected by cervical dysplasia during reproductive years (7-14).

This narrative review aims to discuss perinatal outcomes in women who underwent a surgical treatment of cervical precancerous lesions.

METHODS

Authors searched for the available data on obstetrical complications and perinatal outcomes in women treated for cervical precancerous lesions. We searched PubMed, Medline, EMBASE, and Cochrane Library from inception to July 2023. The key words used were "cervical dysplasia", "cervical intraepithelial neoplasia", "treatment", "pregnancy", "morbidity", "mortality", "pregnancy outcome", "perinatal complication", "obstetrical complication". Only full-length peer-reviewed articles concerning cervical precancerous lesions and perinatal outcomes and complications in women treated for cervical dysplasia were included in the review. Additional articles were identified from the reference section of relevant papers, based on the authors' estimation. We excluded non-English language studies, case reports and studies, book chapters, editorials, and letters.

BRIEF HISTORICAL OVERVIEW

The influence of cold knife conization (CKC) of the cervix on women's reproductive performance has been studied since the 1940s (15). Later studies asserted that CKC was associated with numerous perinatal complications (14, 17, 18, 19).

According to a report published in 1990, data on perinatal complications of large loop excision of the transformation zone (LLETZ) were still unavailable (20). Following initial reports on obstetrical harms caused by LLETZ, several studies indicated that although it was considered to be a small and safe surgical procedure causing a significant decrease in cervical cancer mortality, there was an increased risk of preterm delivery and subsequent complications after LLETZ (6,21). Nevertheless, most of the initial studies on perinatal morbidity associated with LLETZ were observational and conducted on a small number of patients, and the conclusions drawn were equivocal until 2006 when Kyrgiou et al. (18) published a systematic review and meta-analysis. Their publication included a total of 27 retrospective studies, of which ten provided data on the effects of LLETZ, ten on CKC, seven on laser conization, and four on laser ablation. Despite abundant interstudy heterogeneity related to laser conization, the results of the analysis showed that all the excisional procedures were linked to similar pregnancy-related complications. LLETZ was linked to a significantly higher risk of preterm delivery, low birth weight and preterm premature rupture of membranes (PPROM), whereas CKC was associated with a significantly higher risk of preterm delivery, low birth weight, and cesarean section (CS) rate. Importantly, apart from data on the increased rate of perinatal complications associated with cervical excision procedures, this meta-analysis indicated a link between the amount of the excised tissue and unfavorable obstetric outcomes, particularly in relation to cone depth above 10 mm. Moreover, the above-mentioned publication raised the question of perinatal morbidity following treatment with ablative surgical procedures and concluded that these methods also required further evaluation. Later meta-analyses investigated the influence of cervical excisional treatments on pregnancy outcomes (19,22). Two years later, a meta-analysis published by the same research group concluded that CKC and laser conization were connected with an increased risk of perinatal mortality and very low birth weight (<2000gr) in neonates (19). They found a significant association between CKC and perinatal mortality with a relative risk (RR) of 2.87, while LLETZ was also associated with adverse outcomes in relation to perinatal mortality, although without statistical significance - an RR of 1.17. The cited authors estimated that two perinatal deaths occurred in 1000 pregnancies after LLETZ treatment. Bruinsma et al. (22) conducted a meta-analysis aimed to investigate the association between different treatments of cervical precancerous lesions and a risk of preterm birth. These authors reported that excisional treatments of cervical lesions were associated with an increased risk of preterm birth (<37 weeks). Moreover,

the authors concluded that the presence of cervical dysplasia was a *per se* risk factor for preterm birth.

In the following years several researchers evaluated pregnancy-related morbidity associated with surgical treatment of cervical premalignant lesions, thus shedding more light on this topic (2,14,23). Later meta-analyses confirmed previously published results in relation to pregnancy outcomes and complications after excisional treatment of cervical premalignant lesions (14). Recent reports have indicated that even multiple cervical biopsies in women of reproductive age might influence preterm delivery rates in women who have undergone such procedures (24).

PROBABLE CAUSES OF PERINATAL COMPLICATIONS AND THEIR MECHANISMS OF ACTION

Although numerous studies have undoubtedly observed that excisional treatment of cervical precancerous lesions may lead to substantial complications during pregnancy and delivery, it is still not clear what are all the mechanisms that cause these complications (11).

Reduced cervical tissue mass after the treatment is not the only factor causing these complications (25). The removal of the cervical tissue following the use of any of the available excisional treatment techniques leads to structural changes in the uterine cervix. Its weakened mechanical role in supporting pregnancy, sometimes associated with damage in the internal os, together with changes in the cervicovaginal microflora and changed immune milieu of the cervical mucus are the most probable mechanisms causing changes in cervical function during pregnancy and delivery (12, 13, 15, 18, 26, 27). Apart from the procedure itself, it is postulated that sociodemographic, behavioral, and sexual characteristics of women affected by cervical dysplasia represent an additional risk factor for pregnancy complications (19).

Cervical conization is an established risk factor for cervical insufficiency and consequent preterm delivery (28). The removal of the cervical tissue leads to acquired weakening of the cervix and aberrant synthesis of collagen fibers in regenerated cervical stroma and subsequent reduced tensile strength, which is responsible for its premature dilatation in subsequent pregnancies (18, 29, 30).

Apart from the mechanical role of the cervical mucus plug, the antimicrobial activity of cervical mucus is also impeded by excision of cervical glands (31). Additionally, an insufficient amount of cervical mucus due to cervical glands excision contributes to easier migration of bacteria from the vagina (32). Shortening of the uterine cervix increases the risk of ascending infections and PPROM, chorioamnionitis, and the consequent preterm delivery (13). Nevertheless, literature data indicate that PPROM at <32 weeks of gestation is significantly more frequent in women with prior untreated cervical dysplasia than in general population (21).

The genetic basis for an increased risk of cervical precancer and cancer documented by recent genome-wide association studies indicates a possibility of diminished immune defense to HPV infection (33). This might be true for other infections as well, making these women susceptible to pathogens from the vagina causing chorioamnionitis and rupture of the membranes in pregnancy (30).

Vaginal microbiota (VMB) composition in women with cervical dysplasia is different than that in healthy population. Following cervical excision, VMB composition and levels of proinflammatory cytokines remain unchanged (34). This might account for an increased risk of preterm delivery noticed in women with cervical dysplasia, regardless of whether they were treated or not (21,35). This is the case for their risk of overall (<37 weeks of gestation), severe (<32-34 weeks of gestation), and extreme (<28-30 weeks of gestation) preterm delivery (2). Such a baseline risk could be increased with the treatment sequelae on cervical anatomy and physiology. Cervical microbial diversity is also changed following excision of the cervix, and chorioamnionitis represents one of the complications contributing to an increased risk of preterm delivery after cervical conization (2,35).

Cervical conization impairs local immunity as well (13,21). A decrease in the production of immunoglobulin A as well as lysosomal substances and an increased bacterial reproduction in the cervix and prostaglandin levels in the body observed after conizations could lead to preterm contractions of the uterus and, consequently, to a higher risk of premature labor and premature rupture of membranes (PROM) in patients after conization (36,37,38).

PREDICTORS OF OBSTETRIC COMPLICATIONS

Defining possible predictors of perinatal complications is essential for modern prenatal medicine. Hence, identifying women who are at risk of complications following cervical treatment is important for providing them adequate surveillance in pregnancy and during delivery.

So far, there are data indicating that the amount of excised tissue, the type of excision, age at treatment, and period after the operation play a role in the occurrence of subsequent maternal complications, perinatal morbidity, and mortality (2,13,21). Apart from the above-mentioned risk factors, repeated excisions represent an additional risk factor for the occurrence of perinatal complications (21). The effect of multiple treatments has been documented to be substantially higher in relation to preterm delivery (2,24).

Surgical techniques of excision associated with removing more tissue are linked to greater risks of perinatal complications (2). Of all conization techniques evaluated in literature, CKC is steadily associated with

major perinatal complications (19). Thus, a relative risk of preterm delivery is the highest after CKC, followed by laser conization, and the lowest risk is associated with LLETZ (2.70, 2.11 and 2.02, respectively). The use of CKC is accompanied with excision of more tissue than LLETZ. On the other hand, LLETZ excisions might differ substantially, varying from superficial and low volume excisions to deep and large volume ones (14). In relation to these discrepancies, data on pregnancy complications and outcomes may also exhibit different results. In women who had CKC, there is an additional risk of cervical lacerations during delivery due to grossly changed cervical anatomy and scarring. It has been documented that such injuries are eight times more frequent in these women than in general population (39).

Numerous publications investigated the association between the amount of removed cervical tissue and subsequent obstetric complications, and whether there is a predictive value of cone size in predicting unfavorable perinatal outcomes (2,13,40,41). Both cone volume and height have been investigated, as well as postoperative dimensions of the uterine cervix. Preterm delivery risk increases progressively with increasing cone depth (2,21).

Kyrgiou et al. (13) examined if excised cone dimension correlated with pregnancy duration. They measured pre-treatment and post-treatment dimensions and volume of the cervix using magnetic resonance imaging (MRI), three-dimensional transvaginal sonography (3D-TVS), and two-dimensional transvaginal sonography (2D-TVS) to measure cervical size. The dimensions of the cone and its volume were evaluated before formalin fixation. It was concluded that there were extensive variations in pre-treatment cervical dimensions, and the proportion of the excised tissue amount removed from the cervix correlates with the duration of pregnancy.

Cone height <10 mm provides adequate oncological safety, considering the depth of cervical crypts, and avoids obstetrical harms (5). Conization depth below 10 mm does not increase persistence or recurrence of pathological cytology during a 12-18-month follow-up. Also, HPV infection persistence is not influenced by conization depth below 10 mm during 18 months of postoperative follow-up. Greater depth of excision does not ensure more favorable oncological outcomes, while jeopardizing future reproductive performance. On the other hand, a cone depth >10 mm is associated with a significant increase in the rate of premature delivery, while cone depth >20 mm leads to a five-fold increased rate of premature delivery compared to general population (40). Cone height and gestational age at delivery are significantly correlated (17). This negative correlation is more consistent throughout the literature for cone height than it is for cone volume in relation to preterm delivery (17).

Age younger than 25 years at the time of LLETZ excision represents an independent risk factor for extremely early preterm delivery (before 26 weeks of gestation), regardless of specimen height (42). The association between age at conization, live birth rate and term deliveries was also documented for other types of cervical excisions (43). There are literature data showing that younger women had lower cervical regeneration levels when compared to older women (71%-78% and 89.5%-94.5%, respectively) (44).

Women who conceive within two to three months after the treatment are at an increased risk for preterm delivery (45). After CKC, the incidence of premature rupture of membranes (PROM) has been documented to be higher in pregnancies conceived within six months after the operation than in pregnancies conceived after six months (17). Some reports suggest that pregnancy should be postponed until 12 months upon LLETZ due to increased risk of spontaneous abortion in women who conceived less than 12 months after the procedure (46). According to literature, there is a cervical regeneration deficit, meaning that the average cervical length regeneration is 83.4±10.8% and volume regeneration is 87.4±6.1% six months after the LLETZ procedure (44). Some authors advise postponing pregnancy for at least six months after LLETZ and nine months after CKC (27). Postponing pregnancy after the operation reduces the incidence of perinatal complications, and postoperative contraception is generally recommended for the period of at least six months (17).

Ortoft et al. (21) investigated the effect of two conizations on preterm delivery and perinatal mortality in subsequent pregnancies. The frequency of preterm deliveries was 33% in patients who had had two conizations before pregnancy, and 19% of the children delivered by these women had body weight below 2500 grams. A total of 92% of all spontaneous preterm deliveries in this study were associated with PPROM.

OVERVIEW OF PERINATAL COMPLICATIONS

The most significant sequel of cervical conization is preterm delivery, frequently associated with PPROM and chorioamnionitis. Nowadays, preterm birth is a major cause of neonatal mortality and morbidity that may be linked to life-long disability. These together cause significant direct and indirect costs for the healthcare system and entire society. Moreover, worries about impaired reproductive performance lead to overall diminished HRQoL in women of reproductive age treated by cervical excision (7,11).

Data on miscarriage rates are inconsistent. While some studies documented increased abortion rates, others found them to be consistent with those of general population (17). So far, there are no data indicating that first trimester miscarriages are more frequent in women who have undergone treatment for cervical dysplasia (30,47). Some publications report increased rates of second

trimester abortions and ectopic pregnancies in women treated for cervical dysplasia (30,48).

It has been reported that the rate of cervical cerclage application has increased following conization, and it is higher in women treated with CKC than in those who had LLETZ (2). Its usefulness is controversial and routine prophylactic cervical cerclage is not recommended after CKC (17). Moreover, recently published research found that pregnancies after cervical conization were at an increased risk of preterm delivery, regardless of the prophylactic cerclage placement (49).

Women treated for cervical dysplasia with cervical excision are at an increased risk of preterm delivery, and its rates vary depending on the techniques used, mainly based on the amount of cervical tissue, in particular on the length of the excised cervical canal. Excisional surgery (laser, harmonic scalpel, electric knife) for cervical dysplasia is associated with an increased risk of preterm delivery of up to 25.3%, and this risk is increased before 32 weeks of gestation (49). The incidence of preterm delivery after CKC is also higher than in general population of healthy women (17). A relative risk for delivery before 37 weeks of gestation is the highest after CKC (2.70), followed by laser conization (2.11), whereas LLETZ has the lowest relative risk (1.56) (2). The influence of conization is the same for nulliparous and multiparous women. When comparing women with untreated cervical dysplasia and those who mostly had excisional treatment using LLETZ procedure, preterm delivery reported rates ranged from 3.9% to 11.1%, respectively (21). Frequencies of preterm delivery after cervical surgery vary throughout gestation as follows: 2.9% before 27 weeks, 5.7% between 28 and 31 weeks, 4.0% between 32 and 33 weeks, and 12.7 between 34 and 26 weeks (49). The frequency of preterm delivery in women who had two conizations prior to pregnancy is 33%. Following single excisional treatment, 72% of preterm deliveries start with PPROM; following two excisions, 92% of preterm deliveries start with PPROM (21).

The history of cervical conization has been established as a risk factor for PPROM (50). The reported rate after cervical excision is 13.13% (51). It is more frequent before 31weeks of gestation than between 32 and 36 weeks of gestation (49). The risk of PPROM and chorioamnionitis is higher after CKC than after LLETZ (2). Particularly, the incidence of PPROM is significantly higher in women who underwent CKC six months prior to pregnancy than in those who conceived six months or more after surgery (17). Moreover, when compared to healthy pregnant women, PPROM occurs in significantly earlier gestation in women who underwent CKC (17).

Cervical excision with LLETZ increases the rate of vaginal infections and premature rupture of membranes (PROM) (52). A recently reported frequency of PROM following cervical excision is up to 40% (51).

According to some reports, the CS rate is higher in women who had CKC when compared to healthy women, and it is documented to be as high as 36.4% (17). On the other hand, a recent report, despite having documented a significantly higher incidence of labor dystocia after excisional cervical treatment (15.94%), did not find an increased frequency of CSs in comparison with untreated women (51).

The incidence of neonatal complications has been documented to be significantly higher in women after CKC than in general population and is reported to be as high as 15.4% (17). Perinatal mortality after CKC treatment is significantly increased, while the numbers for laser conization and LLETZ, although increased, failed to reach the level of statistical significance in a meta-analysis published in 2008 (19). Cervical excision significantly increases the incidence of adverse neonatal outcomes, i.e., low birth weight, admission to a neonatal intensive care unit (NICU), and perinatal mortality (2). Recent research documented increased incidence of neonatal intrauterine infectious pneumonia following CKC (17). Perinatal mortality rate following single excisional treatment for cervical dysplasia is 1.0%, mainly in babies delivered before 28 gestational weeks (21).

Heinonen et al. (53) observed an increased risk of premature birth in women who were treated with LLETZ regardless the grade of CIN, and even in patients who did not have CIN lesions. The risk of preterm delivery in this study was 7.2%. The frequency of low-birth-weight neonates was also found to be increased.

Simoens et al. (54) found a significantly increased risk of spontaneous preterm delivery associated with cones more than 10 mm deep when compared to untreated women, while this risk was not significantly increased in case of cones ≤10 mm deep. The corresponding rates of spontaneous preterm deliveries were 20.9% and 8.3%, respectively. These data indicate once again that cones ≤10 mm deep are advisable in women of reproductive age to avoid prenatal complications in subsequent pregnancies. Giving priority to oncological safety in these women might lead to an increased rate of obstetrical adverse outcomes.

TOPICS FOR FUTURE RESEARCH

There are insufficient data about preventive measures to minimize adverse pregnancy outcomes after conization (47).

A recent meta-analysis evaluated usefulness of transvaginal cervical cerclage in singleton pregnancies following cervical conization (32). A total of nine studies comprising 3560 patients were included, of whom 605 patients had prophylactic cerclage. This research concluded that prophylactic transvaginal cerclage following conization increases the risk of preterm birth and PPROM. These results are in line with a meta-analysis

published in 2019, which also concluded that transvaginal cerclage placement in singleton pregnancies following conization did not decrease the rate of preterm births. One of the possible explanations is that braided suture as a foreign body influenced the vaginal microenvironment and the immune system (47). In conclusion, prophylactic transvaginal cervical cerclage increases the risk of preterm birth following conization, which is in line with results published later (49). On the other hand, targeted cerclage using monofilament suture material may reduce the incidence of preterm birth (47). This seems like a reasonable option for women adequately screened using transvaginal ultrasonography with cervical length below 25 mm before 24 gestational weeks. True benefits of such an approach require further investigation (30).

Data on other prospective treatments such as pessary and progesterone use are mainly lacking (48, 55). Data on transabdominal cerclage (TAC) placement are available for patients with extremely short cervix after radical cervical operations and repeated conizations, although some authors reported data on its use in patients after cervical myomectomy and conization for cervical dysplasia (56). Both laparotomic, laparoscopic and robotic approach can be used, and it can be performed during pregnancy and preconceptionally (57). This procedure requires at least two additional surgeries and is not widely used in patients treated with cervical conization, although TAC might present a useful tool in rare cases of deep and/or extensive cervical excisions complicated with cervical insufficiency and when other less invasive procedures have failed (58). In order to proclaim it as safe and feasible for this purpose, further research is required.

CONCLUSIONS

The association between cervical excisional treatment and perinatal morbidity and mortality is well established. Only a small number of cervical precancerous lesions will progress to invasive cancer. Different excisional treatments are associated with adverse obstetric outcomes and thus, proper management of these changes in women of reproductive age should be based on an adequate selection of women who are at a real risk of developing cervical cancer compared to those who are not at risk. The latter should be protected from overtreatment and its subsequent sequelae. There is evidence that excision of the uterine cervix in women of reproductive age is associated with a small, but real increase in risk of perinatal complications. Excisional treatment should be used only in women with a clear indication and who would benefit from it. Prior to these procedures, all women of reproductive age must be informed about the possible risks of adverse perinatal outcomes linked to excisional treatment of cervical precancerous lesions. Informed consent regarding the treatment must include information about possible long-term adverse effects regarding prenatal complications. Clinicians should be as conservative as possible when treating young women. Treatment of cervical premalignant lesions must be adjusted to minimize possible perinatal adverse outcomes, to provide maximal oncologic safety, and to minimize the rates of residual disease throughout a woman's life.

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PERINATALNE KOMPLIKACIJE NAKON EKSCIZIONOG LEČENJA CERVIKALNE DISPLAZIJE

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Sažetak

Karcinom grlića materice predstavlja jedan od od najčešćih malignih tumora u ženskoj populaciji. Masovna primena skringa dovela je do značajnog smanjenja njegove incidencije, uz istovremeni porast incidencije premalignih promena grlića materice. Ove promene se uglavnom dijagnostikuju kod žena u reproduktivnom periodu, kada većina njih nije završila sa reprodukcijom. Osnovni cilj hirurškog lečenja premalignih promena je sprečavanje nastanka karcinoma grlića, uz minimalne rizike po reproduktivnu funkciju.

Najvažnije perinatalne komplikacije su prevremeni porođaj, obično udružen sa prevremenom rupturom plodovih ovojaka i horioamnionitisom. Ovo ima za posledicu prematuritet i rađanje dece niske telesne mase na rođenju, što dovodi do povećanja neonatalnog morbiditeta i mortaliteta. Podaci o učestalosti spontanih pobačaja kod lečenih žena su nekonzistentni, ali se smatra da je učestalost spontanih pobačaja u drugom trimestru povećana.

Kao mogući prediktori pojave komplikacija navode se količina ekscidiranog tkiva, vrsta ekscizione tehnike koja je korišćena, životna dob u trenutku operacije i vreme proteklo od tretmana do začeća. Dodatni faktor rizika za nastanak perinatalnih komplikacija su reekscizije grlića materice. Rizik od perinatalnih komplikacija je najveći nakon konizacije nožem, zatim nakon konizacije laserom, a najmanji nakon ekscizije omčicom, što se prevashodno objašnjava razlikama u veličini konusa. Imajući u vidu da sve premaligne promene grlića materice neće progredirati u karcinom, kao i da su sve vrste ekscizionog tretmana povezane sa povećanjem učestalosti perinatalnih komplikacija, adekvatan pristup podrazumeva pre svega odgovarajuću selekciju pacijentkinja, odnosno lečenje samo onih koje su u realnom riziku od nastanka maligne bolesti. Hirurški pristup mora biti prilagođen tako da obezbedi minimalni rizik nastanka perinatalnih komplikacija, maksimalnu onkološku bezbednost i najmanji mogući rizik rezidualne i/ili rekurentne bolesti tokom života žene.

Ključne reči: cervikalna displazija, ekscizija, perinatalni ishod, akušerske komplikacije

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ORIGINAL ARTICLE

The association of R47H variant in the *TREM2* gene and genetic susceptibility to Alzheimer's disease in Serbian population

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Summary

Introduction: Alzheimer's disease (AD) is a chronic neurodegenerative disease, which is clinically manifested by the development of dementia. Studies of genetic susceptibility to AD indicate a whole range of genes and their variants that can potentially influence an individual's susceptibility to develope the disease. AD17 represents a form of Alzheimer's disease associated with mutation(s) in the *TREM2* gene, encoding triggering receptor expressed on myeloid cells 2. The aim of this study was to determine the frequency of R47H variant of the *TREM2* gene in the population of AD patients, to compare the frequency of the variant in the population of AD patients and the control group, and to determine a possible association of a certain genotype with susceptibility to AD.

Material and Methods: The study included 168 consecutive patients with AD and 190 healthy controls. The clinical inerview, neurologic examination, and neuropsychological set of cognitive assessment were performed by neurologists and neuropsychologists in expertise with neurodegenerative deseases. Genotyping of rs75932628, R47H polymorphism of the *TREM2* gene was performed using Real-time Polymerase Chain Reaction and TaqMan® SNP genotyping assay (Applied Biosystem by Thermo Fisher Scientific, USA) according to the manufacturer's recommendations.

Results: In the group of AD patients the frequency of C allele was 98.8%, while the T allele was present in 1.2% of patients. The frequency of the T allele was statistically significantly higher among the AD population than among the control group ($p<0.05$). The frequency of homozygotes without mutation (CC genotype) was 97.62%, while the frequency of heterozygotes for the mutation (CT genotype) was 2.38% among patients with AD, and the frequency of homozygotes without mutation (CC genotype) was 100% among healthy controls.

Conclusion: Our study indicated a possible association of the heterozygous form of the R47H variant of *TREM2* gene with the susceptibility for the developement of AD in Serbian population.

Key words: Alzheimer's disease, polymorphism, prevalence, *TREM2*, R47H

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INTRODUCTION

Alzheimer's disease (AD) is a progressive chronic neurodegenerative disease, which is clinically manifested by the development of dementia (1). As a neurodegenerative disease, AD affects multiple brain functions, which causes a range of signs and symptoms that include a progressive loss of mental and intellectual functions that disrupt daily life, an early occurrence of disorders affecting executive functions, depression, insomnia, anxiety, agitation, and behavioral impairment (2). There is no simple and reliable test applicable for diagnosing AD, thus its course is followed based on clinical observations, cognitive testing, neuropsychological testing along with important neuroradiological procedures (e.g., CT, NMR, PET, PET-CT, etc.). The disease is classified as multifactorial, involving different causes, such as genetic predisposition, environmental factors, and lifestyle habits (3). The most important known risk factor for late onset Alzheimer's dementia is ageing (4). There are several etiopathogenetic mechanisms, and the most supported one is the amyloid hypothesis. Amyloid plaques represent extracellular formations, amyloid – (Aß) peptide being their major component. Aß peptide is produced through the proteolytic processing of amyloid precursor protein (APP) by β and γ secretases. Deposition of Aß peptide in extracellular space (ECS) results in neurotoxicity, it promotes apoptosis, and increases synthesis of oxidative stress mediators (5). On the other hand, neurofibrillary tangles (NFTs) are intracellular insoluble aggregates of hyperphosphorylated tau protein, that affect neurons in different regions of the brain. The result of intracellular deposition NFTs is axonal instability that impairs transport of nutrients along with cell signal communication, neurodegeneration, and apoptosis (6,7).

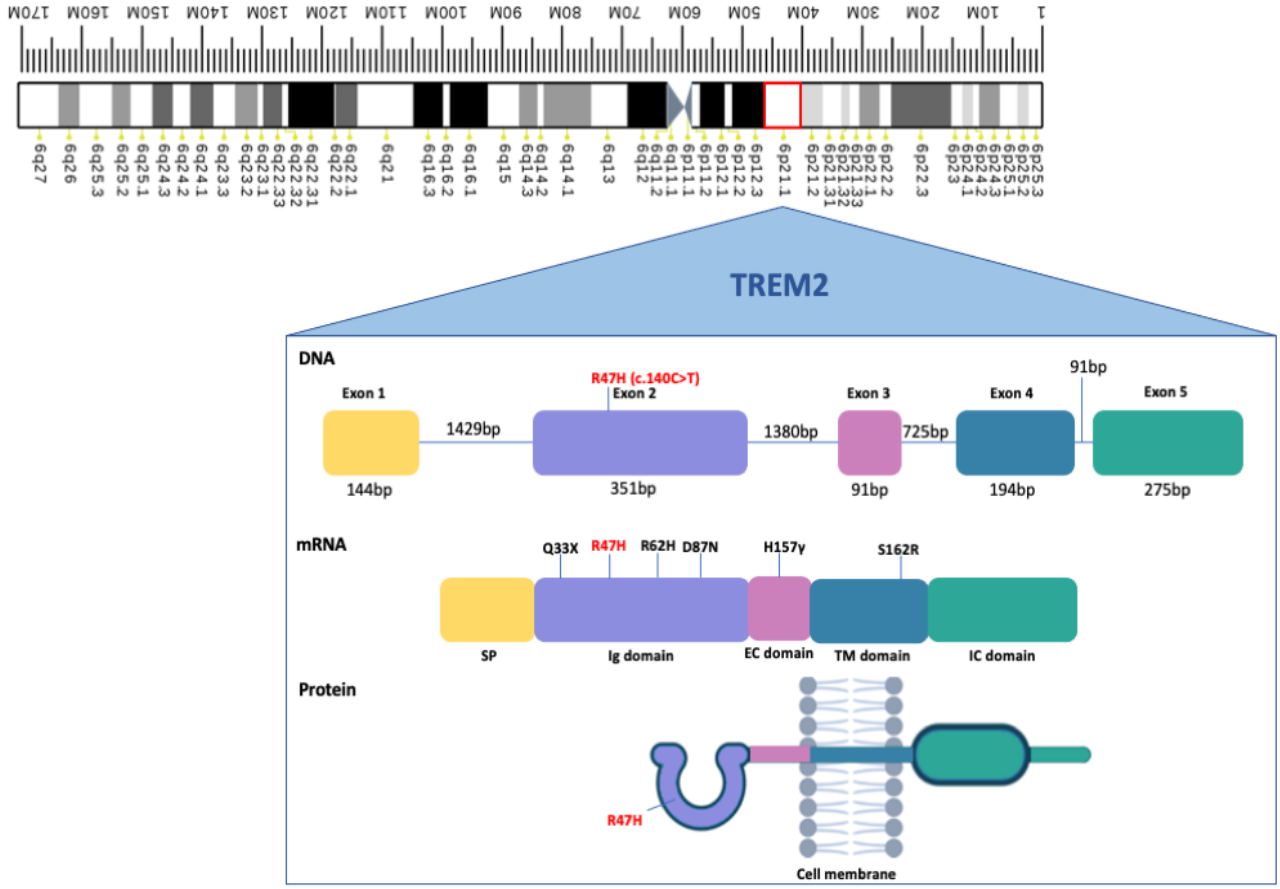

Image 1. Ideogram of chromosome 6 with schematic representation of the human TREM2 gene and protein structure, modified according to Yang et al (11).
The TREM2 gene is located on chromosome 6 in the region 6p21.1. It posses 5 exonic regions (boxes) and 4 intronic regions (lines). The mRNA is shown with transcribed regions, and different colors represent different exonic sequences of the transcription template. Most frequent mutations of the TREM2 gene occur within its exons, some of them being: R47H, Q33X, R62H, D87N, H157γ, S162R, described on the schematic representation of mRNA. Point mutation within the variable region of the TREM2 gene results in the loss of function, decreased ligand binding, disturbances in signal transmission and reduced expressions. The variant R47H of the TREM2 gene implies the existence of point mutation at position (c.140C-T), leading to a modification in the exon 2 domain. During translation, histidine is replaced by arginine at position 47 of the protein. The rs75932628 (R47H) shows an odds ratio (OR) range of 2.0-5.0, and it also has minor allele frequency (MAF) about < 1 % (26)
SP – signal peptide, Ig domain – immunoglobulin domain, EC domain – extracellular domain, IC domain – intracellular domain, TM – transmembrane domain.

Studies of genetic susceptibility to AD indicate a whole range of genes and their variants that can potentially influence an individual's susceptibility to develop the disease. Certain protein-coding genes, such as *TREM2, CD33, CR1, ABCAT, SHIP1, PSENI, PSEN2*, are in close relation with microglia, and they influence immune regulation and inflammatory response. Mutations in these genes are also linked with increased susceptibility for AD (8–10).

Triggering receptor expressed on myeloid cells 2 (TREM2) is a protein coded by *TREM2* gene. The *TREM2* gene is located on an autosomal locus, and it is mapped on the region 6p21.1 (11). TREM2 is a transmembrane glycoprotein which includes 227 amino acids, and consists of an extracellular region, the membrane-traversing segment, and an intracellular region. TREM2 is expressed on the myeloid cells' membrane, granulocytes, macrophages, immature monocyte-derived dendritic cells, osteoclasts, alveolar macrophages, Kupffer cells, and microglia, which are immune cells in the central nervous system (CNS). The ectodomain of TREM2 includes an Ig-like V-type domain. The intracellular region has signal function via DNAX activator proteins. Ectodomain shows affinity to binding ligands such as glycoproteins, lipids, lipopolysaccharides (LPS), high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein A1 (ApoA1), apolipoprotein A2 (ApoA2). apolipoprotein B (ApoB), clusterin or apolipoprotein J (ApoJ), and apolipoprotein E (ApoE) (12).

A large number of frameshift, and missense mutations with different changes in the expression in the *TREM2* gene have been described in association with neurodegenerative diseases (13). Most of mutations were located in a coding sequence, yet those in non-coding regions have also been described. It has been observed that certain polymorphisms of *TREM2* gene can increase the risk of some neurological diseases in particular AD, posterior cortical atrophy (PCA), essential tremor (ET), multiple sclerosis (MS), progressive non-fluent aphasia (PNFA), Parkinson's disease, and Lewy body dementia (14). AB17 is another recognizable entity of Alzheimer's disease that is solely associated with mutations in the *TREM2* gene.

The aim of this study was to determine the genotype of R47H variant of the *TREM2* gene in the population of AD patients, and to compare the frequency of the variant in the population of AD patients and healthy controls, as well as to determine the possible association of a certain genotype with susceptibility to develop the disease.

MATERIALS AND METHODS

Patients and methods

The study comprised 168 consecutive patients with Alzheimer's disease, and 190 healthy controls. Clinical diagnosis of AD was assesed according to the valid diagnostic criteria (15). A clinical interview, a neurological examination, and neuropsychological testing were performed by a neurologist, with subspecialization in neurodegenerative dementia, and a clinical neuropsychologist, respectively. All subjects fulfilled the same examination protocol. For the purposes of our study global cognitive assesement by Mini Mental State Examination Test (MMSET) and Goldman score were investigated.

The MMSET systematically assesses the cognitive status of a patient. This test provides an insight into eleven domains of cognitive functioning, including temporal orientation, spatial orientation, immediate orientation, attention/concentration, calculation, delayed recall, naming, verbal repetition, verbal comprehension, writing, reading a sentence, and constructional praxis. The maximum number of points is 30, and a score of 23 or less indicates a cognitive impairment.

A Goldman score (GS) was assigned to each subject according to family medical history in order to indicate the pattern of inheritance. Goldman score 1 denotes the presence of at least three affected individuals in two generations on the genealogical tree (where one person is a first-degree relative to the other two affected individuals); Goldman score 2 is a family aggregation of three or more family members with dementia who do not meet the criteria for score 1; Goldman score 3 refers to the existence of one affected family member with early-onset dementia; Goldman score 3.5 is assigned if the disease has occurred after the age of 65 in the affected family member (late-onset dementia); Goldman score 4 is in case of unknown medical history or in families with insufficient data (16).

Genetic investigation

During hospitalization, every patient was venepuctured to obtain 10 ml of peripheral blood. After sampling, sodium citrate was added to the blood at a concentration of 0.38% (w/v), and then the samples were stored at -20°C until the analysis. Genomic DNA was isolated from the peripheral blood leukocytes using the *PureLink™ Genomic DNA Mini Kit (Life Technologies, USA)*. Genotyping of rs75932628, R47H polymorphism of the TREM2 gene was performed using *Real-time Polymerase Chain Reaction (qPCR)* and *TaqMan® SNP genotyping assay (Applied Biosystem by Thermo Fisher Scientific, USA*). The reaction mix in the total volume of 15 µl consisted of 5 µl Taqman genotyping master mix, 0.75 µl Taqman SNP assay (20x), a total of 1 µl DNA sample and 5.75 µl water.

PCR temperature profile was as follows: one cycle of initial step of 10 min at 60°C to activate the chemically modified *Taq DNA polymerase,* followed by 40 cycles of denaturation for 15 s at 95°C, and subsequent hybridization and extension at 60°C for 1 minute. The initial phase of data processing from molecular analysis was

performed by Life Technologies Real Time PCR software, which shows allelic discrimination data results as a scatter plot of allele 1 (VIC® stain) versus allele 2 (FAM® stain). Each reaction well of the plate is represented as a single point on the graph, which allows the identification for the following genotypes of the R47H *TREM2* gene variant: CC – homozygous without mutation, CT – heterozygous for mutation, TT – homozygous for mutation.

Statistical analyses

For data analysis, selective methods of descriptive statistics were used: measures of central tendency (arithmetic mean, median), variability measures (standard deviation, range), and structure indicators (absolute and relative numbers). In order to compare differences in allele frequency, Pearson's chi-squared test with Yates correction or Fisher's exact test were employed. To test the statistical significance of the association between most frequent alleles and clinical presentation, an odds ratio (OR) was calculated with 95% confidence interval (CI). The Mann-Whitney U Test was used to evaluate statistically significant differences among MMSET and Goldman score. Spearman correlation coefficient was computed to assess the relationship between the MMSET score and the level of education. The criteria for the significance of the statistical differences were $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

RESULTS

Demographic and clinical characteristics

The study included 168 consecutive patients with the Alzheimer's disease (mean age of onset 57.6±6.7 years, 54.8% for women and 45.2% for men), and 190 healthy controls. The youngest patient was diagnosed with AD at the age of 35, while the oldest patient was diagnosed with AD at the age of 83. The duration of the disease from the time of diagnosis to the inclusion in the study was 3.6±2.1 years. The early onset form of AD occurred in 91.7% of patients, while the late onset form occurred in 8.3% of patients. The average years of education of our patients are shown by class intervals. Among patients, 33% had 0-8 years of education, 42.9% had 8-12 years of education, and 23.2% had more than 12 years of education. The presence of neurological and non-neurological comorbidities occurred in 39.9%, while 60.1% of respondents had only cognitive issues as clinical presentation.

Analyzing the Goldman score, we determined that 4.2% of patients had GS 1; 1.8% had GS 2; 8.3% had GS 3; 22.6% of patients had GS 3.5; and finally, 63.1% of patients received score 4 **(Table 1)**.

Table 1. Demographic and clinical characteristics of AD patients

Variable		AD
The size of the sample (n)		168
[a] Age (years)		57.63±6.71
[a] Time elapsed from onset to diagnosis (years)		3.64±2.12
[b] Gender	Male	76 (45.2%)
	Female	92 (54.8%)
[b] Level of education	0-8 years	57 (33.9%)
	8-12 years	74 (42.9%)
	>12 years	39 (23.2%)
[b] Goldman score	1	7 (4.2%)
	2	3 (1.8%)
	3	14 (8.3%)
	3.5	38 (22.6%)
	4	106 (63.1%)
[b] Comorbidities	Positive	67 (39.9%)
	Negative	101 (60.1%)
[b] Clinical presentation	Early onset (Early AD)	154 (91.7%)
	Late onset (Late AD)	14 (8.3%)

[a] Mean (± SD)
[b] absolute frequency (relative frequency in %)

MMSE test scores varied greatly among patients. The MMSE test score ranged from 3/30 to 28/30, and the average value of the score was 15.43±6.60. About 50.6% of patients had the MMSE test score <15 **(Table 2)**.

Table 2. MMSET score in AD patients

Variable			Value
[b] The size of the sample (n)			168
[a] MMSET score			15.43±6.60
[b] MMSET score	28-30	Normal	4 (2.4%)
	25-27	Minimal cognitive impairment	11 (6.5%)
	21-24	Mild cognitive impairment	32 (19.1%)
	16-20	Moderate cognitive impairment	36 (21.4%)
	<15	Severe cognitive impairment	85 (50.6%)

[a] Mean (± SD)
[b] absolute frequency (relative frequency in %)

The difference in MMSE test score values among different genotypes of the R47H variant of AB patients did not reach statistical significance (p=0.3786). The comparison of the Goldman score among different genotypes of the R47H variant did not reveal any statistically significant differences (p=1) **(Table 3)**.

A higher score of the MMSE test score at the beginning of the disease was statistically significantly associated with a higher level of education in patients (r=0.3144; p=0.00003; r2=0.0988). Patients with higher educational level exhibited significantly higher MMSE test score values at the beginning of the disease **(Image 2)**.

Table 3. The MMSET score and Goldman score among different genotypes of the R47H variant of AD patients

Score	AD (genotype CC)	AD (genotype CT)	p
	Mean	Mean	
MMSET	**The size of the sample, n=164**	**The size of the sample, n=4**	
	15.5	12.5	**0.3786**
Goldman	**Sthe sze of the sample, n=164**	**The size of the sample, n=4**	
	4	4	**1**

For comparing statistical significance in difference of MMSET and Goldman score *Mann-Whitney U Test* was used (*p<0.05, **p<0.01, ***p<0.001).

Molecular genetics analysis

In the group of AD patients, the frequency of the C allele was 98.8%, while the T allele was present in 1.2% of patients. Within the control group, the frequency of the C allele was 100%, while the T allele was not found in any of the healthy subjects. The frequency of heterozygous carriers of the R47H variant of the TREM2 gene in the group of patients differed significantly from the frequency in the control healthy population of Serbia (p<0.05; OR 0.10; 95% CI 0.01-1.81) **(Table 4)**.

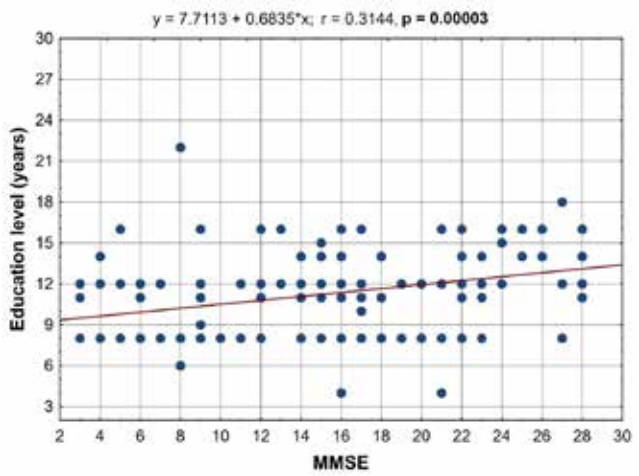

Image 2. Correlation between MMSET score and years of education in AD patients
A bivariate scatterplot with a smooth regression line shows the interrelationship between the MMSET score and the level of education expressed in years of education. Correlation analysis was performed using the Spearman Rank test, which shows the existence of a statistically significant positive relationship between the examined variables. The level of statistical significance was p<0.001.

The frequency of homozygotes without mutation (CC genotype) was 97.62%, the frequency of heterozygotes for the mutation (CT genotype) was 2.38% **(Image 3)**.

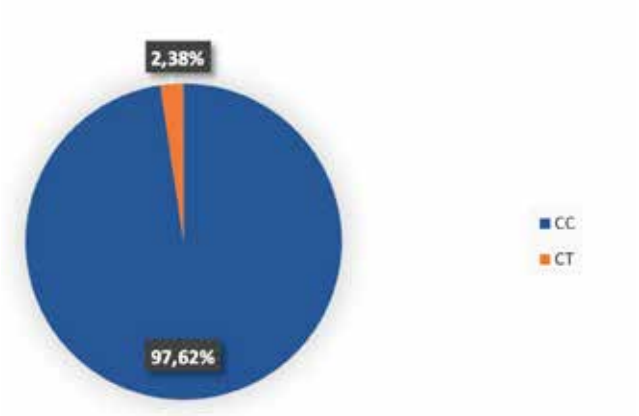

Image 3. Frequency of CC, CT genotype of variant R47H among patients with AD

DISCUSSION

According to our knowledge, this research is the first original investigation which analyzed distribution of alleles of R47H variant of the *TREM2* gene in patients with AD.

Recent clinical studies have shown that certain variants of the TREM2 gene could have a significant impact on the occurrence of dementia, in addition to the development of clinical presentation typical for AD (1,8,10).

In our study group, cognitive performance on the MMSET among subjects with a low level of education was shown to be worse compared to subjects with a higher level of education. These findings could be explained by cognitive reserve theory. Interestingly, as brain development

Table 4. Frequencies of C and T alleles of the R47H *TREM2* gene variant among AD patients and controls

Allele	AB	Control group*	Odds ratio (95% confidence interval)	p
	Allele frequency (%)	Allele frequency (%)		
C				
c.140C	98.8	100	0.10 (0.01-1.81)	***0.0329**
T				
c.140C>T	1.2	0	0.10 (0.01-1.81)	***0.0329**

* Healthy population of Serbia, 190 persons
In order to assess the statistical significance of the difference in relative allele frequency (%) Fisher's exact probability test was used (*p<0.05, **p<0.01, ***p<0.001).

proceeds, cognitive reserve tends to increase primarily early in life through young adulthood, and then tends to deplete later in life. However, as formal education affects cognitive abilities, and consequently the achievement of certain values on the MMSET, people with a lower level of education can potentially be misidentified as being at risk of developing dementia (17,18). An early onset of AD (between 30–50 years of age) is associated with the existence of autosomal dominant mutations within the genes encoding amyloid precursor protein (APP), presenilin-1 (PSEN1) and presenilin-2 (PSEN2) (19–21). Palonev and colleagues (Palonev J, et al. 2002) showed that the mutated homozygous form of the R47H variant of the *TREM2* gene led to the dysfunction of the TREM2 protein, which is associated with the development of various forms of dementia, indicating a great importance of the TREM2 signaling molecule in brain immune homeostasis (22). A meta-analysis by Lu Y, et al. pointed out that the R47H variant of the TREM2 gene increased the risk of developing AD up to 2.70 times (23). Research of Cruchaga et al, which examined the association of the R47H variant with the progression of AD, showed that there was an association of this variant with a faster progression of AD along with the findings of high levels of hyperphosphorylated tau in the cerebrospinal fluid of AD patients. Later studies reported similar results (24, 25). Furthermore, two independent studies indicated that there was an association between the rare heterozygous form of the R47H variant of the TREM2 gene with the development of AD, and subsequent studies confirmed this observation in the population of Spain and France. Benitez et al. also analyzed the frequency of the R47H heterozygous variant among AD patients of the Spanish population, and it was estimated to be 7/550 (1.27%) (26,27). In one genome-wide association study conducted on 3550 AD patients and a large number of controls in Iceland, Jonsson et al. found a significant association between the T allele of the R47H variant of the TREM2 gene (rs75932628) and the risk of developing the disease (p= 3.42×10(-10); OR 2.92; 95% CI 2.09-4.09); this association was stronger when healthy control subjects were older than 85 yrs; there were four subjects who were homozygous for the TT genotype mutation, while AD was confirmed *postmortem* in the two of them (28). Yet, one association study of Ma J et al. conducted in 279 patients with late-onset AD and 346 controls in Chinese population concluded that no T allele of rs75932628T was found neither in patients nor in controls (29). High-throughput sequencing of the TREM2 gene in 988 patients with late-onset AD and 1354 healthy controls of Chinese origin by Jiang et al. identified four rare coding variants, and then showed that the H157Y variant (rs22342555) represented a risk factor for the development of the disease (30).

Among our AD patients there were 4 heterozygous patients (genotype CT), as well as 164 homozygotes without mutation (genotype CC), while homozygotes for a mutation (genotype TT) were absent. The frequency of allele C was 97.62%, and the frequency of allele T was 2.38% among patients with AD **(Image 3)**. Within the control group, the frequency of allele C was 100%, while the T allele was absent. Compared to the healthy population, the heterozygous form of this mutation was found significantly more often among patients with AD **(table 4.)**.

CONCLUSION

Currently, neurologist dealing with AD lack a causal therapeutic intervention for their patients. The etiological cause of AD is notably diverse, encompassing both early-onset familial and late-onset sporadic variants, each bearing a distinct genetic basis. Early-onset familial AD pathogenesis is tangled and intertwined with an autosomal dominant inheritance pattern governed by three salient genes: *APP*, *PSEN1*, and *PSEN2*. The most ubiquitous sporadic form of AD, typically manifesting subsequent to the age of 60, has consistently, across a multitude of empirical investigations, been linked to the involvement of a single gene, the apolipoprotein E (*APOE*) gene. While extensive investigations have probed the mechanistic roles played by these genes in the pathogenesis of Alzheimer's disease (AD), the biological basis governing the progression of AD has yet to be definitively characterized. Notably, a hypomorphic variant identified within the microglial receptor *TREM2*, denoted as R47H, has demonstrated a marked escalation in the risk for AD. This study which was conducted on Serbian peatients indicated this possible influence of the heterozygous R47H variant of the *TREM2* gene in susceptibility to develop the disease.

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STUDIJA POVEZANOSTI VARIJANTE R47H GENA *TREM2* SA GENETIČKOM PODLOŽNOŠĆU ZA ALCHAJMEROVU BOLEST U POPULACIJI SRBIJE

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Sažetak

Uvod: Alchajmerova bolest (AB) je hronično neurodegenerativno oboljenje, koje se klinički ispoljava razvojem demencije. Zastupljenost genetičkih faktora rizika kao i njihova uloga u patogenezi AB nisu do kraja razjašnjeni. Istraživanja u oblasti genetičke podložnosti za AB ukazuju na postojanje čitavog niza gena i njihovih varijanti, koje potencijalno mogu uticati na sklonost pojedinca da razvije AB. AB17 je entitet Alchajmerove bolesti koji se povezuje sa mutacijama u genu *TREM2* koji kodira okidački receptor mijeloidnih ćelija tip 2.

Cilj ovog istraživanja bilo je ispitivanje učestalosti varijante R47H gena *TREM2* u populaciji obolelih od AB, poređenje učestalosti ispitivane varijante u populaciji ispitanika i zdravoj populaciji, kao i utvrđivanje rizika za oboljevanje kod nosilaca određenog genotipa.

Materijal i metode: U studiju je uključeno 168 konsekutivnih ispitanika sa dijagnozom AB i 190 zdravih kontrola. Klinički intervju, neurološki pregled i neuropsihološki set testiranja obavljeni su od strane neurologa i neuropsihologa koji se bave neurodegenerativnim bolestima. Genotipizacija rs75932628, R47H polimorfizma *TREM2* gena vršena je korišćenjem metode *PCR* u realnom vremenu (engl. *Real-time Polymerase Chain Reaction, qPCR*) i *TaqMan® SNP genotyping* eseja (*Applied Biosistem by Thermo Fisher Scientific, USA*).

Rezultati: U grupi ispitanika učestalost alela C iznosila je 98,8%, dok je T alel bio zastupljen kod 1,2% pacijenata. Učestalost T alela bila je statistički značajno veća u populaciji obolelih nego u kontrolnoj grupi ($p<0,05$). Učestalost homozigota bez mutacije (genotip CC) iznosila je 97,62%, učestalost heterozigota za mutaciju (CT genotip) iznosila je 2,38% unutar grupe obolelih od AB, dok je učestalost homozigota bez mutacije (genotip CC) iznosila 100% unutar zdrave kontrolne grupe.

Zaključak: Naša studija ukazala je na mogući uticaj ređeg alela varijante R47H gena *TREM2* na razvoj AB u našoj populaciji.

Ključne reči: Alchajmerova bolest, polimorfizam, prevalenca, *TREM2*, R47H

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REVIEW ARTICLE

Celiac disease in children

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Summary

Celiac disease is a multisystemic autoimmune disease induced by gluten in wheat, rye, and barley. It is characterized by polygenic predisposition, high prevalence in members of the Caucasian race (1%), especially in close relatives (5-15%), very heterogeneous expression, and frequent association with other autoimmune diseases (3-10%), as well as selective deficiency of IgA and Down, Turner, and Williams syndromes. The basis of the disease and the key finding in its diagnostics is symptomatic or asymptomatic inflammation of the small intestinal mucosa, which is resolved by a gluten-free diet. Accordingly, the basis of the treatment involves an elimination diet, so the disorder itself, if timely recognized and adequately treated, is characterized by an excellent prognosis.

Keywords: celiac disease, children, clinical forms, diagnostics, therapy

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INTRODUCTION

Celiac disease (CD) is a systemic autoimmune disease induced by gluten found in wheat, rye, and barley in genetically predisposed individuals (1, 2, 3). It occurs in all population groups and, most often, in members of the Caucasian race (1:100). (3-6). Like other autoimmune diseases, it is more frequent in females than in males (1.5:1 to 2:1) (7, 8, 9). It is prevalent in first- and second-degree relatives (5-15%) (10). With slightly lower frequency (3-10%), it also occurs in patients with other autoimmune diseases, selective IgA deficiency, and Down, Turner, and Williams syndromes (1, 3, 6, 11-15).

Non-specific inflammation of the small intestinal mucosa resolved on a gluten-free diet represents the main feature of the disease and the basis of its diagnosis (1, 3, 6, 16, 17). In addition to damage of the small intestinal mucosa, which can be symptomatic or asymptomatic, the disease is also characterized by numerous extraintestinal manifestations and, in cases diagnosed too late or treated inconsistently, by potentially severe complications (3, 4, 9, 18-24).

PATHOGENESIS

The pathogenetic basis of CD is a polygenic predisposition and exposure to gluten (3, 25). In addition to gluten, gastrointestinal infections, alterations in the gut microbiota, some medications, and other factors play an important role in the onset of the disease, which explains its incomplete prevalence in monozygotic twins (83-86%) (3, 10, 11, 25, 16). Evidence point to the principal role of polygenic inheritance in the occurrence of the disease, its highly variable frequency in different populations, as well as its high presence in identical twins and first-degree relatives (~10%) (10, 11, 13, 26). The HLA class II genes DQ2 and DQ8 (6p21.32), present in 98-99% of patients, play a central role in hereditary predisposition to the disease (6, 10, 25, 27). HLA DQ2 molecules are registered in 85-95% of patients, and HLA DQ2 in 5-15% of patients (28, 29). However, apart from HLA DQ2 or DQ8 genes and exposure to gluten, the presence of one or more of approximately 40 non-HLA genes that have been verified so far is indispensable for the onset of the disease (3, 11, 13, 25).

The importance of DQ2 and DQ8 glycoproteins present on antigen-presenting cells (dendritic cells and macrophages) in the pathogenesis of CD is reflected in their ability to activate intestinal CD4+ T-cells after binding with deaminated gluten polypeptide hydrolysates (2, 3, 11). The deamidation of gluten hydrolysates, which increases their binding affinity to HLA DQ2 and DQ8 molecules, is performed by tissue transglutaminase (tTG). Proinflammatory cytokines released by activated CD4+ T-cells activate intraepithelial cytotoxic CD8+ T-cells, which lead to enterocyte apoptosis and infiltrative or infiltrative-destructive inflammation of the small intestine mucosa. They also lead to the differentiation of B lymphocytes into plasma cells, as well as to the production of antibodies against gluten peptides and autoantibodies to tTG, endomysium and other body structures (10, 25).

THE ENTEROPATHY

Enteropathy, a structural injury to the small intestine mucosal layer, mainly affects the duodenum and the proximal jejunum, and this process severity progressively decreases towards the ileum (30). Sometimes, however, this mucosal lesion may only affect the duodenal bulb (1, 6, 16). The three primary, distinctive forms of this inflammatory process in the mucosa of the small intestine, defined by modified Marsh criteria, are infiltrative (I), infiltrative-hyperplastic (II), and destructive (III) (31). In the infiltrative form, there is stromal lympho-plasmocytic infiltration, accompanied by an increase in the number of intraepithelial γ/δ lymphocytes. At the same time, alterations in the height of the intestinal villi and crypt depth do not occur. In the second form, in addition to the more pronounced infiltration, hyperplasia of the crypts is observed. In contrast, in the third (destructive) form, the additional, accentuated infiltration and hyperplasia of the crypts, with shortening and/or loss of villi, occurs. According to the degree of mucosal damage, destructive enteropathy is further classified into partial (IIIa), subtotal (IIIb), and total (IIIc) **(Figure 1)**. Apart from this, the fourth form of damage may also occur, and it is characterized by complete atrophy of the villi, but with no crypt hyperplasia and typical signs of mucosal inflammation.

CLINICAL FORMS OF THE DISEASE

Considering the aspect of manifestation, there are two primary clinical forms of CD: symptomatic and asymptomatic (subclinical) (1, 2, 16). Within the framework of symptomatic disease, forms with classical and non-classical clinical presentation are distinguished (1, 2, 16). The characteristics of classical celiac disease are chronic diarrhea, malabsorption, and consequent malnutrition. Extraintestinal manifestations are most conspicuous when it comes to the clinical presentation of the non-classical disease (1, 2, 16, 22, 23). The classical celiac disease form most often occurs in infants and young children. The non-classical disease form occurs in later ages and in adults (11, 16, 23). In the symptomatic form of the disease, the autoantibodies, anti-transglutaminase (AtTG), and anti-endomysial (EMA) antibodies, as well as the HLA DQ2 and DQ8 genotype, are almost regularly registered in addition to the evident enteropathy (1, 2, 12, 27, 29). However, despite the presence of all these indicators,

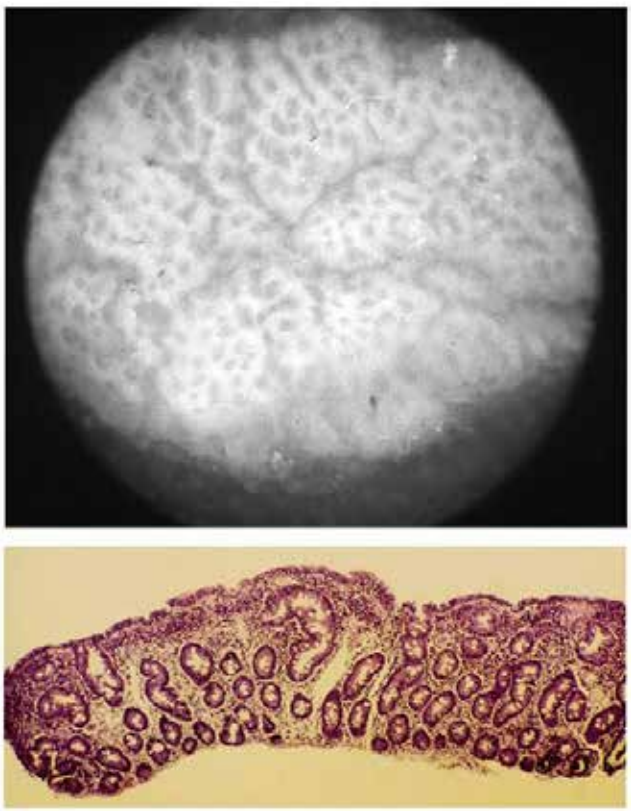

Figure 1. The stereomicroscopic and pathohistological appearance of the mucosa of the small intestine with the most severe degree of damage (Marsh IIIc). The stereomicroscopic image shows the lack of villi with crypt openings, and the pathohistological image shows hyperplasia of the crypts with pronounced lymphoplasmacytic infiltration of the lamina propria. (Original recordings by the authors.)

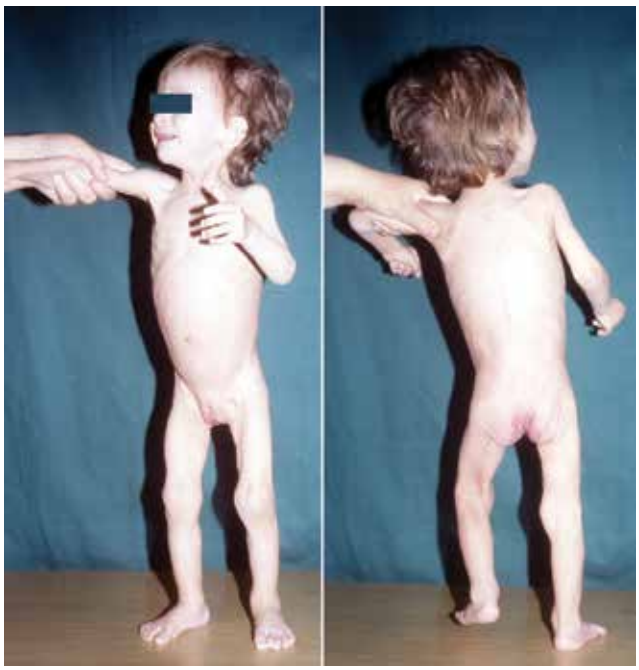

Figure 2. A 20-month-old girl with the classical clinical form of celiac disease. In addition to the typical clinical aspect, there is a noticeable loss of fat and muscle tissue in the gluteal region ("tobacco bag phenomenon") and perianal erythema due to secondary lactose intolerance. (Original recordings by the authors with parental permission.)

in most cases, CD does not manifest itself for a long time, and this form of the disease is called subclinical ("silent celiac disease") (1, 2). In addition, potential CD has an asymptomatic character, which differs from the previous one in the normal appearance of the small intestine mucosa (1, 2, 27). In a significant number of patients with potential CD, enteropathy is also registered later (1).

In children of the youngest age (9-36 months), CD almost regularly occurs in the classical clinical form (9, 16). It is characterized by a relatively short period after the introduction of gluten into the menu, a gradual onset, and a progressive course manifested by chronic diarrhea, anorexia, occasional vomiting, abdominal distension, apathy, and irritability (16). Because of insufficient intake and malabsorption of nutrients, global malnutrition occurs, accompanied by sideropenic anemia, a loss of fat tissue, and reduction of bone and muscle mass **(Figure 2)** (32, 33)**.** In most severe cases, secondary lactose intolerance, isolated hypertransaminasemia ("celiac hepatitis"), and sometimes the appearance of hypoproteinemic edema are registered (20, 33). Within the first 6-9 months upon birth, the disease usually has a rapid and severe clinical course. In rare cases, the so-called "celiac crisis" is characterized by total gastrointestinal insufficiency followed by severe dehydration, metabolic acidosis, meteorism, drastic weight loss, and hypoproteinemic edema (9).

The onset and course of the disease in preschool children are predominantly non-classical (atypical) (16). Compared to an earlier age, gastrointestinal disturbances are less often present or completely absent. Recurrent abdominal pain and constipation, sometimes diarrhea, and often sideropenic anemia, poor appetite, malnutrition, stagnation, stagnation in longitudinal growth, and a change in the child's personality are encountered.

When it comes to symptomatology of the disease in later childhood and adolescence, mono or oligosymptomatic extraintestinal manifestations are dominant (16). In addition to the manifestations seen in preschool age, there are others, such as maturation delay, enamel hypoplasia, recurrent aphthous stomatitis, chronic malaise, dermatitis herpetiformis, osteopenia, arthralgia, myalgia, cerebellar ataxia, polyneuropathy, epilepsy, and others (3, 6, 16, 22, 34).

Although the classical form of the disease is the most often described and best-studied entity, nowadays it is known that it represents only the "tip of the celiac iceberg" and that the largest number of patients, both children and adults, are those with non-classical and subclinical forms of the disease (16)**.**

CELIAC DISEASE AND OTHER DISEASES

Besides the frequently observed presence of celiac disease among patients' close relatives, particularly first-degree relatives, this pathology is commonly associated with

other coexisting autoimmune diseases (3-10%), such as type I diabetes mellitus, thyroiditis, Addison's disease, rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, Sjögren's disease, autoimmune diseases primarily affecting the liver, IgA nephropathy, myasthenia gravis, psoriasis, dilatative cardiomyopathy, and autoimmune pericarditis. (1, 6, 11-15). Approximately the same prevalence of the disease occurs in selective IgA deficiency, as well as in Down, Turner, and Williams syndromes (1, 3, 13).

DIAGNOSIS

The diagnosis of CD is based on an enterobiopsy with pathohistological examination of the mucosa of the small intestine (1, 3, 6, 16, 17). Biopsies are obtained from the duodenum using an upper gastrointestinal endoscopy, whereby 1 or 2 from the bulb and ≥4 from the distal duodenum (1, 3, 16). Such, i.e., multiple enterobiopsies, are necessary because the histological changes may be patchy in distribution and confined to the duodenal bulb. In order to provide adequate samples for pathohistological analysis, the correct orientation of the biopsies is required.

Recommendations of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published in 2012, unlike previous ones, consider that enterobiopsy is not necessary in patients with symptoms and/or signs consistent with CD, and in addition, they have IgA titer antibodies to tissue transglutaminase (AtTG-IgA) ≥10 times above the upper reference value, positive antiendomysial antibodies of the same class (EMA-IgA) and "celiac HLA" (DQ2 and/or DQ8) (1). Clinical recovery of the patient and the disappearance of AtTG are part of the confirmation of the disease, i.e., the justification for the introduction of a gluten-free diet without a previous enterobiopsy. This attitude in the diagnosis of CD is based not only on high sensitivity and specificity of AtTG-IgA as a serological marker of the disease (>95%), but also on the highly significant correlation of their titer with the degree of damage to the mucosa of the small intestine, as well as the almost inevitable correlation (>98%) presence of HLA DQ2 and/or DQ8. An additional difference compared to the previous position is that even in children under two years of age with an exact diagnosis of CD, a gluten provocation test with pathohistological analysis of the small intestine mucosa are not required. However, in patients in whom a gluten-free diet was introduced without a previous enterobiopsy, as well as in cases where the morphological damage of the mucosa was not typical, or the samples were inadequate for a reliable interpretation, the final confirmation or exclusion of CD is based on enterobiopsy and pathohistological findings during the gluten provocation test. Since it can jeopardize the quality of permanent teeth, this procedure is not recommended before the age of six and because of the side effects related to the child's growth and development during puberty.

ESPGHAN, as part of the additional modification of the criteria for the diagnosis of CD, adopted in October 2019 and published in January 2020, does not consider enterobiopsy with pathohistological analysis of the small intestine mucosa samples necessary even in asymptomatic patients with a serum level of AtTG-IgA class ≥10 times above the upper reference level values and positive EMA-IgA (27). Also, bearing in mind the almost absolute association of CD and HLA DQ2 and/or DQ8 in these patients, as well as in those whose diagnosis was established by enterobiopsy, testing in this sense is not necessary. However, in all other cases, the diagnosis of CD requires strict adherence to the 2012 criteria. It is additionally recommended that, as part of the initial serological screening for CD, with prior verification of normal serum IgA for age, AtTG-IgA should be used, and not EMA and antibodies to deamidated gliadin peptide (AtDGP). However, if it is a suspected patient with IgA deficiency, tests based on IgG class antibodies (AtDPG, EMA or AtTG) should be used to this purpose. If there is a discrepancy between the level of AtTG-IgA and the pathohistological findings, it is necessary to re-evaluate the result of the biopsy or consult another pathologist. Patients with elevated serum levels of AtTG-IgA and EMA-IgA in whom normal or minimally damaged small intestine mucosa (Marsh 0/I) was registered require strict monitoring.

Except in the above-mentioned exceptions, serological tests for CD have no diagnostic value (1, 6). Hence, they are primarily used for detection of asymptomatic and non-classical forms of the disease and in the assessment of the consistency of the elimination diet in patients in whom it has been established (1). When interpreting serological screening, it should be kept in mind that it can be positive even without the characteristic damage of the small intestinal mucosa, which is also found in other autoimmune diseases and in other pathological conditions (1). Contrary to this, due to the immunological immaturity of children under two years of age, AtTG may be negative despite evident enteropathy (6, 16). For this reason, when screening children younger than two years of age for CD, the IgA TTG test should be combined with deamidated gliadin peptide (IgA and IgG) (6, 16).

THERAPY

Patients with CD should adhere to a gluten-free diet for life (1, 3, 6, 16, 17). Most of those with a symptomatic form of the disease, especially the classical one, during the initial phase of treatment, require the correction of micronutrient deficits, primarily iron, and folate, and sometimes temporary restriction of lactose (6, 33). In patients with "celiac crisis", in addition to correcting hydro-electrolyte and acid-base imbalance and removing

edema, semi-elemental and additional parenteral nutrition is applied, and exceptionally rarely short-term glucocorticoid therapy (9, 35).

PROGNOSIS

The prognosis of timely recognized and adequately treated CD is excellent (11, 31). Delayed recognition of the disease or non-compliance with the elimination diet, however, can lead to severe consequences, including serious complications, both during growth and development, and those that manifest in adulthood, such as enteropathy-associated T-cell lymphoma, small bowel adenocarcinoma, osteoporosis, infertility, and others (3, 9, 11, 18, 21, 23).

CONCLUSION

Celiac disease denotes a genetically predisposed autoimmune pathology whose onset is triggered and precipitated by the intake of gluten found in wheat, barley, and rye. Most often, it affects Caucasians, it frequently occurs in close relatives, and it should be noted that it is in some cases accompanied by other autoimmune diseases, a selective deficit of IgA antibodies, as well as Down, Turner, and Williams syndromes. The highlight of celiac disease is the non-specific small intestinal mucosal inflammation that completely ceases and is resolved upon introducing a gluten-free diet. Besides enteropathy, which can be either symptomatic or asymptomatic, celiac disease is also characterized by various extraintestinal manifestations. Timely recognition and proper treatment provide excellent prognoses in patients with this condition.

Author contributions

ZL and NR contributed to study design, data analysis and interpretation and they also drafted the manuscript. VR, MM, SD, and BB contributed to data analysis and interpretation, and provided critical review of the intellectual content of the manuscript. PR contributed to data collection, data analysis and interpretation. All the authors approved the final version of the manuscript before submission.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

None to declare.

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CELIJAČNA BOLEST KOD DECE

Zoran Leković[1,2], Vladimir Radlović[1,2], Marija Mladenović[3], Siniša Dučić[1,2], Bojan Bukva[1,2], Petar Rosić[1], Nedeljko Radlović[4]

Sažetak

Celijačna bolest je multisistemsko autoimunsko oboljenje indukovano glutenom pšenice, raži i ječma. Karakteriše je poligenska predispozicija, visoka prevalencija kod pripadnika bele populacije (1%), posebno kod bliskih srodnika (5-15%), veoma heterogena ekspresija i česta udruženost sa drugim autoimunskim bolestima (3-10%), kao i selektivnim deficitom IgA i Daunovim, Tarnerovim i Vilijamsovim sindromom. Osnova bolesti i ključni nalaz u njenoj dijagnostici je simptomatsko ili asimptomatsko zapaljenje sluzokože tankog creva koje se povlači na dijeti bez glutena. U skladu s tim, osnovu lečenja čini eliminaciona dijeta, tako da poremećaj, ako se blagovremeno prepozna i adekvatno leči, takođe karakteriše odlična prognoza.

Ključne reči: celijačna bolest, deca, klinički oblici, dijagnostika, terapija

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REVIEW ARTICLE

Attitudes towards death and end-of-life care

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Summary

End-of-life care represents a unique segment of palliative care. In the end-of-life period, the task of the involved healthcare professionals is to accompany patients during their last days, weeks or months of life to the moment of their death. The way people die has changed profoundly over the past 70 years. Health care is now the main context in which many people encounter death. The focus is on clinical interventions in the end-of-life period with the aim of defeating death, while a broader context and the significance of dying is neglected. Progressive medicalization of dying has inevitably led to changing attitudes towards death and dying in both general population and healthcare professionals. There is a struggle among healthcare professionals and individuals as well to accept the inevitability of death. There has been a growing interest in examining attitudes towards death and dying, in order to achieve a greater acceptance of death with repercussions on adequate planning and implementation of end-of-life care. A thorough understanding and estimation of attitudes to death and dying, both among general population and among health professionals, is required for the development of an effective strategy to promote end-of-life care. Due to the upcoming examination of attitudes to death and dying in Serbia, as well as exploring their influence on attitudes to end-of-life care, this paper presents the current knowledge in this area.

Key words: dying, death, end-of-life care

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INTRODUCTION

Comprehensive and available palliative care should be one of the key components of health care. According to the new, consensus-based definition of palliative care from 2020, it represents active, holistic care of individuals across all ages with serious health-related suffering due to severe illness, especially of those who are close to the end of life (1). The goal of palliative care is to alleviate suffering and improve the quality of life of patients, their families, and their caregivers (2). End-of-life care represents a unique segment of palliative care. In the end-of-life period, the task of doctors and other involved health care professionals is to accompany patients during the last days, weeks or months of their life to the moment of their death: during this common journey, their symptoms are treated, the family of the dying patient is supported, they are enabled, if possible, to stay and die in the place they chose, they are helped in their fears and preoccupations. Hence, high-quality palliative care is based on a close physical and emotional contact with dying patients and their relatives (3).

DEATH AND DYING IN THE 21ST CENTURY

The way people die has changed profoundly over the past 70 years. Death comes later in life and the dying process is often significantly prolonged (4). Death and dying have been displaced from the family and community setting (where they used to take place relying on traditional knowledge and skills) to the health care system. Health care is now the main context in which many people encounter death. Futile and inappropriate treatment can continue into the final hours of life. Due to technological progress and its impact on medicine, especially in terms of mastering the possibilities of long-term life support, defining death has become complex and the technology that accompanies death has become even more sophisticated (5). Death and dying have become unbalanced in high-income countries and increasingly so in middle- and low-income countries - the focus is on clinical interventions in the end-of-life period with the aim of defeating death, while a broader context and the significance of dying is neglected (6). Therefore, especially in the context of acute health-care setting, death and dying are viewed as a clinical problem (the object of treatment/cure, although it is impossible to have a cure since the dying process is irreversible), reduced to a series of biomedical tests and markers with the application of various, often invasive and expensive, medical interventions (intubation, mechanical ventilation, artificial nutrition) that continue until the final hours of life with minimal attention to the patient's suffering (7). Recognition of dying is often made very late, if at all. This progressive medicalization of dying is not accompanied by adequate attention and treatment of the very diverse symptoms of dying patients with relatively cheap, evidence-based methods within the framework of the palliative care system, i.e., end-of-life care – The World Health Organization estimates that globally only 14% of people in need can access such care (8).

ATTITUDES TOWARDS DEATH

The progressive medicalization of dying arose on the wave of technological development, as well as the transfer of dying to hospitals and the health care system. "The experiment of making mortality a medical experience" is only a few decades old and has inevitably led to changing attitudes towards death and dying in both general population and healthcare professionals (7). The current prevalent attitude towards dying, especially in high-income countries (dying is not allowed, it is fought against!) is shaped through perceiving medicine as extremely powerful with the desire to defeat death, creating a delusion that we are able to control nature and not that we are part of it. There is a struggle within the health care system (and among individuals as well) to accept the inevitability of death. Dying is less and less present within families and communities. The roles of families and communities have receded as death and dying have become unfamiliar and skills, traditions, knowledge, and confidence in supporting death, dying, and grieving have been lost. Death has become invisible to the family (as it takes place in hospitals), and dependence of both families and patients on the health care system in the process of dying is increasing. The social and cultural contexts of dying essential to providing meaning, connection, and long-term support for those who grieve are at risk of disappearing. Health care cannot replace the sense of coherence, rituals, and traditions nor can it replace long-term mutual support that family and community provide to those who are dying and grieving (6).

IMPACT ON END-OF-LIFE CARE

Death and dying have become unbalanced and contradictory in high-income countries – health care has a central role in the care of dying with often excessive and futile end-of-life care interventions associated with increased suffering and higher costs. On the other hand, this imbalance is even more striking in middle- and low-income countries, since the rich receive excessive care while the poor receive little attention or relief of suffering, often with no access to opioids (9). Hence, the disadvantaged and powerless suffer most from the imbalance in the care provided to those dying and grieving.

Acknowledging that the changed way of dying over the past 7 decades has also changed the attitude towards death and dying with significant repercussions on the

suffering of dying patients and palliative care at the end of life, the statement of the Lancet Commission on the Value of Death entitled "Bringing death back to life" published in January 2022, set 5 principles of realistic utopia – new visions of what death and dying could be like, i.e. how to rebalance death, dying and grieving (6). The five principles are: 1) the social determinants of death, dying, and grieving are tackled; 2) dying is understood to be a relational and spiritual process rather than simply a physiological event; 3) networks of care lead support for people dying, caring, and grieving; 4) conversations and stories about everyday death, dying, and grief become common; 5) death is recognized as having value. Therefore, death and dying must be recognized as not only normal, but valuable (6).

In recent years there has been a growing interest in examining attitudes towards death and dying, not only because of the attempt to rebalance death and dying and bring about a greater acceptance of death, but also in the context of adequate planning and implementation of end-of-life care (type of interventions, decisions on the type of treatment) which aims to respond to the suffering and problems of the dying. The knowledge of the basics of end-of-life care is often insufficient in countries with underdeveloped palliative care, and very often the contents of end-of-life care are not integrated into curricula.

In the available literature, the studies are oriented mainly towards the examination of attitudes towards death in health professionals and to a lesser extent in general population. One of the most frequently used questionnaires for assessing attitudes towards death is the Death Attitude Profile-Revised DAP-R (10). Death is still a taboo in traditional Serbian culture. Diagnosing an incurable disease (especially in the end-of-life period and when death is approaching) generally leads to hiding the truth from the patient, both by health professionals and family members. A similar cultural pattern is present in China, yet previous Chinese surveys have shown that more than half of the citizens want to be informed when faced with terminal illness, and 92,9% of advanced cancer patients hoped to be informed of their real condition by doctors and family members (11,12). A cross-sectional study conducted in China (published in 2019) examined the influence of death attitudes measured by the Chinese version of the Death Attitude Profile-Revised DAP-R-C and showed that attitudes towards death also influenced attitudes towards the end-of-life care. Namely, fear of death and death avoidance showed a negative correlation with attitudes towards the end-of-life care. That is, those healthcare professionals with a greater fear of death and the dying process, and those who avoided thinking about dying as much as possible, had a less positive attitude towards caring for dying individuals (13). Moreover, the same Chinese study found that although community healthcare providers had positive attitudes towards end-of-life care, they also agreed that most terminally ill patients did not want to be informed of advanced disease and they were inclined to conceal the truth, reflecting the "denial of death" as a still prevalent mindset and attitude towards death (13). Previously conducted research also showed that the attitudes towards death of healthcare professionals, students and even patients can be changed through education about the dying process and have a positive effect in terms of reducing the fear of death and better care for dying patients (14,15). A study conducted in Jordan on a student population (nursing faculty) also showed a negative impact of fear of death and death avoidance measured by the same instrument on the care of dying patients (16). Similar results, where attitudes to death were also measured by the DAP-R questionnaire, were shown by a Singaporean study, demonstrating a positive effect of a two-day workshop on end-of-life care on changing attitudes towards death and caring for dying patients (17).

There is a clear need for developing end-of-life care in Serbia as one of the important part of palliative care. At the same time, attitudes to end-of-life care, death and dying in Serbia are unknown both among healthcare professionals and general population. The Serbian version of the Death Attitudes Profile Revised (DAP-RSp) Instrument is now available and should enable the assessment of attitudes towards death in healthcare professionals and in general population (18).

The DAP-R questionnaire was developed by Wong et al. with the aim of evaluating and measuring attitudes towards death and it includes 32 questions that form 5 dimensions. Each of the 5 dimensions measures particular types of attitudes towards death as a distinctive multidimensional theoretical construct: Fear of Death (negative thoughts and emotions related to death and the dying process), Death Avoidance (avoidance of thinking and speaking of death in order to diminish unpleasant feelings), Neutral Acceptance (acceptance of death as an integral part of life), Approach Acceptance (implies a belief in a happy afterlife), and Escape Acceptance (understanding death as an alternative to escaping current suffering) (10).

A relevant application of this questionnaire lies in its ability to assess different attitudes towards death and dying and provide a detailed understanding of how people react when faced with the dying process – for example during the care of terminally ill or dying patients, which is the case in the end-of-life care setting. Attitudes towards death characterized by acceptance are fundamentally adaptive and are associated with less anxiety and a greater meaning in life (10,19, 20, 21, 22, 23).

CONCLUSION

In Serbia palliative care delivery has not yet fully emerged in medical practice. End-of-life care skills are

not sufficiently present among physicians dedicated to the treatment of terminally ill and dying patients. There is now ample evidence that attitudes towards death and dying can shape healthcare professionals' approach to end-of-life care. Non-affirmative attitudes towards death and dying (e.g., fear of death) can be significant obstacles in the development of the meaningful end-of-life care. A more profound understanding and estimation of attitudes to death and dying, both among general population and healthcare professionals, should be a prerequisite for the development of an effective strategy to promote end-of-life care. Caring for the dying is a gift. Much of the value of death is no longer recognized in the modern world, but rediscovering this value can help care at the end of life and enhance living (6).

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STAVOVI O SMRTI I ZBRINJAVANJE NA KRAJU ŽIVOTA

Dragana M Marić [1, 2]

Sažetak

Zbrinjavanje na kraju života (*end-of-life care*) predstavlja poseban i jedinstven segment palijativnog zbrinjavanja. U periodu završetka života, visoko kvalitetno palijativno zbrinjavanje zasniva se na bliskom fizičkom i emocionalnom kontaktu sa onima koji umiru i njihovim porodicama. Način na koji ljudi umiru se radikalno promenio tokom poslednjih 70 godina. Smrt i umiranje izmešteni su iz okvira porodice i zajednice u zdravstveni sistem u okviru kojeg se primenjuje često uzaludno i neprimereno lečenje čak i u poslednjim satima života. Fokus je na kliničkim intervencijama na kraju života sa ciljem da se pobedi smrt, dok se širi kontekst i značaj umiranja zanemaruju. Ova progresivna medikalizacija umiranja dovela je do promene stavova prema smrti i umiranju kako u opštoj populaciji tako i kod zdravstvenih radnika. Čini se da je aktuelan stav prema umiranju (umiranje se ne dopušta, protiv njega se bori) oblikovan shvatanjem medicine kao izuzetno moćne, sposobne da porazi smrt, stvarajući zabludu da smo u stanju da kontrolišemo prirodu, a ne da smo njen deo. Priča o umiranju u 21. veku postala je je neizbalansirana i kontradiktorna. Raste interesovanje za ispitivanje stavova prema smrti i umiranju, kako bi se postiglo veće prihvatanje smrti, povratila izgubljena ravnoteža, a istovremeno omogućilo adekvatno planiranje zbrinjavanja na kraju života. Temeljno razumevanje i procena stavova o smrti i umiranju, kako među opštom populacijom tako i među zdravstvenim radnicima, potreban je preduslov za razvoj i planiranje efikasne strategije za promovisanje zbrinjavanja na kraju života. Zbog predstojećeg ispitivanja stavova o smrti i umiranju u Srbiji kao i istraživanja njihovog uticaja na stavove o zbrinjavanju na kraju života, u ovom radu prikazana su aktuelna saznanja u ovoj oblasti.

Ključne reči: smrt, umiranje, zbrinjavanje na kraju života

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CASE REPORT

The treatment of diffuse large B-cell non-Hodgkin's lymphoma in pregnancy: a case report and a literature review

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Summary

Aim: Hodgkin's lymphoma (HL) is the most common type of lymphoma diagnosed during pregnancy while the occurrence of non-Hodgkin's lymphoma (NHL) is rare because the peak incidence of disease occurs after reproductive age.

Case report: We present a case of 36-year-old woman, in the 13th week of pregnancy, admitted to our department for suspected thyroid lymphoma. She presented with neck swelling, respiratory distress, and dysphagia. After biopsy, histopathological analysis led to the diagnosis of primary diffuse large B-cell mediastinal NHL. The recommendation of multidisciplinary medical team was to start the treatment immediately with R-CHOP for a total of 6-8 cycles. Five years have passed since the beginning of the treatment, the disease is still in remission and the child is at normal level of growth for their age.

Conclusion: Lymphomas in pregnancy represent a challenge for the medical team as well as for the patient. With early diagnosis and appropriate therapy, despite all the risks, it is possible to bring mother into remission without endangering the offspring.

Key words: non-Hodgkin's lymphoma, pregnancy, chemotherapy

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INTRODUCTION

Lymphomas are heterogeneous malignant diseases of the lymphatic system. Hodgkin's lymphoma is the fourth most common malignancy diagnosed during pregnancy and some reports estimate its frequency in approximately 1 in 6000 pregnancies (1). Non-Hodgkin lymphomas occur later, affecting between 0.2 and 0.7 of 100.000 pregnant women (2). However, in the latest studies, the incidence of 1 to 5 cases per 100.000 pregnancies was reported. This can be explained by the trend of delaying first childbirth in developed countries (3). It appears that NHL associated with pregnancy often has an aggressive histology, with diffuse large B-cell lymphomas being most common. It is believed that hormonal and immune changes during pregnancy can act as a trigger and affect the course and prognosis of the disease (4).

CASE PRESENTATION

This report joins a small number of diffuse large B-cell lymphoma cases complicating pregnancy which resulted in survival of the mother and a normal full-term fetus. Thirty-six-year-old woman was admitted to our department for suspected thyroid lymphoma. The patient noticed a fast-growing lump in the neck region, a few weeks before hospital admission, together with respiratory distress and dysphagia. She was 13 weeks pregnant, had proven thrombophilia (FV Leiden heterozygote V), and a previous miscarriage in the 9th week of pregnancy. Her hormonal status was within the reference range, and she had unremarkable medical and family history. Laboratory evaluation demonstrated normal blood counts and inflammatory markers.

MRI revealed conglomerates of enlarged lymph nodes, on both sides of the neck, which compressed the surrounding thyroid tissue that appeared to be infiltrated by the primary tumor located in the anterior mediastinum. There were no enlarged axillary or abdominal lymph nodes or hepatosplenomegaly.

The patient underwent surgery, a tumor infiltrating subhyoid muscles was identified and biopsy led to the diagnosis of primary diffuse large B-cell mediastinal non-Hodgkin's lymphoma.

A team of specialists from general obstetrics, hematology, oncology, and neonatology was assembled to discuss the patient's care plan. Given that the patient was in the second trimester of pregnancy and in the second stage of disease (IIA), the recommendation of multidisciplinary medical team was to start the treatment promptly, with R-CHOP therapy, for a total of 6-8 cycles. Namely, the standard for treating diffuse large B-cell lymphoma is a combination regimen containing cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone with or without rituximab.

After receiving the first cycle of therapy NMR showed dramatic response with tumor regression by 50%. Thereafter, another five cycles of R-CHOP therapy were administered at three-week intervals. The patient's pregnancy was regularly monitored by a gynecologist during the administration of R-CHOP therapy, and it successfully ended with the birth of a healthy baby (Apgar score 9/10) in the 37th week of pregnancy by caesarean section. The delivery in the 37th week balanced the risk of adverse outcomes associated with preterm birth, with the risk of disease progression.

On the 40th day after delivery, a PET scan was performed, where scar tissue was detected in the area of previous tumor mass. Thereafter, the patient was regularly monitored every six months. Five years have passed since the beginning of the treatment and the disease is still in remission. The child has been repeatedly seen by a pediatrician and his growth and developmental status are completely normal.

DISCUSSION

Simultaneous pregnancy complicates the treatment of a malignant disease affecting not only the mother but also the fetus. The goal of treatment is not only to bring the mother into remission, but also to ensure a safe course of pregnancy and healthy offspring. When it comes to non-Hodgkin lymphoma, current clinical practice is based largely on case reports and small case series. The treatment decision is influenced by the type and malignant potential of lymphoma, the stage of the disease, the associated maternal diseases, the stage of pregnancy, side effects, and contraindications of therapy.

It is advised that in patients at low risk, i.e., patients with indolent NHL (follicular lymphomas and small lymphocytic lymphomas) therapy should be postponed until the end of the first trimester to minimize the risk to the fetus. However, the majority of NHL diagnosed during gestation includes aggressive forms (large B-cell lymphomas, mantle cell lymphoma, mature T cell and NK cell lymphomas, Burkitt's lymphoma), and most patients with this form of lymphomas should be treated without delay with intensive combination chemotherapy (1,2).

Most cytotoxic agents cross the placenta due to their low molecular weight. The teratogenicity of these chemotherapeutic agents largely depends on the timing of exposure and almost all of them have been documented to be teratogenic in animal models (5). Chemotherapy during the first trimester of pregnancy may increase the risk of spontaneous abortions, fetal death, and major malformations, as chemotherapy could interfere with organogenesis (3,6).

Although the available literature has not shown CHOP chemotherapy administered during the second and the third trimester to adversely affect the fetal outcome, perinatal risks are present. A higher frequency of

miscarriages, premature births, intrauterine fetal growth retardation and low birth weight was documented, compared to pregnancies of healthy women (6,7).

Lately, much attention has been drawn to the use of immunotherapy in pregnancy as a form of lymphoma treatment. The CHOP regimen usually in combination with rituximab, a monoclonal anti-CD20 antibody, has been commonly used for treating patients with diffuse large B-cell lymphoma. Not many cases of rituximab administration during pregnancy have been reported so far, usually for treatment of different autoimmune diseases. According to these reports the use of rituximab, even in the first trimester of pregnancy, was not associated with an increased risk of adverse fetal outcome (8,9). However, in the latest report from the International network of cancer, infertility and pregnancy, the administration of rituximab during the second and the third trimester of pregnancy (from 13 to 35 weeks) in 36 cases resulted in five neonatal complications (three cases of neonatal systemic infection and one case of neonatal neutropenia), and three maternal infections. In this series rituximab was not used as a single drug, but in combination with chemotherapy, so it is difficult to determine whether complications are due to the use of rituximab or other chemotherapeutical agents (10). In a 2001 study, Aviles and Neri evaluated morbidity in children exposed to chemotherapy in utero. The series included 84 children whose mothers were exposed to chemotherapy during pregnancy to treat a variety of hematologic malignancies. In this group, there were 32 mothers suffering from NHL and nine of them were treated with R-CHOP regimen. Six of their children developed late toxic complications in the form of severe infections. Although it was difficult to prove that rituximab therapy was the unique cause, the authors ceased to use it in the therapy of diffuse NHL in pregnancy (11).

Another major concern regarding the lymphoma treatment during pregnancy is postnatal development of children exposed to chemotherapy in utero and their long-term fertility. In the previously mentioned study, all children of the 32 mothers treated for NHL have had normal physiological, physical, and mental development during the nineteen-year-long observation. This study also partly addressed the issue of fertility; all children showed normal sexual development and 12 of them had become parents (11). A more recent study compered neurocognitive development of children exposed to chemotherapy in utero (n=35) with unexposed children (n=22) and confirmed that there were no significant differences (12).

CONCLUSION

To sum up, the diagnosis of lymphoma in pregnancy poses challenges for the patient and her family as well as for the medical team. Informed consent of the patient is required, and all treatment decisions should be made by a multidisciplinary medical team. Based mainly on small series and case reports it could be said that although the course of diffuse large B-cell lymphoma during pregnancy is often progressive and rapid, treatment with R-CHOP therapy can be considered after the first trimester, with reassuring maternal and fetal outcomes.

Authorship

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

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LEČENJE DIFUZNOG B-KRUPNOĆELIJSKOG NON-HOČKIN LIMFOMA U TRUDNOĆI: STUDIJA SLUČAJA I PREGLED LITERATURE

Stepanović Boban[1], Tausanović Katarina[1,3], Kesić Vesna[2,3], Živaljević Vladan[1,3]

Sažetak

Uvod: Hočkinov limfom je najčešće dijagnostikovan tip limfoma u trudnoći, dok je pojava non-Hočkin limfoma u trudnoći retka, budući da se oni najčešće javljaju nakon reproduktivnog perioda.

Prikaz slučaja: Prezentujemo slučaj tridesetšestogodišnje pacijentkinje koja je primljena u Kliniku za endokrinu hirurgiju u trinaestoj nedelji trudnoće zbog sumnje na postojanje limfoma štitaste žlezde. Pacijentkinja se javila sa simptomima respiratornog distresa, otežanog gutanja i otoka vrata. Nakon otvorene biopsije postavljena je histološka dijagnoza primarnog difuznog B-krupnoćelijskog non-Hočkin limfoma. Zaključak multidisciplinarnog stručnog tima je bio da se što pre započne sa R-CHOP terapijom, u šest do osam ciklusa. Od početka lečenja je prošlo pet godina, bolest je i dalje u remisiji, a dete se normalno razvija.

Zaključak: Limfomi u trudnoći predstavljaju izazov, kako za medicinski tim tako i za pacijenta. Uz ranu dijagnozu i odgovarajuću terapiju, uprkos svim rizicima, moguće je postići remisiju bolesti, bez ugrožavanja ploda.

Ključne reči: non-Hočkin limfom, trudnoća, hemioterapija

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REVIEW

Giant cell myocarditis in modern clinical practice

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Summary

Giant cell myocarditis is a rare but extremely severe disease with a frequent need for heart transplantation and a high mortality rate. To this day, the most common reason for the occurrence of this disease has not been precisely determined, but its frequent association with autoimmune diseases speaks in favor of autoimmune etiology. Clinically, it is presented to the greatest extent by symptoms and signs of acute heart failure and ventricular rhythm disturbances, which are registered in as many as half of the patients. Arrhythmias can be accompanied by the occurrence of palpitations, repeated syncope, and even sudden cardiac death. A severe degree of acute heart failure in hemodynamically unstable patents who respond inadequately to the administered therapy for heart failure and refractory heart rhythm disorders and/or conduction disorders that are common in these patients should always raise suspicion of giant cell myocarditis. Given that changes in electrocardiogram, echocardiography and positive biomarker values are not strictly specific for this disease, the diagnosis of giant cell myocarditis is most often established by endomyocardial biopsy, especially in patients with the fulminant form of the disease. Timely endomyocardial biopsy enables not only quick and accurate diagnosis, but also early administration of immunosuppressive therapy, which greatly improves the outcome in these patients. Pathohistological verification of this disease is important so as to rule out cardiac sarcoidosis and other granulomatous infectious and non-infectious diseases. Given that in a large number of patients the disease has a fulminant course, early and rapid diagnosis, application of inotropic stimulation and mechanical circulatory support in hemodynamically unstable patients and adequate modern therapeutic regimen can largely reduce mortality in these patients, which is still high despite modern diagnostics and therapeutic progress of medicine

Key words: fulminant myocarditis, acute heart failure, hemodynamic instability, endomyocardial biopsy, mortality

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INTRODUCTION

Myocarditis is an inflammatory disease of the heart muscle caused by infection, drug-mediated activation of the immune system, or the activity of other harmful agents (1). The classification of myocarditis is made according to the etiological factor that causes it, the stage in which the disease has been diagnosed, the severity of the disease, the dominant symptoms, and the pathohistological findings (2). In acute myocarditis (AM), symptoms usually appear a month before the diagnosis (2,3). It occurs more often in men (76,6%-82%) and predominantly in younger population (the highest frequency is found in the population between 23 and 50 years of age) (4,5). Apart from adults, AM is not rare in children either (6). There is also evidence of a possible genetic predisposition in the development of myocarditis (7). Pathohistologically, according to the Dallas criteria, AM is classified as active myocarditis characterized by the presence of an inflammatory infiltrate in the myocardium (14 leucocytes/mm^2 including up to 4 monocytes/mm^2 with the presence of CD 3 positive T-lymphocytes 7 cells/mm), necrosis and/or by degeneration of adjacent cardiomyocytes (8). Myocarditis can be pathohistologically divided into lymphocytic, giant cell (GCM), eosinophilic, and granulomatous myocarditis (9).

In a certain number of patients, the acute form of the disease can be asymptomatic but it can also manifest with a variable clinical picture, ranging from mild infection symptoms to the development of severe heart failure, and even sudden cardiac death. The form of the disease that develops rapidly, has a progressive course with rapid development of heart failure and shock, and requires frequent use of inotropic stimulation or mechanical circulatory support is referred to as fulminant myocarditis (10). According to the Lombardy registry, approximately 73,4% of patients have an uncomplicated form of the disease that is not accompanied by heart failure and malignant rhythm disorders (11).

Most patients complain of general symptoms (fever, weakness) and chest pain (82%-97% of patients), which can have an anginal character or resemble the pain experienced in acute pericarditis (sharp pain worsened during inspiration and in lying position) (11,12). Signs and symptoms of heart failure (fatigue, shortness of breath, orthopnea) are especially common in fulminant myocarditis. Due to rhythm disturbances, patients often report palpitations and syncope, especially in cases of fulminant myocarditis. Gastrointestinal and respiratory symptoms are also not uncommon, especially when the course is a viral infection, and they occur in approximately 80% of patients about 2 weeks before the diagnosis of myocarditis is established (10).

To establish the duagnosis of myocarditis, due to its heterogeneous clinical presentation, it is important to take a detailed medical history regarding prior infections, medication use, travel to endemic areas, alcohol abuse, and other illicit substances.

Non-invasive diagnostic tests (ECG, biomarkers, and echocardiography) are recommended for all patients in whom myocarditis is suspected (3). These tests have high sensitivity but low specificity in patients with myocarditis (13-15). According to the ITAMY study, ECG changes occur in approximately 96% of those affected, elevated levels of inflammatory markers and hsTnI in as many as 99% of those affected, while segmental kinetics impairments of the ventricle, as assessed by echocardiography, are observed in 21% of myocarditis patients (13). Although the final diagnosis of myocarditis is established through endomyocardial biopsy, there is a growing use of cardiac magnetic resonance (CMR) imaging (sensitivity 87.5% and specificity 96.2%), which nowadays represents the non-invasive diagnostic gold standard (16,17). Its application is recommended in all hemodynamically stable patients, and for better diagnostic accuracy, it is best performed within the first 2 to 3 weeks from the onset of symptoms (2). In addition to diagnosing myocarditis, CMR has proven to be a method of choice for monitoring the course of the disease and assessing clinical outcomes (18).

GIANT CELLULAR MYOCARDITIS

Giant cellular myocarditis represents a severe heart muscle condition characterized by inflammation mediated by the activation of T lymphocytes (19). Although the etiology of the disease is unknown, there are indications suggesting the involvement of autoimmune processes. Given that this is a very serious condition, timely diagnosis and early initiation of immunosuppressive therapy are of immense importance.

Epidemiological Characteristics

The results of studies conducted in Japan, England, and India have shown that the incidence of Giant Cell Myocarditis (GCM) is 0.0075, 0.025%, and 0.051%, respectively (20-22). It most commonly occurs in individuals who are in their fourth decade of life, although cases of GCM have been described in the pediatric population as well (23-26). There are documented cases of GCM in infants as young as a few months old and in patients over 70 years of age (27). When considering all forms of myocarditis, a higher prevalence is observed in males; however, GCM has not shown a significant difference in incidence based on gender distribution (28). The non-fulminant form of GCM occurs with a frequency of up to 4% while the fulminant form of the disease is more common, ranging from 3.6% to 14.5% (29-31). Various cardiovascular diseases have shown a circadian rhythm of occurrence both during the day and in different periods of the year, sometimes without a clear cause-and-effect relationship

(32). The frequency of GCM varies depending of the time of year, and the results of an autopsy study by Okada R. and colleagues, considering the annual fluctuation in GCM incidence, suggest a predominantly infectious etiology of this condition (21, 33, 34).

Histopathological Findings, Potential Etiological Factors, and Associated Conditions in GCM

Active myocarditis, which is the basis of every acute myocarditis, is histopathologically characterized by the presence of an inflammatory cellular infiltrate in the myocardium along with concurrent degeneration and/or necrosis of cardiomyocytes (8). Interstitial tissue edema is often present along with fibrotic changes. GCM is characterized by the presence of a diffuse or multifocal mononuclear infiltrate alongside multinucleated giant cells and eosinophils (35). Myocyte damage and necrosis are always present. The presence of poorly formed granulomas is possible (36). Clearly formed granulomas exclude the diagnosis of GCM (35). In 5-10% of cases, GCM is diagnosed as extracardiac granulomatous inflammation in lymph nodes or the liver (37). It is pathologically crucial to differentiate between well-organized granulomatous lesions found in cardiac sarcoidosis and a diffuse non-granulomatous infiltrate seen in GCM. The first to highlight this histopathological difference between these two clinical entities was Tesluk H. in 1956 (38). In case of cardiac sarcoidosis, the presence of granulomas and the absence of necrosis are important differential diagnostic criteria. Non-caseating granulomas may be composed of epithelioid cells that can occasionally fuse to form Langhans giant cells (36). However, Cooper LT Jr. and colleagues emphasize that the presence of granulomatous infiltrate in lymph nodes or other organs, or the presence of sarcoidosis, does not exclude the possibility of GCM coexistence (35). In addition to sarcoidosis, other infectious and systemic granulomatous diseases can represent a major pathohistological differential diagnostic problem. Infectious agents such as tuberculosis, cryptococcus, measles, syphilis and fungal infections can lead to granulomatous changes in the myocardium, especially in immunocompromised patients (39-42). Myocardial involvement is seen in 6-44% of patients with Wegener's granulomatosis (43). Rheumatic carditis is characterized by Aschoff nodules and MacCallum plaques (44). Granulomas have also been described as a reaction to a foreign body in patients with implanted permanent pacemaker (45).

When it comes to lymphocytic myocarditis, the most common etiological factor for its occurrence is a preceding viral infection with cardiotropic viruses. In case of GCM, there have also been reported cases or an association between this type of myocarditis and infections involving Coxsackie type B and Parvovirus B19 viruses (46,47). Myocardial damage accompanied by elevated hsTnI values is often registered in patients with Covid-19 infection (48). Amiri A. and colleagues described a case of GCM in a patient with Covid-19 infection (49). Apart from direct damage to cardiomyocytes in some viral infections, GCM can also be associated with autoimmune systemic disease, which is why a detailed medical history regarding symptoms and signs related to other systems of organs is important (50). Involvement of autoimmune processes and molecular mimicry may be significant for disease progression in patients with myocarditis.

It has been shown that patients with GCN have significantly higher titers of antibodies to myosin and their cross-reactivity with adrenergic receptors compared to a healthy individual (51). Animal models have demonstrated the development of GCM through the induction of an autoimmune process involving myosin and CD4+T lymphocytes (52,53). The association of inflammatory bowel disease (IBD) with GCM has been most commonly described. Myocarditis associated with IBD occurs in the acute phase of the disease (67%) and it more frequently affects young males (72%), predominantly presenting with an infarct-like state (58%) (54). Shivaraj et al. investigated adult IBD patients from 2010 to 2014, and found that myocarditis was present in 0.01% patients, specifically 0.018% in ulcerative colitis, and 0.009% in Crohn's disease (55). GCM has been reported to coexist with Guillain-Barre syndrome, myasthenia gravis, Takayasu arteritis, pernicious anemia, orbital myositis, systemic lupus erythematosus, autoimmune hepatitis, etc. (3,56-59). When it comes to tumors, the association of GCM with the occurrence of Thymoma and with lymphomas is most often described (59,60).

A series of cases of GCM associated with hypersensitivity to drugs (Minocycline, Cefadroxil monohydrate; Phenytoin, primidone;) was presented by Daniels PR and associates. Cases of GCM after the administration of certain vaccines are not rare either (61,62).

Data on the association of the above-mentioned diseases come primarily from individual cases or series of cases described in literature, so the true cause-and-effect association of this disease with other autoimmune and malignant disorders is still unknown.

Clinical picture of GCM

Myocarditis is considered to be the cause of 4.6-14% of sudden cardiac deaths in young people, and therefore this disease should be considered even when the symptoms of the disease are mild (63-65). Unlike lymphocytic myocarditis, where prodromal symptoms in the form of respiratory or gastrointestinal infection accompanied by fever are often present, they are much less pronounced when it comes to GCM (11,66). Most common symptoms in as many as 78% of patients with GCM are orthopnea, dyspnea, fatigue, which are caused by heart muscle weakness (31). Along with the symptoms of heart failure, signs of heart failure are also often present (auscultatory presence

of late inspiratory crackles over the lungs, presence of the third heart sound, peripheral edema, distended neck veins, enlarged liver, etc.). It is important to recognize in time the signs of hemodynamic instability/shock when the patient is tachycardic, with cold extremities, which all indicate poor cardiac output. In such cases, timely application of inotropic stimulation with mechanical circulatory support is necessary. Patients suffering from GCM who initially present with signs and symptoms of heart failure have a high risk of death and, in case of a positive outcome, a great need for a heart transplant (67).

Chest pain occurs in about 19% of GCM patients (68). The pain can be similar to that experienced by patients with acute coronary syndrome, but it can also have a pericardial character. Palpitations occur due to ventricular and supraventricular arrhythmias, and the substrate for their formation is active inflammation of the heart muscle, infiltration of the myocardium by giant cells, and the development of a residual scar of the myocardium as a consequence of chronic inflammation. About 14% of patients with GCM have ventricular tachycardia on admission, which is accompanied by palpitation, syncope, and if it degenerates into ventricular fibrillation even sudden cardiac death (68, 69). The prevalence of ventricular rhythm disorders in GCM is up to 55%, of which episodes of non-sustained VT are most common (69).

The results of the study by Ghaly M. and colleagues suggest that disorders in implementation are registered in as many as 27% of patients with GCM (69). On the other hand, Okura Y. et al. showed that atrioventricular blocks were reported in 15% of patients with GCM, in contrast to approximately 50% in patients diagnosed with cardiac sarcoidosis (68). Studies have also shown that the risk of arrhythmias and sudden cardiac death does not necessarily correlate with severity of myocardial inflammation and that recovery does not necessarily correlate with the disappearance of arrhythmias. A multicenter study of GCM survivors found that 23% of patients had recurrent VT during a follow-up of 5.5 years, but mostly in patients who had a malignant cardiac rhythm disorder at initial presentation (70).

Diagnostic procedures

Given the polymorphism of the clinical picture, the first step in establishing an accurate diagnosis of this disease is to correctly take anamnestic data on previous viral infections, febrile conditions, and associated diseases. It is necessary to take a detailed history of previous diseases, with a special focus on autoimmune diseases, with which it has been shown that there is an overlap of up to 20% (71). It is important to note that in patients with myocarditis in whom the signs of heart failure appeared suddenly and did not respond as expected to the applied treatment measures, GCM should always be considered. Given that these patients can benefit significantly from a specific form of treatment compared to other forms of myocarditis, the timely decision to perform an endomyocardial biopsy can be crucial.

ECG

The largest number of patients with GCM has changes in electrocardiogram. A retrospective analysis of 1.814 ECGs of 274 consecutive patients with acute myocarditis aged ≥13 years, who were hospitalized in two centers between August 2007 and November 2019, showed that as many as 91.6% of patients have ECG abnormalities (72). The common ECG findings are ST-T wave changes, Q waves, QT interval prolongation, QRS prolongation, low voltage of QRS, atrioventricular block (AVB), bundle branch block (BBB) and ventricular tachycardia (VT), atrial fibrillation and other supraventricular arrhythmias (72-74). Considering that none of the mentioned changes are pathognomonic for GCM, the sensitivity of ECG is low (47%) (75). Changes in the form of ST segment elevation occur in 24-73% of cases depending on the results of various studies (74). In these patients, especially if they present with chest pain, it is important to make a clear distinction in relation to acute coronary syndrome. In these patients, ST elevation occurs more often when the pericardium is affected. In these cases, ST elevation is usually concave upwards and occurs in a large number of leads and is not accompanied by reciprocal ST depression as in acute myocardial infarction (76). T wave inversion is a common finding in electrocardiogram of these patients (77). When it comes to the fulminant form of GCM, the frequency of ventricular rhythm disorders and conduction disorders is higher. Atrioventricular blocks are registered in about 30% of GCM patients and their appearance in the electrocardiogram indicates a more severe form of the disease (72, 78). About 30% of patients have VT on admission, while VF occurs with a frequency of about 3% of patients (68). In addition to ventricular rhythm disorders, supraventricular heart rhythm disorders have also been described with a much lower frequency. Atrial fibrillation occurs due to myocarditis involving the left atrial wall (left atrial GCM), its dilation and changes in the structure of its wall (edema) (74, 79).

In all patients with myocarditis, if it is impossible to stop arrhythmias with antiarrhythmic drugs, GCM should always be considered. The occurrence of complete atrioventricular block in electrocardiogram of GCM patients must encourage us to rule out long-term diseases with GCM, such as Lyme disease (44%) or cardiac sarcoidosis (30%). The incidence of the association of these two diseases with GCM is much higher than in other forms of myocarditis (74, 80).

Biomarkers in myocarditis

There are no specific biomarkers that would confirm the diagnosis of GCM. As with other forms of myocarditis,

the values of inflammatory markers, markers of cardiomyocyte degradation and markers of heart dysfunction are elevated. Elevated CRP values and accelerated erythrocyte sedimentation are registered in 80-99% of patients with myocarditis (3). Given that elevated values of both CRP and erythrocyte sedimentation are present in many infectious and non-infectious diseases, their positive values have low specificity. However, there are studies that indicate a positive correlation between the level of CRP and the extent of myocardial damage assessed by CMR (81). The results of a retrospective study in which hs-CRP values were compared in patients with fulminant GCM and fulminant lymphocytic myocarditis showed that patients with GCM had lower hs-CRP values and that the cut-off value of hs-CRP- and of 11.71 mg/L was proposed as a cut-off point for differentiating the diagnosis in patients with fulminant GCM and fulminant lymphocytic myocarditis (82).

Highly sensitive TnT values are usually elevated in at least a third of patients with GCM, but specificity of hsTnT values for this disease is low considering that positive values are registered both in ischemic heart diseases and in many other cardiac and non-cardiac diseases (83, 84). The results of the joint study by Gilotra NA and colleagues showed that hsTn levels are not important in establishing a diagnosis or in assessing severity of the disease in patients with GCM (85). An early increase and a sudden decrease in hst TnI is associated with the reduction of the inflammatory process in the myocardium and suggests a good prognosis for the patient (86).

Increased BNP and NT-pro BNP concentrations in patients with GCM may be explained by the development of left ventricular dysfunction and volume overload, which is often seen in patients with GCM (87, 88). Ukena et al. showed that in patients with myocarditis NT-proBNP in the highest quartile (>4.225 ng/ml) was predictive for cardiac death or heart transplantation (hazard ratio 9.2; 95 % confidence interval 1.7–50; p = 0.011) (89).

Novel inflammatory biomarkers under investigation include tumor necrosis factor-α, interleukins, interferon-γ, serum-soluble Fas, and soluble Fas ligand levels. Elevation of these markers portends a worse prognosis (90).

Considering a possible autoimmune nature of myocarditis, elevated autoantibodies to heart components can often be found (in as many as 60% of patients) (91). The level of cardiac- and inflammatory-associated miR-21 was significantly elevated during the acute phase of myocarditis (92). Serology for possible causes of myocarditis is suggested in case of specific cases, especially if HIV or Borrelia burgdorferi is suspected (2). However, it was shown that results of serological tests for viruses did not correlate with endomyocardial biopsy findings (93). Given the association of GCM with many systemic diseases, it would be useful to perform immunological tests (antinuclear antibodies, anti-dsDNA antibodies, c ANCA, anti Scl 70 antibodies, etc.).

Echocardiographic findings

Echocardiography is a non-invasive cardiac diagnostic method that should be performed in all patients with suspected GCM. In addition to its diagnostic significance, echocardiography plays a major role in monitoring and prognosis of GCM patients. In patients with GCM, wall thickening and decreased wall motion can typically be seen, which are not present in the revascularization region of a certain coronary artery. Most often, this finding occurs due to myocardial edema and is reversible. Not infrequently, the presence of pericardial effusion is registered by echocardiography. In about 75% of patients with GCM, we have a reduced left ventricular ejection fraction (LVEF), which is an important diagnostic criterion that can lead to the diagnosis of GCM (78, 94). In a series of 51 patients with GCM, the mean LVEF was 41%: 72% had LVEF <50%, and 52% had LVEF <35%. LV dilatation was absent in 72% (71).

In recent years, the speckle-tracking method was increasingly used in the field of echocardiography for diagnosing myocarditis. In addition, this imaging technique had a role in predicting deterioration and overall event-free survival in patients with myocarditis (95). Apart from the assessment of the left ventricular diameter and LVEF, right ventricular systolic function should also be assessed in terms of right ventricular size, tricuspid annular plane systolic excursion (TAPSE), tricuspid systolic velocity (RV s'), right ventricular fractional area change (RVFAC) (3).

In case of an isolated form of atrial GCM, almost all patients have severe atrial dilatation, mitral/tricuspid regurgitation, and often mural thrombi due to the presence of hypokinesia of the atrial wall and atrial fibrillation (79).

Cardiomagnetic resonance

Cardiomagnetic resonance is the non-invasive gold standard in hemodynamically stable patients with myocarditis. CMR is recommended in patients with clinically suspected AM or in patients with chest pain, normal coronary arteries, and raised troponin, for differential diagnosis of ischemic versus nonischemic origin (2, 96). Sensitivity and specificity of CMR for diagnosing myocarditis is high (87.5% and 96.2%) (16, 97).

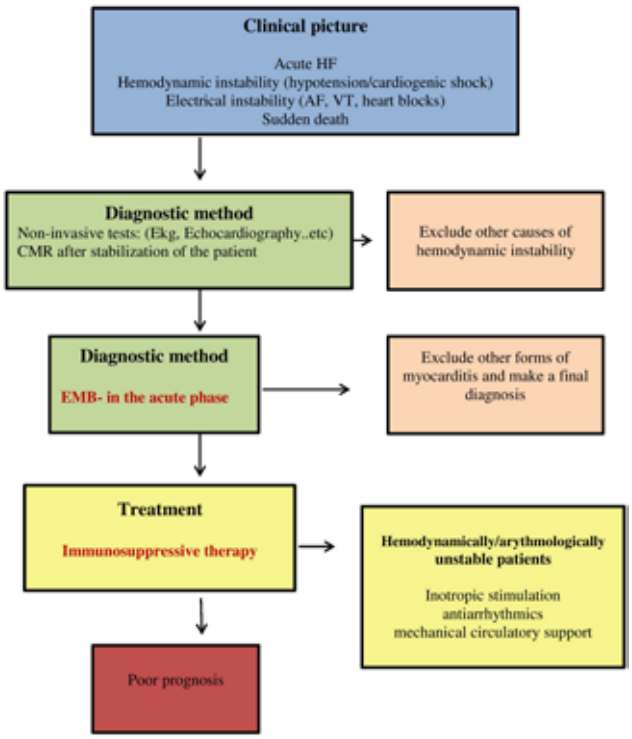
The study by Yang I. and colleagues, which was performed in patients with GCM, showed late gadolinium accumulation (LGE) in both ventricles, multilayer LGE was frequent, and most involved areas were the RV side of the septum, subepicardial LV, the anterior wall, and subendocardial RV wall. (98, 99). The largest number of patients with GCM present with multiple patchy areas of transmural LGE in addition to diffusive subendocardial LGE. In the study by Li JH and associates, CMR was compared in patients with GCM and lymphocytic AM. Subendocardial and transmural LGE characteristic of

GCM while LGE was predominantly subepicardial or missing in lymphocytic myocarditis ([86%] vs [38%]; P = 0.04) (99). Similar findings were shown by I Pöyhönen P and colleagues (100). These authors also compared the results of CMR in patients suffering from GCM and cardiac sarcoidosis and indicated a great difference in the results of CMR in these two groups of patients.

In a large number of patients, GCM is accompanied by severe general condition with the development of the fulminant form of the disease, and often CMR cannot be performed, so EMB is used as the basic diagnostic tool.

Endomyocardial biopsy

Endomyocardial biopsy is indicated in patients with suspected AM, especially those with acute HF, cardiogenic shock, left ventricular dysfunction, refractory ventricular arrhythmia, or conduction system disorders (30). The diagnostic value of endomyocardial biopsy in acute myocarditis is maintained within 2–4 weeks upon the onset (2, 3). Specifically, in case of GCM, within 2–4 weeks upon the onset, the diagnostic sensitivity is 80%,



Abbreviations: HF - hearth failure; AF - atrial fibrillation; VT - ventricular tachycardia; EMB - endomyocardial biopsy; CMR - cardiac magnetic resonance;

Figure 1. Fulminant myocarditis: clinical picture, diagnosis, treatment and prognosis

and the positive predictive value is 71% (101). Kandolina R and colleagues showed that with repeated EMB, sensitivity increased to as much as 93% for establishing the correct diagnosis (78). Of course, in such cases, the risk of periprocedural complications also increases. For the diagnosis of cardiac sarcoidosis, the sensitivity of EMB is only about 30% (102). Liang J and colleagues showed that electrogram-guided EMB targeting sites with abnormal or low-amplitude electrograms may increase a diagnostic yield for detecting abnormal pathological findings. The risk of cardiac perforation (0.2%) and vascular (1.2%) or embolic (0.4%) complications from catheter manipulation during electrophysiological studies is low (103, 104) **(Figure 1.)**

Treating patients with GCM

Given that a large number of patients with GCM present with severe weakness, it is important to use all the drugs recommended in the ESC guidelines for treating heart failure in these patients. Furthermore, in patients with the fulminant form of the disease and the development of cardiogenic shock, it is necessary to apply inotropic and vasopressor support as soon as possible. The administration of inotropic stimulation should not last long, it was shown that long-term use of large doses of noradrenaline dramatically increased in-hospital mortality in patients with myocarditis (105). In most severe cases, the application of mechanical circulatory support is advised. Mechanical circulatory support can be used regardless of initiation of immunosuppressive therapy. Different MCS devices (veno-arterial extracorporeal membrane oxygenation (V-A ECMO); intra-aortic balloon pumps (IABPs); percutaneous ventricular assist devices TandemHeart; ProtekDuo; Impella microaxial flow catheters) are effective for temporary hemodynamic stabilization and can serve as a bridge-to-transplant in patients with fulminant myocarditis (91). The study by Li S et al. showed that earlier introduction of IABP provided effective circulatory support for fulminant myocarditis patients in shock. In addition, the application of IABP also decreased the rate of in-hospital deaths. Moreover, within the same study it was shown that the combination of IABP and ECMO provided enough circulation support. All patients recovered after 3–5 days of support (105). And many other studies have shown that the combined application of mechanical circulatory support methods (V-A ECMO with IABP, EC-IVAC or LV impeller) was safer and more effective than the application of ECMO alone in patients with fulminant myocarditis (106, 107).

Patients with myocarditis have a high risk of heart rhythm disturbances and conduction regardless of the height of LVEF, and their treatment should be in accordance with the leading recommendations for the treatment of arrhythmias (108, 109). It is important to note that many patients with GCM are resistant to the use of high doses of antiarrhythmics. In these patients, sometimes temporary pacing may also be effective for suppressing an electrical storm (3). The occurrence of recurrent malignant heart rhythm disorders (ventricular tachycardia/ventricular fibrillation) may require

antiarrhythmic medications such as amiodarone and/or implantation of a cardioverter defibrillator for secondary prevention if meaningful survival >1 year is expected (71). Cardiac device implantation for the management of ventricular arrhythmias should be evaluated after resolving reversible acute myocarditis, generally 3–6 months after the initiation of the acute phase (91). In patients with GCM, given the high rate of the occurrence of ventricular rhythm disorders, even during disease regression, placement of an implantable cardiac defibrillator (ICD) is generally recommended in all patients including those with full recovery of LVEF (2).

Nowadays, combined immunosuppressive therapy is inevitably used in patients with GCM. The combined administration of 1 or 2 immunosuppressants with steroids is usually used. A prospective multicenter study investigated the use of immunosuppressive therapy in patients with acute endomyocardial biopsy-proven GCM. The results of serial endomyocardial biopsies showed that 4 weeks upon the start of the treatment, the degree of necrosis and cellular inflammation decreased ($p = 0.001$) (24). The results of a meta-analysis conducted on 1534 patients with acute GCM showed that intravenous administration of immunoglobulin significantly reduced in-hospital mortality (OR: 0.44, 95% CI 0.17 to 0.71, $P < 0.001$) and improved LVEF (OR: 1.73, 95% CI 1.34 to 2.13, $P < 0.001$) (110). Discontinuation of immunosuppressive therapy was correlated with fatal recurrence of GCM. It is recommended to use the steroid Methylprednisone (10 mg/kg/day) for 3 days in the acute fulminant phase of GCM with continuation of chronic administration of Prednisolone (1.0 mg/kg/day with dose titration to about 5 mg/day) for a year. The use of cyclosporine and tacrolimus is also recommended. Alternative regimens may include high-dose corticosteroids along with cyclosporine and azathioprine (1.5 to 2 mg/kg/day) (71). Azathioprine is a purinergic antagonist that is used in combination with calcineurin inhibitors like Tacrolimus. In previous studies, a 3-drug combination of steroids, cyclosporin, and azathioprine was used (111). The duration of immunosuppressive therapy must be well considered due to a possible recurrence of the disease even after 8 years upon the diagnosis of GCM.

Prognosis of the disease

The results of the study by Ammirati E. and colleagues showed that fulminant presentation of diseases, giant-cell histology, QRS interval >120 ms on electrocardiography, the presence of cardiac arrest and advanced atrioventricular block were significantly associated with a poor outcome in patients with AM within 60 days of placement disease diagnoses (31). Histological subtype of the disease (especially GCM type compared to lymphocytic and eosinophilic type) is the main independent predictor of poor outcome in patients with fulminant myocarditis both in the period up to 60 days of follow-up and after 3 years of follow-up (overall log-rank $p < 0.0001$) (31). The use of immunosuppressive therapy prolongs the survival of patients with GCM. In patients with GCM, the need for a heart transplant is more common, and disease recurrence occurs in 20% to 25% of patients after heart transplantation (112). The results of the study by Kandolin R. and associates showed that about 52% of patients survived in the five-year follow-up period from the moment of GCM diagnosis (79). However, with more frequent application of heart transplantation, the cumulative survival rate of GCM patients has been prolonged, so after 1, 5 and 10 years it is 94%, 82%, and 68%, respectively (113).

CONCLUSION

Giant cell myocarditis is one of the most severe forms of myocarditis, often accompanied by a fulminant course of the disease. In patients with the development of acute heart failure accompanied by hemodynamic instability and frequent occurrence of malignant arrhythmias that are poorly controlled by modern antiarrhythmic drugs, this disease should always be suspected. The majority of patients are diagnosed with EMB, which is the gold diagnostic standard when it comes to this disease. A correct and rapid diagnosis in patients with GCM is extremely important in order to administer an adequate drug therapy regimen (modern heart failure therapy, inotropic stimulation, immunosuppressive therapy) to the combined application of various types of mechanical circulatory support and an early decision for heart transplantation. Considering a constant topicality of this topic, the high rate of disease recurrence and high early and long-term mortality, this paper should help doctors in daily clinical practice both with a faster diagnosis and with the adequate selection of therapy, with the aim of reducing the mortality rate in patients with GCM.

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GIGANTOCELULARNI MIOKARDITIS U MODERNOJ KLINIČKOJ PRAKSI

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Sažetak

Gigantocelularni miokarditis predstavlja retko ali izrazito teško oboljenje sa čestom potrebom za transplantacijom srca i visokom stopom mortaliteta. I do današnjeg dana najčešći razlog pojave ovog oboljenja nije precizno odredjen, ali njegova česta udruženost sa autoimunim bolestima govori u prilog autoimune etiologije. Klinički se prezentuje u najvećoj meri simptomima i znacima akutnog srčanog popuštanja i ventrikularnim poremećajima ritma koji se registruju kod čak polovine obolelih. Aritmije mogu biti praćene pojavom palpitacija, ponavljanim sinkopama pa i naprasnom srčanom smrću. Težak stepen akutne srčane insuficijencije kod hemodinamski nestabilnih bolesnika koji neadekvatno reaguju na primenjenu terapiju za srčanu insuficijenciju i refraktorni poremećaji srčanog ritma i/ili poremećaji u sprovođenju koji su česti u ovih bolesnika uvek treba da pobude sumnju da se radi o gigantocelularnom miokarditisu. S obzirom na to da promene u elektrokardiogramu i ehokardiografiji kao i pozitivne vrednosti biomarkera nisu strogo specifični za ovo oboljenje dijagnoza gigantocelularnog miokarditisa se najčešće postavlja endomiokardnom biopsijom, naročito kod bolesnika sa fulminantnom formom bolesti. Pravovremena endomiokardna biopsija omogućava ne samo brzo i precizno postavljanje dijagnoze, već i ranu primenu imunosupresivne terapije koja u mnogome poboljšava ishod kod ovih bolesnika. Patohistološka verifikacija ovog oboljenja važna je u cilju isključivanja kardijalne sarkoidoze i drugih granulomatoznih infektivnih i neinfektivnih oboljenja. S obzirom da veliki broj bolesnika ima fulminantan tok bolesti, rana i brza dijagnostika, primena inotropne stimulacije i mehaničke cirkulatorne potpore kod hemodinamski nestabilnih bolesnika, adekvatan moderan terapijski režim u mnogome mogu smanjiti mortalitet kod ovih bolesnika koji je i dalje visok uprkos dijagnostičkom i terapijskom napretku savremene medicine.

Ključne reči: fulminantni miokarditis, akutna srčana insuficijencija, hemodinamska nestabilnost, endomiokardna biopsija, mortalitet

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ORIGINAL ARTICLE

Primary split thickness skin grafting for hand and finger defects: do not hesitate

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Summary

Introduction: Hand injuries often result in soft tissue defects. The treatment of these defects belongs to the most difficult challenges in reconstructive surgery. There are numerous options for covering soft tissue defects, including flaps and skin grafts**.**

Material and methods: This retrospective observational study included seventeen patients with 24 skin defects of the hand, who were treated by primary split-thickness skin graft (STSG) in a single center. The average follow-up period was 6 months**.** The age of the patients ranged from 36 to 80 years. The majority of patients (n=16) were males, and one patient was female. Skin defects varied in size from 7x8mm to 39x40mm. Primary goals were STSG survival, recipient site infection, and donor site morbidity. Secondary goals were cosmetic appearance and time needed for complete wound healing.

Results: All 24 wounds healed successfully in a mean of 28,11±9,94 days. There were no graft infections. Partial graft loss occurred in one case. There was no major donor site morbidity reported. Six patients described the cosmetic result as good (score 3), 10 patients ddescribed it as acceptable (score 2), and one patient described it as poor (score 1).

Conclusion: Split thickness skin graft is an excellent option for immediate treatment of hand and finger skin defects. This method is simple, has less consequences than secondary grafts, requires minimum equipment and can sometimes be done in the emergency room, without hospitalization. Therefore, there is no need to be afraid of primary skin grafting.

Keywords: hand, finger, soft tissue defects, primary reconstruction, split-thickness skin graft

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INTRODUCTION

The skin of the hand is specifically designed to provide tactile input from the environment and it must be resistant to numerous factors and forces (1). Therefore, restoration of the skin coverage is extremely important and it must provide a good aesthetic result and the earliest and maximal recovery of function (2, 3).

Hand injuries are extremely common in home and industrial setting. These injuries are of particular interest because they often result in soft tissue defects. The treatment of these defects represents one of the most difficult challenges in reconstructive surgery.

Tissues should be replaced as soon as possible, but not necessarily at the time of injury. The aim of the initial treatment is to provide primary wound healing whenever possible, because it minimizes inflammation and reduces the length of hospital stay (4). In an ideal situation, the primary procedure is definitive and early wound closure and rapid healing are obtained (2,3).

There are numerous options for covering soft tissue defects, including flaps and skin grafts. A flap is a healthy tissue with its own blood supply, attached to the donor site by a pedicle (4). Skin grafts are avascular, therefore their survival depends on the ingrowth of blood vessels from the recipient area.

When deciding upon the most suitable method of tissue replacement, each case must be accessed individually and various factors must be considered – age, sex, general health and previous condition of the hand, as well as the patient's social and economic status (5).

MATERIAL AND METHODS

Seventeen patients with 24 skin defects of the hand were treated by primary split-thickness skin graft (STSG) between January 2017 and August 2020 in a single center. Each patient underwent a complete evaluation which consisted of preoperative clinical and radiological assessment, prescription of antibiotics and tetanus prophylaxis. The procedure was done in axillary block anesthesia. The hand and arm were prepared and draped above the elbow. The njured hand was inspected thoroughly for tissue viability and integrity of tendons and neurovascular structures. Firstly, meticulous debridement and irrigation were performed. The injury dictated whether other procedures had to be done. Fractures and fracture-dislocations had to be stabilized. In our cases, only Kirschner wires (K wires) were used. Tendon and nerve repair were performed if needed. Homeostasis was secured by cauterization. The exact size of the skin defect was measured by a surgical ruler and traced with a sterile marking pen on the donor site. Depending on the size of the defect, an STSG was taken using a dermatome or a Humby knife. A petroleum gauze was firmly applied on the donor site, covered with an iodine solution soaked gauze, and then tightly bandaged.

The graft was fenestrated by a surgical blade, applied to the soft tissue defect and secured by peripheral sutures (Dafilon® 4-0 nylon). Petroleum gauze was applied and gently molded around the edges of the defect. The hand was immobilized using a plaster splint and maintained in position. The dressing was not changed for three days. The stitches were removed on the 14th postoperative day. The immobilization was removed after the wound had completely healed. If there were any associated injuries (tendon lesions and/or fractures), the immobilization was prolonged. When the cast and K wires were removed, physical therapy was introduced.

The average follow-up period was 6 months. Patients' age ranged from 36 to 80 years with a mean age of 56,76±13,6 years. There were sixteen male patients and one female patient. They sustained their injuries by industrial machines (10 patients), agriculture machines (two patients) and other (five). Skin defects varied in size from 7x8 mm to 39x40 mm. Wound localization, associated injuries, the length of hospital stay and STSG size are shown in **Table 1**.

Primary measures were STSG survival, recipient site infection and donor site morbidity. Secondary measures were cosmetic appearance and time needed for complete wound healing. The criteria for the wound to be considered healed included complete epithelialization, no wound drainage, as well as the patient being allowed to wash their hands. Patients were asked to rate their cosmetic outcome on a 3-point scale, developed by the authors. The score of 0 denotes patient's unhappiness; the score of 1 denotes poor appearance; the score of 2 denotes acceptable appearance, and the score of 3 denotes total satisfaction with cosmetic results.

RESULTS

The purpose of this paper was to report the clinical results of immediate treatment of hand defects based on the hypothesis that primary STSG shortened the surgery time and the length of hospital stay, the number of interventions needed with no additional surgery skills or expensive equipment required. This was a retrospective observational study without a control arm. All 24 wounds healed successfully in a mean of 28,11±9,94 days without further surgical interventions. There were no graft infections. Partial graft loss occurred in one case over the perichondrium. There was no donor site morbidity reported, except for slight hypopigmentation in seven cases and hyperpigmentation in six cases. Six patients described the cosmetic result as good (score 3), 10 patients described it as acceptable (score 2), and one patient described it as poor (score 1). None of the patients reported any wound drainage, tissue disintegration, xerosis, scaling or pruri-

Table 1. Patient data

Patient	Age	Side /L-left R-right/	Cause	Skin defect	Associated injuries	Hospital length of stay */days/*	STSG size */mm x mm/*
1	71	L	Grinder	Radial side of 3rd finger and radial and volar side of 2nd finger	Fracture	9	27x13 68x21
2	36	L	Explosive device	Mangled hand with dorsal skin destruction	Fracture, tendon lesion,	19	20x13 34x28
3	45	L	Printing press	Ring avulsion, 3rd finger	Fracture	6	15x22
4	36	R	Planer machine	Tip of 2nd, medial and distal phalanges 3rd and 4th and distal phalanx 5th finger	Tendon injury	8	7x8, 38x13, 12x7, 34x11
5	55	R	Traffic accident	Dorsal skin avulsion of the proximal phalanx of 2nd finger MCP joint	Fracture, tendon injury	10	39x40
6	36	R	Corn grinding machine	Mangled index finger, amputation of thumb's distal phalanx	Fracture	11	37x19
7	80	L	Carpentry machine	Mangled distal phalanges from II to V finger	Fracture	8	21x7, 7x9, 9x8
8	63	L	Circular saw	Thumb's distal phalanx.	Fracture, tendon lesion	11	26x12
9	47	R	Circular saw	Dorsal over PIP joint, middle finger	Fracture, tendon lesion	12	14x21
10	54	L	Metal pipe	Proximal and middle phalanges, index finger	Fracture, tendon lesion	9	42x20
11	61	L	Fall	Medial and distal phalanges, 4th finger	Fracture	9	46x32
12	72	R	Planer machine	Dorsal, medial phalanx, PIP joint of 3rd finger	Fracture	12	28x16
13	57	R	Corn picker	Volar side, 3rd finger's distal phalanx	Fracture, tendon lesion	22	23x16
14	80	L	Sickle	Dorsal, PIP joint, index finger	Fracture, tendon lesion	9	13x21
15	58	L	Circular saw	Mangled hand with index finger amputation. STSG over the 1st web space	Fracture, tendon lesion	10	46x30
16	62	L	Circular saw	2nd and 3rd finger amputation, partial amputation of the 4th finger. STSG for fingertip of the thumb	Fracture, tendon lesion	6	9x7
17	52	R	Circular saw	2nd finger	Fracture	10	66x17

tus at the 6-month mark following their injuries, when the last control assessment was conducted.

DISCUSSION

The first skin transplantation was performed by Reverdin in 1869 (6). In 1929 Brown introduced his technique of STSG and was the first to differentiate between full-thickness and epidermal (Thiersch) grafts (6). Since then, there have been no significant changes in the basic principles.

Even though the only indication for the use of skin grafts mentioned in this paper is hand trauma, there are numerous other indications suggested in literature (2). Generally, they can be divided into two main groups, primary and secondary. Primary skin grafting was described in traumatic wounds. Secondary grafting is taken into consideration for granulating wounds (2).

Primary Thiersch graft use has been described in literature for treating hand and finger defects, fingertip skin defects, donor defects of hypothenar flaps, palm and finger defects following the release of Dupuytren's contracture, skin avulsion of the upper and lower extremity, crush injuries of the foot, severe open fractures and mangled extremities (split-thickness skin excision technique) (7-13). Also, in coverage of the vascular pedicle in free tissue transfer, extensive traumatic skin loss and surgical

wounds after scar or neoplasm excision, biofilm-associated infections in chronic diabetic ulcers and even in the treatment of chronic osteomyelitis alongside surgical debridement (2,14-16).

A consensus has not been reached about primary wound closing. It is obvious that primary coverage is not indicated in crush injuries and wounds with a high risk of infection such as farm injuries, as well as those with necrotic tissue (12). Many papers disagree with the role of primary STSG in hand injuries, as they are not suitable for exposed tendons, bones and joints (17,18). Elliott and colleagues advised against skin grafting in finger and thumb tips, stating that the procedure resulted in donor site morbidity, delayed mobilization, poor sensation and esthetics (19). Instead, they opted for healing by secondary intention, full-thickness and venous flaps, with the advent of full-thickness skin graft because of better skin quality (19). Numerous authors also mention sanatio per secundam as a good option for finger injuries (1, 20, 21). Others disagree, and Patton found that spontaneous healing took one to three months to heal enough for the patient to go back to work and the finger may have decreased function (10). Pros and cons of STSG versus full-thickness skin graft have been a subject of discussion for a long time. Krister prefers full-thickness over split-thickness skin grafts in fingertips because STSG is difficult to hold in place and it leaves a sensitive scar after healing (22). STSG can survive in a less vascularized bed, no suture of the donor site is necessary and it is easier to take because there is no hematoma forming due to meshing (6,8,13). On the other hand, STSG gives greater contractures post-operatively, especially on the flexor side of the joint, worse cosmetic results than full-thickness and less resistance on shear stress (6,13). Wood prefers full-thickness grafts on areas where scarring would result in a significant loss of function and poor cosmetic result, such as the hand (17). The donor site of full-thickness heals quicker with less pain and a smaller scar than STSG. Beasley stated that there was no significant difference between a full-thickness graft and a very thick STSG (4).

We agree with the philosophy that the fresh wound is an adequate site to be covered with healthy donor tissue (12). As Pshenisnov and colleagues stated, emergency coverage in hand injuries results in the most rapid bone healing, fewer surgical interventions, shorter hospital stay and the lowest infection rate (18). The use of STSG as a primary treatment in traumatic hand wounds is not a new idea. Many papers describe this method as superior to alternatives in providing skin coverage with minimum morbidity, and without the need to wait for clean healthy granulations suitable for skin grafting (7-10,15). On the other hand, with delayed coverage there is a higher potential risk of secondary infection, and it may result in prolonged hospitalization, which has economic and psychosocial consequences (11, 12).

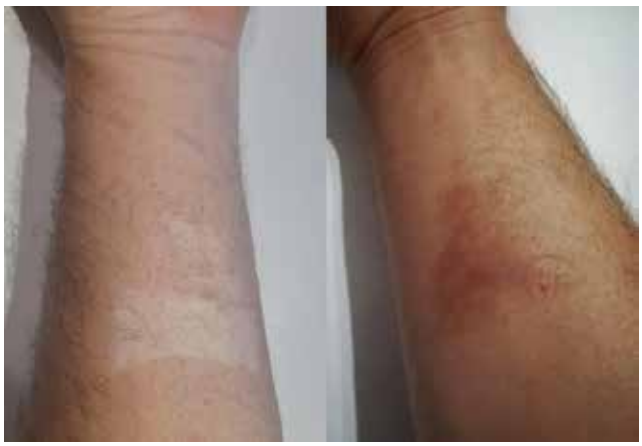

Figure 1. Hypo- and hyperpigmentation of the donor site

STSG may be taken from any area of the body (6). When deciding upon the donor site for hand defects, important factors to consider are the absence of hair, similar skin color and texture, dermal thickness, and potential donor site morbidity. Tissues near the recipient site will obviously be the best match (4, 17). The most common donor sites are thigh, inner aspect of the arm, forearm and hypothenar eminence (2,7,14,15,22). In almost all of our cases, the front of the forearm was used, except in one patient in whom the size of the defect dictated using a larger donor site, so we used anterolateral aspect of the thigh. The clear advantage of using the forearm is that it requires no additional preparation or drape and the procedure is usually done in regional anesthesia so no other type of anesthesia is needed. We did not have any donor site morbidity, except a slight hypo- or hyperpigmentation of the skin, also described by other authors and presented in **Figure 1** (6,17).

There are different types of instruments for removing STSG. The most commonly used are hand-held skin knife and the electrical dermatome. The choice of instrument depends on the size of the defect and the surgeon's experience (23)**.** The procedure can be done with minimum equipment and in the emergency room, without hospitalization. We only used a dermatome in one case, and a Humby knife in others.

STSG can be meshed or not. When the skin is perforated, an increased area can be covered, exudate and hematoma can be drained and graft modeling on irregular surfaces is better, although the result may be pebbled and less aesthetically pleasing (15, 17, 21). According to some authors, meshing even promotes angiogenesis (24, 25). In all the cases in our study, we perforated the STSG with a surgical blade, given that the largest defect was still too small for a mesher.

The recipient site must have effective blood microcirculation. Therefore, skin grafts can be applied on fascia, muscle, periosteum, paratenon, perichondrium, granulation surface and adipose tissue (6). In our paper, the graft was applied on finger pulp in 11 cases, paratenon in eight cases, muscle in two cases and on the periosteum and

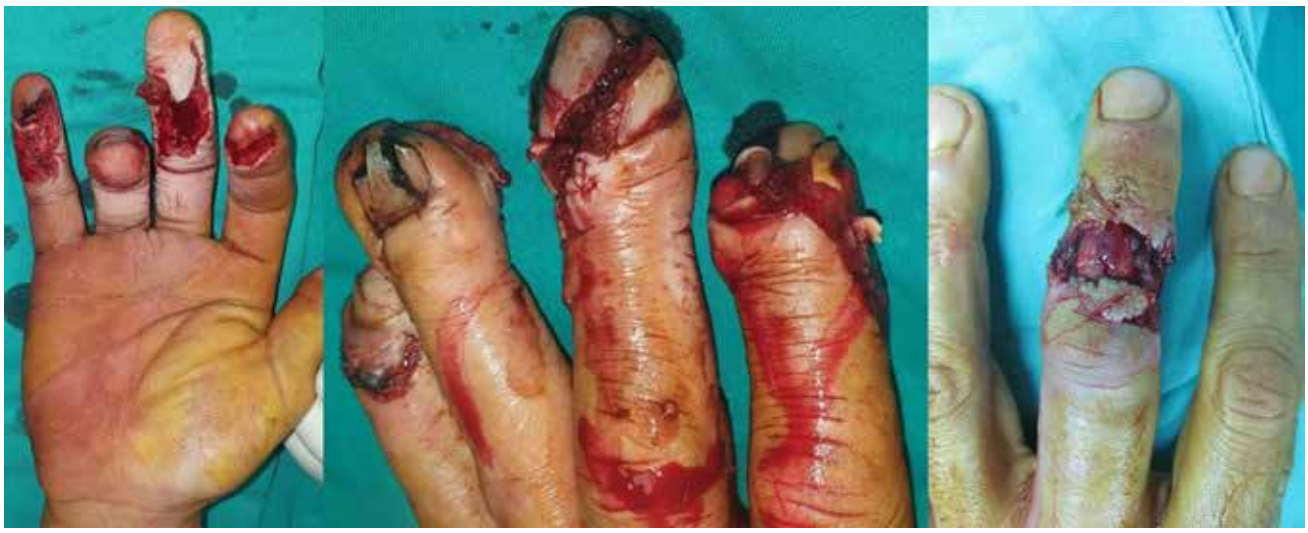

Figure 2. Recipient site examples

perichondrium in three cases. Some of the recipient sites from this paper are shown in **Figure 2**. Even a mangled hand can be a good recipient site **(Figure 3)**.

The most common causes of graft failure are infection and haematoma leading to mechanical separation (2, 17). Post-operative care is crucial for skin graft success (8). Failure can sometimes be caused by inadequate fixation of the graft (2). Fixation is performed through the margin by suturing (14, 17). In all the cases in our study, the grafts were sutured. The graft should cover the whole defect. The limb must be splinted, especially around joints

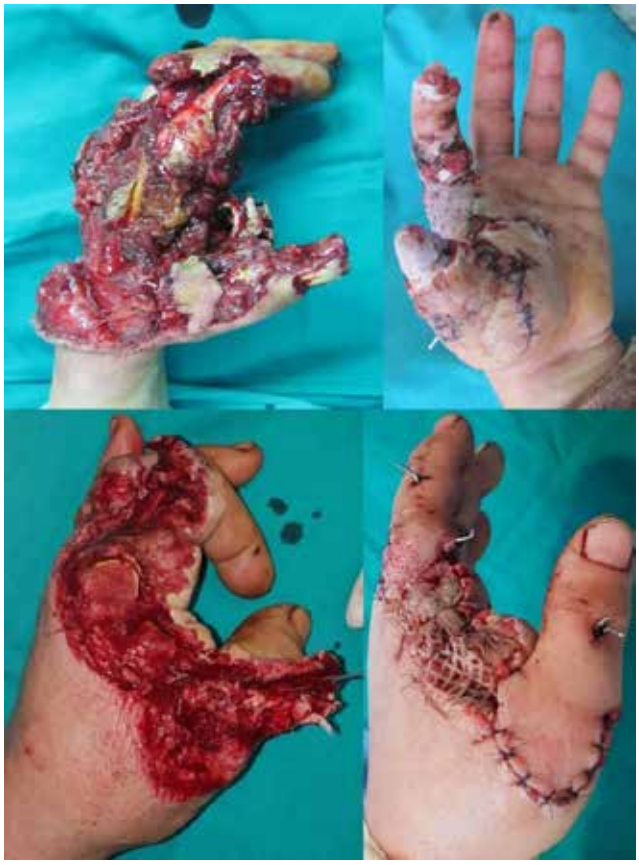

Figure 3. The use of STSG in a mangled hand

(2). By decreasing the movement of the dressings, a graft is protected from shear stress and trauma (21). The patient must be informed about the protection of the graft and donor area (10). According to Rank, fixation and firm pressure are more important for primary graft take than the local blood supply (2).

The average healing time in our patients is 28,11±9,94 days, which is similar to the findings of other authors (8-10). Out of 24 defects, partial graft loss occurred in one case, in which the graft was applied on the perichondrium. Patton describes one graft failure and Mosher a few cases of partial loss out of 40 patients (8, 10). Rank used primary STSG in three cases for fresh trauma, and his original research included numerous different indications for STSG (2). Results shown referred to the total number of cases. Complete graft take was achieved in 59% and incomplete in 36% (2). Innis describes the use of STSG in six severe hand injuries and the graft take was 90-100% (11). We had no major donor site morbidity, as is seen in other papers as well (8,10). The cosmetic appearance was assessed on a scale of 0-3 and most of our patients (n=10) rated it as acceptable (58,82%). 35,29% rated the result as good and 5,88% as poor. Cosmetic results are shown in **Figure 4**. We found no similar data for primary STSG in the available literature. Schenck used a similar tool, but for full-thickness grafts (3).

Hand defects can also be covered with various flaps. For smaller finger or fingertip defects, there are different available options, such as V-Y advancement flap, cross finger flap, Moberg or thenar flap (1,8,26-31). For defects with exposed bone and tendons, a dorsal metacarpal artery flap or island flap can be used (28, 32-34). In recent years, there have been more papers describing the use of perforator flaps, venous free flaps and even the use of free vascularized toe pulp and partial toe transfers (28,35-40). Flaps also cause greater donor site morbidity, may necessitate sacrifice of a peripheral artery and result in a longer hospital stay (39). With all of these flaps and techniques in mind, we must ask ourselves whether primary

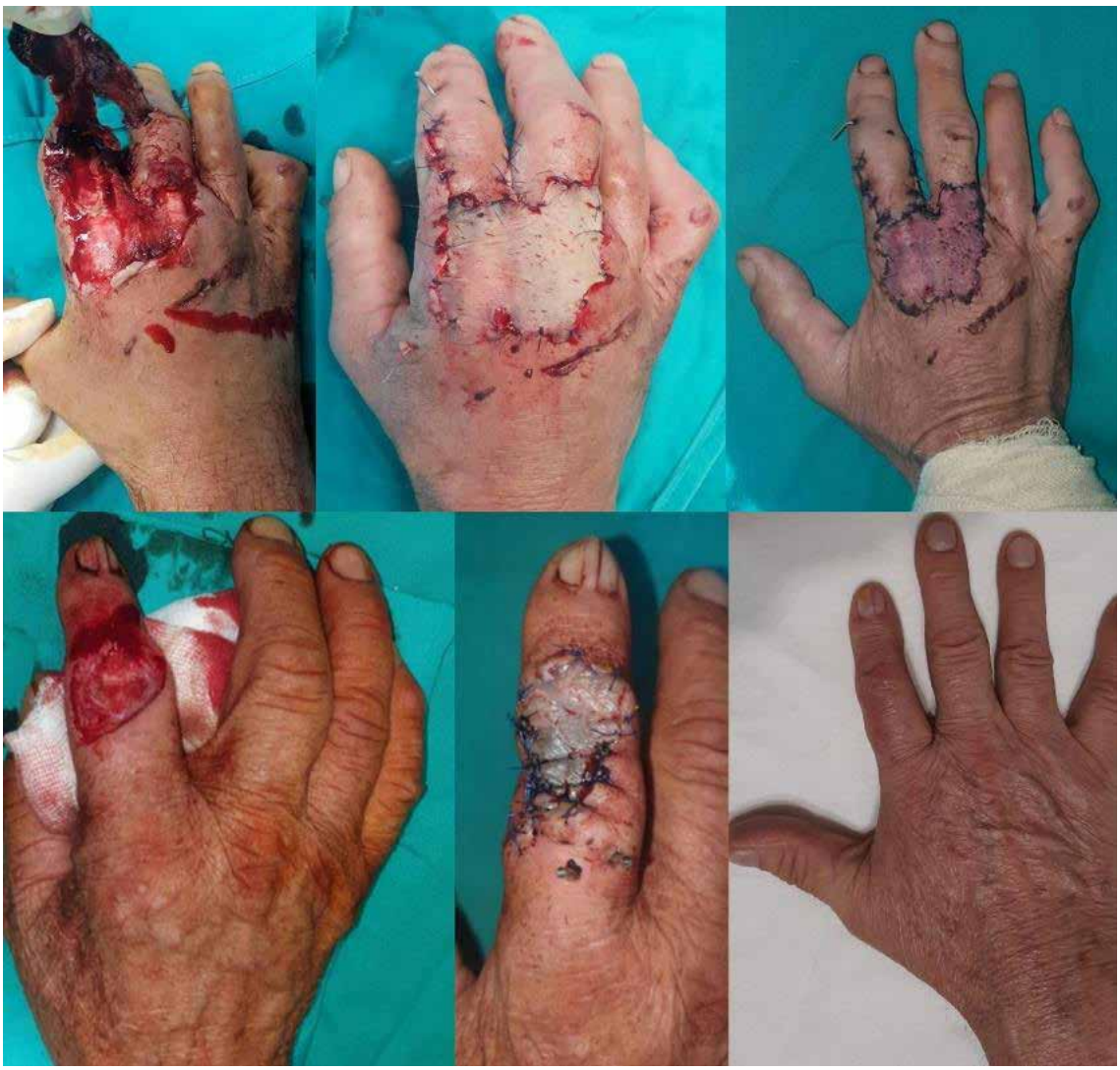

Figure 4 - Preoperative, postoperative and follow-up state

STSG still has a place in the management of hand soft tissue defects.

There are no recent papers that describe primary STSG use. All the literature concerning this subject was published 40 to 80 years ago, before the introduction of various flaps, intraoperative Doppler use and advancement of microsurgery technique (2, 5, 8-11). Although flaps are a powerful tool in a surgeon's hand, they require specially trained surgical staff and the procedure itself is more complicated and significantly longer. We think that even in modern times, STSG, as a less invasive method, lower on reconstructive ladder, can still be used with similar outcome.

CONCLUSION

A fresh wound after surgical debridement is an ideal bed for skin grafting. Many risks associated with delayed treatment can be avoided by primary coverage. The method is simple, it is easy to learn and requires minimal equipment so it can be done in the emergency room. The wound heals more quickly, the hospital stay is shorter and the functional result is better with earlier return to work. Even with all the new techniques available, STSG still has an important place on the reconstructive ladder and there is no reason for any diffidence in managing skin defects.

CONFLICT OF INTEREST

We know of no conflict of interest associated with this publication, and there has been no financial support for this work that could have influenced its outcome.

AUTHOR CONTRIBUTIONS

According to the authors, the following contributed to the paper: conceptualization and design of the study: Sladjana Matic. Katarina Gambiroza and Petar Vukman, who collected the data, performed the statistical analysis, and created the figures. Sladjana Matic and Tomislav Palibrk carried out the analysis and interpretation of the

findings. Sladjana Matic and Darko Milovanovic prepared the draft manuscript. Katarina Gambiroza edited the manuscript's grammar. Petar Vukman and Mihailo Ille provided technical and administrative help during the writing. An article revision of the scientific content was performed by Mihailo Ille. The final draft of the manuscript was approved by all authors after they had evaluated the findings.

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PRIMARNI GRAFT PARCIJALNE DEBLJINE KOŽE ZA POKRIVANJE MEKOTKIVNIH DEFEKATA ŠAKE I PRSTIJU: NE OKLEVAJTE

Matić Sladjana[1,2], Gambiroza Katarina[1], Vukman Petar[1], Milovanović Darko[1,2], Palibrk Tomislav[1,2], Ille Mihailo [1,2]

Sažetak

Uvod: Povrede šake često uzrokuju defekt mekih tkiva, čije lečenje predstavlja jedan od najtežih izazova u rekonstruktivnoj hirurgiji. Postoje brojne opcije za pokrivanje mekotkivnih defekata, uključujući flapove i kožne graftove.

Materijal i metode: Sprovedena je retrospektivna opservaciona studija, koja je obuhvatila 17 pacijenata sa 24 kožna defekta šake, koja su lečena primarnim graftovima parcijalne debljine kože u jednom medicinskom centru. Prosečan period praćenja je bio šest meseci. Starost pacijenata se kretala od 36 do 80 godina. Bilo je 16 pacijenata muškog pola i jedan pacijent ženskog pola. Kožni defekti su varirali po veličini, od 7x8mm do 39x40mm. Primarni ciljevi su bili preživljavanje grafta, infekcija recipijentnog mesta i morbiditet donorskog mesta grafta. Sekundarni ciljevi su bili kozmetički rezultati i potrebno vreme za kompletno zarastanje rane.

Rezultati: Svih 24 rana je sraslo u prosečnom periodu od 28,11±9,94 dana. Nije došlo do pojave infekcije grafta ni kod jednog pacijenta. U jednom slučaju je došlo do parcijalnog gubitka grafta. Nije prijavljen značajan morbiditet donorskog mesta. Šest pacijenata opisuju kozmetički efekat kao dobar (skor 3), 10 pacijenata kao prihvatljiv (skor 3) i jedan pacijent kao loš (skor 1) .

Zaključak: Graft parcijalne debljine kože predstavlja odličnu opciju za inicijalni i definitivni tretman defekta kože prstiju i šake. Ovakav način lečenja je jednostavan, nosi manje posledica od sekundarnog pokrivanja defekta, zahteva minimalnu medicinsku opremu i ponekad se može uraditi u okviru hitnog prijema, bez potrebe za hospitalizacijom pacijenta. Ne treba oklevati u primeni ovakvog načina pokrivanja defekta, ukoliko postoji takva klinička indikacija.

Ključne reči: šaka, prsti, defekt mekih tkiva, primarna rekonstrukcija, kožni graft parcijalne debljine

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Simpozijum „Stremljenja i novine u medicini“ Medicinskog fakulteta u Beogradu

Appendix sa apstraktima

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Tradicionalni simpozijum „Stremljenja i novine u medicini“ Medicinskog fakulteta u Beogradu, održava se svake godine u nedelji svečanosti koja se organizuje povodom Dana fakulteta 9. decembra.

Specijalni broj časopisa “Medicinska istraživanja“ prati simpozijum u obliku Knjige sažetaka.

Ovogodišnji simpozijum “Stremljenja i novine u medicini“ održava se od 4. do 8. decembra 2023. godine.

Članovi Organizacionog **odbora simpozijuma„Stremljenja i novine u medicini“**

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Prof. dr Katarina Paunović
Doc. dr Darko Antić

Sekretar
Dr sc. Viktorija Popović

Tehnički sekretar
Tijana Tošić

Sadržaj

MINI SIMPOZIJUM: NOVINE U DIJAGNOSTICI I TERAPIJSKOM PRISTUPU INFEKCIJAMA U JEDINICI INTENZIVNOG LEČENJA

MINI SIMPOZIJUM: 100 GODINA KATEDRE ZA HIGIJENU NA MEDICINSKOM FAKULTETU - OD NARODNOG ZDRAVLJA DO MEDICINSKE EKOLOGIJE

MINI SIMPOZIJUM: NOVI TRENDOVI U DOKTORSKOJ EDUKACIJI U OBLASTI BIOMEDICINSKIH NAUKA U EVROPI

MINI SIMPOZIJUM: 100 GODINA INSTITUTA ZA HEMIJU U MEDICINI „PROF. DR PETAR MATAVULJ“ MEDICINSKOG FAKULTETA U BEOGRADU

MINI SIMPOZIJUM: MAGNETNA REZONANCA SRCA: DA LI JE BUDUĆNOST SVETLA?

MINI SIMPOZIJUM: STO GODINA KLINIKE ZA NEUROLOGIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE - EVOLUCIJA NEUROLOŠKE MISLI TOKOM JEDNOG VEKA

MINI SIMPOZIJUM: 100 GODINA KLINIKE ZA PSIHIJATRIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE

MINI SIMPOZIJUM: LAJMSKA BOLEST: NOVA SAZNANJA I AKTUELNE DILEME

MINI SIMPOZIJUM: ŠTA, KADA I KAKO – PROCES DONOŠENJA ODLUKE U URGENTNOJ HIRURGIJI, KONTROVERZE I STREMLJENJA

MINI SIMPOZIJUM: NARKOMANIJA, ASFIKSIJE, SAMOUBISTVO – NOVI PATOFORENZIČKI ASPEKTI

MINI SIMPOZIJUM: DELO PROF. DR ALEKSANDRA Đ. KOSTIĆA – POVODOM 130 GODINA OD ROĐENJA I 40 GODINA OD SMRTI

MINI SIMPOZIJUM: SAVREMENI PRISTUP RADIOLOGIJI DOJKE: DIJAGNOSTIČKE I BIOPSIJSKE PROCEDURE

MINI SIMPOZIJUM: SINHRONIZOVANO DO CILJA – 20 GODINA KOHLEARNE IMPLANTACIJE NA KLINICI ZA OTORINOLARINGOLOGIJU I MAKSILOFACIJALNU HIRURGIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE

MINI SIMPOZIJUM

NOVINE U DIJAGNOSTICI I TERAPIJSKOM PRISTUPU INFEKCIJAMA U JEDINICI INTENZIVNOG LEČENJA

Univerzitetski klinički centar Srbije, Beograd

Služba za bolničku epidemiologiju i higijenu ishrane

EPIDEMIOLOŠKI NADZOR NAD BOLNIČKIM INFEKCIJAMA U JEDINICI INTENZIVNOG LEČENJA URGENTNOG CENTRA

Vesna Mioljević

Infekcije uzrokovane mikroorganizmima otpornim na većinu dostupnih antibiotika, (MDRO) su u porastu u poslednjih deset godina. Otpornost bakterija na antibiotike javlja se I kao posledica neracionalne upotrebe antibiotika. Infekcije uzrokovane MDRO predstavljaju veliki terapijski problem. Prisustvo MDRO u bolničkoj sredini uz postojanje faktora rizika za prenošenje omogućavaju širenje MDRO u bolničkoj sredini. Prenošenje MDRO najčešće se javlja na odeljenjima intenzivne nege ali se može javiti i na svim drugim odeljenjima bolničke ustanove.

Preporuke Centra za kontrolu i prevenciju bolesti, Altanta, www.cdc.gov/getsmart/healthcare.

Mere prevencije i kontrole MDROs mogu se grupisati u nekoliko kategorija: racionalna upotreba antibiotika, primena standardnih i kontaktaktnih mera prevencije, edukacija i dekolonizacije. Nadzor je važna komponenta kontrole MDRO koja omogućava otkrivanje novonastalih MDRO, praćenje epidemioloških trendova i merenje efikasnosti primenjenih mera.

Mere prevencije i kontrole širenja MDRO u zdravstvenim ustanovama moraju biti deo svakodnevne rutinske prakse.

Ključne reči: MDRO, prevencija, epidemiologija

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BRZA IDENTIFIKACIJA UZROČNIKA INFEKCIJA I ANTIBIOTSKE REZISTENCIJE U JEDINICI INTENZIVNOG LEČENJA

Snežana Jovanović

Antimikrobna rezistencija (AMR) je jedna od najozbiljnijih pretnji javnom zdravlju, čemu je doprinela, pored ostalog, prekomerna upotreba i/ili zloupotreba antimikrobnih lekova (AML) kod ljudi i životinja, neprimenjivanje mera prevencije infekcije. AMR ima mnoge štetne posledice uključujući: teži tok bolesti, otežano lečenje, dužu hospitalizaciju, povećane troškove zdravstvene zaštite, preopterećen sistem javnog zdravlja.

U rutinskom radu mikrobiološke laboratorije primenjuju za ispitivanje osetljivosti na AML testove kao što su bujon mikrodilucija, disk difuzija i gradijent test. Nekoliko komercijalnih sistema je pojednostavilo i automatizovalo identifikaciju mikroorganizama i ispitivanje AMR. Mnoge metode u roku od nekoliko sati mogu omogućiti identifikaciju patogena i prisustvo gena ili proteina rezistencije (amplifikacija nukleinske kiseline, sekvenciranje genoma, hibridizacija, imunodijagnostičke metode, masena spektrometrija). Prednost molekularne dijagnostike u odnosu na klasične metode je da se za kraći vremenski period mogu identifikovati mikroorganizmi i geni rezistencije, sa kulture i iz kliničkih uzoraka.

Prema dostupnim podacima, otprilike 50% terapije u bolničkim uslovima se započinje pogrešnim antibioticima i bez prethodne detekcije patogena. Uprkos značajnom napretku u dijagnostičkim tehnologijama, mnogi pacijenti sa infektivnim bolestima se i dalje leče empirijski. Zbog toga je, naročito u JIL, neophodno brzo identifikovati etiološki agens, utvrditi da li se radi o virusnoj, gljivičnoj ili bakterijskoj infekciji, omogućiti brzo i pristupačno ispitivanje osetljivosti na AML. Ogromna raznolikost antimikrobnih lekova i mehanizama rezistencije komplikuje ispitivanje osetljivosti. Ali, ove metode nam daju odgovore na praktična pitanja: koji antibiotik je efikasan i u kojoj dozi bi ga trebalo primeniti u terapiji. To omogućava kliničkom mikrobiologu brzo davanje smernica kliničkom lekaru za pravovremenu dijagnozu bolesti, a time i ordiniranje adekvatne antimikrobne terapije. Time bi se nepotrebna upotreba antibiotika mogla svesti na minimum, bolje bi se kontrolisalo širenje rezistencije na antibiotike, a lečenje i oporavak pacijenata u JIL, bilo bi uspešnije.

Ključne reči: antimikrobna rezistencija, antimikrobna terapija, JIL, infekcija

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PROCESI PURIFIKACIJE KRVI KOD BOLESNIKA U SEPSI

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Sepsa je životno ugrožavajuća organska disfunkcija koja nastaje usled neadekvatnog imunskog odgovora domaćina na infekciju. Vodeći je uzrok smrti u jedinici intenzivnog lečenja. Godinama unazad razne strategije kontroli-

sanja imunskog odgovora u sepsi su primenjivane, ali ni jedna nije krucijalno promenila tok lečenja (kortikosteroidi, imunoglobulini).

Purifikacija krvi je ekstrakorporalna terapija koja menja imunski odgovor (smanjuje hiperinflamatorni odgovor) na infekciju neselektivnim uklanjanjem inflamatornih medijatora, endotoksina ili oba. Suštinski, purifikacija krvi ima zadatak da "resetuje" imunski odgovor. Ekstrakorporalna purifikacija krvi se postiže različitim procesima: difuzijom (hemodijaliza), konvekcija (hemofiltracija), kombinacijom difuzije i konvekcije (hemodijafiltracija) i apsorpcijom. Prva tri procesa se odvijaju separacijom preko membrane, dok se apsorpcija rastvora zasniva na uklanjanju čestica iz plazme pomoću čvrstog sredstva (sorbenta). Sorbenti "zarobljavaju" čestice i onemegućavaju njihovo vraćanje u ekstrakorporalni krug, a potom u cirkulaciju. Trenutno, postoji više vrsta sorbenta i glavna karakteristika je da su neselektivni (uklanjanju i proinflamatorne i antinflamatorne citokine). Do sada je više studija pokazalo benefit primene ekstrakorporalne hemoperfuzije (značajan pad proinflamatornih citokina, brže postizanje hemodinasmke stabilnosti), ali za sada bez značajnog smanjenja mortaliteta. Problem hemoperfuzije je što delom aporbuju i antiinflamatorne citokine i antibiotike. Sepsa je predominanto profinflamatorno stanje, te se očekuje da aposorpcija proinflamatorinih citokina bude viša u odnosu na antiiflamatorne. Kada započeti hemoperfuziju? Danas predominira stav da purifikaciju krvi bi trebalo započeti rano, pre nastanka organske disfunkcije (uključujući i razvoj akutne bubrežne insuficijencije). Trajanje terapije je sledeće pitanje, kao i kada prestati terapiju i kada je ponovo započeti. Čini se da njeno trajanje zavisi od kliničkog stanja bolesnika, kao i njen prekid. Hemoperfuzija je suportivna terapija i paralelno sa njenom primenom uvek lečiti uzrok sepse.

Purifikacija krvi u sepsi pokazuje obećavajuće rezultate, ali su neophodna dalja istraživanja kako bi se njen značaj potvrdio i svrstala u vodič za lečenje sepse.

Ključne reči: sepsa, purifikacija krvi, sorbetni, hemoperfuzija

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PROCENA BOLA U JEDINICI INTENZIVNOG LEČENJA I UTICAJ BOLA NA POSTOPERATIVNI DELIRIJUM

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Uzroci bola u JIL mogu biti mnogobrojni a najčešće su posledica same operacije, prisustva raznih medicinskih sredstava (CVK, tubus, dren...), posledica nege ili proceduralnih dijagnostičkih ili terapijskih postupaka (bronhoskopija, drenaža, pinkcija...). Bol je jako teško proceniti kod pacijenata u JIL a posebno onih koji su na mehanikoj ventilaciji pa se zato koriste skale za procenu bola od kojih je jedna od najboljih Critical Care Pain Observation Tool. Kod sediranih pacijenata gde je smanjena vrednost BIS-a odnosno QCON-a može se primeniti i procena jačine bola pomoću QNOX parametra, što predstavlja novinu u praćenju intenziteta bola i pokušaj njegove objektivizacije. Bol je jedan od predisponirajućih faktora za nastanak postoperativnog delirijuma (POD) a tu su jos i hipoksija, infekcija, nepokretnost, kognitivni poremećaji, poremećaj sna, slaba ishrana, oslabljena čula kao i primena velikog broja lekova. Postojanje depresije, postojanje preoperativnog bola kao i postoperativno pojačanje bola su pozitivni prediktori za nastanak POD. Ono sto nam otežava tumačenje uticaja bola na delirijum jeste povezanost dejstva poremećaja odnosno preklapanje uticaja kognicije, poremećaja sna i intenziteta bola. Obzirom na patofiziološko preklapanje inflamacije i bola, analgetici sa antiinflamatornim dejstvom dobijaju na značaju u prevenciji delirijuma kod pacijenata u akutnom postoperativnom periodu. Treba istaći i da neki analgetici mogu dovesti do delirijuma ali pokazalo se da je nedovoljna terapija bola značajniji faktor rizika za postoperativni delirijum nego lečenje lekovima koji potencijalno izazivaju delirijum. Veoma je značajno i praćenje moždane aktivnosti pacijenta jer preterana supresija aktivnosti moždane kore dovodi do pada indeksa Burst Supresion Ratio (BSR) a pokazalo se da BSR ima dobru korelaciju sa pojavom delirijuma. Naime, što je veća i u trajanju duža supresija moždane kore to je veća šansa za pojavu POD.

Ključne reči: delirijum, analgezija, postoperativni bol

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NOVINE U DIJAGNOSTICI I TRETMANU AKUTNOG PANKREATITISA

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Akutni pankreatitis i dalje predstavlja veliki izazov u savremenoj medicini sa raznolikom etiologijom, uključujući kalkulozu žučne kesice, alkoholizam i metaboličke poremećaje. Sve se više kao etiološki faktor ističe i endoskopska retrogradna holangiopankreatografija.

Najteži oblik bolesti, akutni nekrotizirajući pankreatitis, karakteriše sterilna nekroza koja se može komplikovati razvojem sindroma sistemskog inflamatornog odgovora, infekcije, kao i multiorganskim popuštanjem, sa visokom stopom smrtnosti.

Klinička slika je slična blažim oblicima bolesti – javljaju se bol u abdomenu, distenzija trbuha, mučnina i povraćanje, povišena temperatura, kardiorespiratorno pogoršanje, promene mentalnog statusa.

Dijagnoza se postavlja na osnovu kliničke slike, laboratorijskih parametara (povišena lipaza i amilaza u serumu), kao i radioloških nalaza (kompjuterizovana tomografija abdomena sa kontrastom). Za dijagnozu se koriste i inter-

ventne hirurške procedure**:** abdominalna punkcija, laparotomija ili dijagnostička laparoskopija.

Najpoznatiji skoring sistem za određivanje težine pankreatitisa predstavljaju RANSON kriterijumi, dok je najviše u upotrebi BISAP skor, koji uzima u obzir 5 parametara merenih u prvih 24 sata od nastanka simptoma (urea >25 mg/dl, poremećen mentalni status, postojanje sindroma sistemskog inflamatornog odgovora, starost preko 60 godina i prisustvo pleuralnog izliva) i sa velikom tačnošću predviđa smrtnost.

Specifična terapija odnosi se na uvođenje inhibitora pankreasne sekrecije i proteolitičkih enzima, ali nije dokazan njihov pozitivni uticaj na mortalitet. Nespecifična terapija obuhvata primenu nesteroidnih antiinflamatornih lekova uz eventualnu epiduralnu anesteziju za kupiranje bola. Akcenat je na adekvatnoj nadoknadi tečnosti i potpori vitalnih organa (mehanička ventilacija pluća, hemodinamska potpora, potpora bubrežne funkcije). Antibiotska terapija je rezervisana za slučajeve koji se komplikuju infekcijom, a antibiotici izbora su iz grupe karbapenema. Raniji stavovi o prekidu peroralnog unosa danas su napušteni, a enteralnu ishranu je potrebno započeti odmah po stabilizaciji pacijentovog stanja putem nazojejunalne sonde. Što se tiče hirurškog lečenja, zlatni standard predstavlja debridman retroperitonealnog nekrotičnog pankreasnog tkiva.

Akutni pankreatitis je oboljenje koje zahteva multidisciplinarni pristup dijagnostici i lečenju, kako bi se umanjio moebiditet i mortalitet.

Ključne reči: intenzivna terapija, hirurgija, multiorgansko popuštanje

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INFEKCIJE U PEDIJATRIJSKOJ JEDINICI INTENZIVNOG LEČENJA

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Infekcije povezane sa zdravstvenom zaštitom (HAI) i dalje su važan izvor morbiditeta i mortaliteta širom sveta. HAI je definisana kao infekcija koja se javlja 48 sati nakon hospitalizacije ili 10 dana nakon otpuštanja. Pacijenti hospitalizovani u jedinicama intenzivne nege su sklonije HAI zbog teške kliničke slike, stanja i posledične invazivne procedure. Definitivna dijagnoza infekcije je često otežana te je lečenje odloženo i često na početku empirijsko.

Razumno i optimalno lečenje antibiotikom kod pacijenata u pedijatrijskim jedinicama intenzivne nege (PICU) je od suštinskog značaja u svetlu približavanja „postantibiotske ere“ sa još manje opcija antibiotika za decu u poređenju sa odraslima.

Najčešće izolovani mikroorganizmi su *Staphylococcus aureus*, koagulaza negativni *staphylococci*, *enterococci* i *Candida spp.* Od gram negativnih sojeva to je *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter* a od virusa *Rotavirus* i *respiratory syncytial virus.*

Takođe se često javljaju rezistentni mikroorganizmi *methicillin resistant Staphylococcus aureus (MRSA), methicillinresistant coagulase-negative staphylococci (MRCNS), vancomycin-resistant enterococci (VRE), Extended spectrum beta-lactamases (ESBL)-positive Klebsiella and E. coli,* i *carbapenem-resistant Pseudomonas and Acinetobacter.*

Faktori rizika su prematuritet, mala porođajna težina, urođene anomalije i imunosupresija,hronične bolesti,upotreba sedativnih lekova,operacija,invazivne intervencije i procedure kao npr infuzije, transfuzije krvi, parenteralna ishrana, prisustvo nazogastričnog, centralnog venskog, urinarnog katetera i mehanička ventilacija.

HAI je jedan od najčešćih uzroka povećanja mortaliteta i morbiditeta u PICU. Treba poštovati stroga pravila asepse tokom lečelja i nege.

Poštovanje protokola o bezbednosti pacijenata, zajedno sa redovnim mikrobiološkim kontrolama i upravljanjem antibioticima je neophodno.

Oko 50% HAI se može sprečiti ako se prati asepsa i razumna upotreba uređaja i opreme.

Ključne reči: infekcija, pedijatrija, intenzivna, rezistencija

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“MECHANICAL POWER” (MP) U MEHANIČKOJ VENTILACIJI

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Mehanička ventilacija je procedura podrške respiratornom sistemu u toku opšte anestezije u operacionoj sali ili je deo terapijske strategije u jedinici intenzivnog lečenja kod bolesnika sa akutnim ili hroničnim plućnim oboljenjem kada spontanim disanjem nije moguće održati adekvatnu razmenu gasova. Mehanička ventilacija može da bude invazivna (preko endotrahealnog tubusa) ili neinvazivna preko maski (“full face”, “total face”) ili preko posebnih kaciga za neinvazivnu ventilaciju. Bilo da se govori o neinvazivnoj ili invazivnoj mehaničkoj ventilaciji ona se sprovodi uz pomoć mehaničkog ventilatora koji generiše pozitivan pritisak. Međutim, sama mehanička ventilacija pozitivnim pritiskom može da ošteti pluća što se naziva ventilacijom indukovano oštećenje pluća (VILI – ventilation induced lung injury).

Kako bi se izbegao nastanak oštećenja pluća mehaničkom ventilacijom 2016. godine je osmišljen koncept nazvan “Mechanical power”. Ovaj koncept se zasniva na principima prvog zakona termodinamike, polazi od klasične jednačine kretanja koja se primenjuje u mehaničkoj ventilaciji sa ciljem da kvantifikuje doprinos respiratorne frekvence, “driving” pritiska, protoka, disajnog volumena i pozitivnog end-ekspiratornog pritiska (PEEP) u generisanju energije u jedinici vremena koja se za vreme mehaničke ventilacije isporučuje plućnom parenhimu.

Kako bi se složena matematička formula prevela u jednostavnu formulu za svakodnevni klinički rad, predloženo je i validirano više jednostavnijih formula. Formule se razlikuju za volumenom i pritiskom kontrolisanu ventilaciju. Jedna od formula za volumenom kontrolisanu ventilaciju je formula predložena od L. Giosa i saradnika:

$$\text{Mechanical Power} = [\text{VE} \times (\text{Peak Pressure} + \text{PEEP} + \text{F}/6)]/20$$

Mechanical power (MP) se izražava u J/min, VE je minutni volumen (L/min), Peak pritisak je vršni pritisak u disajnom sistemu (cm H2O), PEEP je pozitivni endeksipratorni pritisak (cm H2O) i F je protok u L/min. Svi parametri iz jednačine su dostupni na ekranu mehaničkog ventilatora, pa izračunavanje ove formule ne predstavlja poseban problem.

Ukoliko se sprovodi mehanička ventilacija nekim od pritiskom kontrolisanih modova ventilacije, jednačina za izračunavanje MP:

$$\text{Mechanical Power} = 0{,}098 \times \text{RR} \times \text{Vt} \times (\text{PEEP} + \Delta\text{Pinsp})$$

U ovoj formuli RR je broj respiracija u minutu, Vt disajni volumen izražen u litrima, PEEP –pozitivni end-ekspiratorni pritisak i ΔP je "driving" pritisak.

Postavlja se pitanje kakva je praktična primena saznanja o MP i koje su to vrednosti do kojih je mehanička ventilacija bezbedna po bolesnika? Više animalnih i humanih studija su pokazale da su vrednosti MP za vreme mehaničke ventilacije veće od 17 J/min udružene sa daljim oštećenjem pluća i povećanim mortalitetom.

Ključne reči: mehanička ventilacija, oštećenje pluća, mechanical power

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NOVINE U ANTIBIOTSKOJ TERAPIJI

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Razvoj savremenog pristupa u dijagnostici i tretmanu kritično obolelih pacijenata omogućio je kraći boravak u bolnici kao i smanjen mortalitet od teških formi kritičnih bolesti. Međutim, problem rezistencije bakterija i drugih agenasa nije se do sada uspešno rešio. Bakterije stalno menjaju način na koji uspevaju da opstanu u organizmu, adaptirajući se prvenstveno rezistencijom i na najnovije antibiotike. Drugi načini adaptacije i time povećane rezistencije su i sinergizam kao i interakcija sa drugim bakterijama čime se, kao u biofilmu, sprečava prodor antibiotika do samih uzročnika infekcije. Radi lakšeg prepoznavanja epidemiološke situacije i praćenja razvoja rezistencije, bakterije se dele na rezistentne, multirezistentne i na panrezistentne kod kojih se in vitro primećuje rezistencija na sve antibiotike. U modernom pristupu termana kritično obolelih pacijenata sa sepsom, na primer, razvojem i usavršavanjem dijagostičkih intervencija, lakše se otkriva fokus infekcije ali je terapija i dalje neadekvatna. Još odavno se zna da se više od 40% antibiotika empirijski daje pogrešno, što zbog neadekvatnie implementacije protokola, što zbog neadekvatnog doziranja leka čime se dodatno povećava rizik od povećanja rezistencije. Suvremeni protokoli naglašavaju zato iskustvo i znanje, ali i odgovornost, kliničara kao i važnost poznavanja farmakokinetike i farmakodinamike antibiotika i primene tog znanja kog kritično obolelih pacijenata kod kojih su ti parametri značajno promenjeni. Uvećan "treći prostor", često kod sepse, značajno smanjuje koncentraciju antibiotika u krvi. Na koncentarciju leka utiče i multiorganska disfunkcija, naročito akutna bubrežna insuficijencija.

Noviji antibiotici značajno povećavaju osetljivost bakterija, na primer, smanjujući minimalnu inhibitornu koncentraciju leka. Takođe, znanje o sinergističkom delovanju više antibiotika omogućava da se kombinacijom novijih i starijih antibiotika postigne zadovoljavajući efekat. Uprkos mnogim novinama i dalje je rezistencija veliki problem, zato je timski pristup lečenja kritično obolelog pacijenta još uvek najznačajniji kada je u pitanju efikasnost tretmana.

Ključne reči: sepsa, rezistencija, antibiotici

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INFEKCIJE KOD POLITRAUMATIZOVANIH BOLESNIKA

Adi Hadžibegović, Jovana Stanisavljević, Bojan Jovanović

Godišnje 312 miliona ljudi doživi traumu, od čega se više od 90% povreda dogodi u srednje razvijenim i nerazvijenim zemljama. Trauma predstavlja vodeći uzrok smrti u starosnoj grupi ljudi mlađih od 40 godina. Pored povreda koje rezultiraju smrtnim ishodom, registruje se i veliki broj nefatalnih povreda koje nose značajne posledice kao što je smanjenje kvaliteta života.

Unapređenje dijagnostičkih procedura, kao i intenzivnog lečenja bolesnika koji su preživeli tešku trauma doveli su do smanjenja mortaliteta usled teških povreda ali i do većeg procenta komplikacija njihovog lečenja. Smrtni ishod kod traumatizovanih bolesnika najčešće je posledica povrede glave, krvarenja, sepse i multiorganske disfunkcije. Vreme nastanka smrti kod bolesnika koji su doživeli tešku trauma ima trimodalnu distribuciju: neposredni, rani i kasni. Među najčešćim "kasnim" uzrocima smrti navode se infekcija i sepsa. Bolesnici koje prežive inicijalnu traumu u velikom su riziku za nastanak bolničke infekcije, sepsa i septičnog šok. Smatra se da disbalans između proinflamatornih i antinflamatornih procesa aktiviranih traumom na račun anttinflamatornih procesa dovodi do

kompenzatorne imunosupresije i povećane osetljivosti povređenih bolesnika na infekciju.

Incidencija bolničkih infekcija u grupi traumatizovanih bolesnika kreće se od 9-50% u zavisnosti od težine povreda bolesnika uključenih u studiju i nadzora nad infekcijama. Incidencija je značajno viša nego incidencija bolničkih infekcija u opštoj populaciji

hospitalizovanih bolesnika gde iznosi 4,9%. Faktori rizika koji doprinose nastanku bolničkih infekcija kod bolesnika sa traumom su oni koji su vezani za bolesnika (pol, starost i komorbiditeti) i oni vezani za trauma (težina povrede, broj povreda, hitna operacija, primena masivne transfuzije krvi itd.). U studijama se povrede kičme ili ekstremiteta predstavljaju kao značajan faktor rizika, kao i hipotenziju na prijemu, tupe povrede, Glazgov koma skor, splenektomiju, gojaznost.

Ključne reči: infekcija, politraumatizovani bolesnici, teška trauma, sepsa

Medicinski fakultet, Univerzitet u Beogradu

Urgentni centar, Univerzitetski klinički centar Srbije, Beograd

NOVINE U DIJAGNOSTIFIKOVANJU I TRETMANU GLJIVIČNIH INFEKCIJA

Jovana Stanisavljević

U jedinici intenzivnog lečenja prevalencija gljivične infekcije iznosi oko 16% i udružena je sa visokm morbiditetom i mortalitetom (>40%). *Candida albicans* je i dalje najčešći uzročnik gljivične infekcije u JIL. Zabrinjavajuće je da se poslednjih decenija na globalnom nivou menja epidemiološka slika u korist non-albucans vrsta rezistentnih na azole (*Candida glabrata, Candida tropicalis, Candida parapsilosis,* i *Candida krusei*). To je dovelo do razvoja bržih dijagnostičkih metoda (detekcija gljivičnih biomarkera: (1,3)-β-D-glukan, mannan antigen i anti-mannan antitela, galaktomanan, detekcija DNK patogena pomoću tehnologije T2 magnetne rezonance i nanotehnologije (T2MR) preko kandida T2 panela) i novih bezbednijih antimikotika- ehinokandina koji ne ispoljavaju neželjenje efekte azola (hepatotoksičnost, inhibicija citohroma P450, produžetak QT intervala) ili nefrotoksičnost Amfotericina B.

Jedan od najvećih problema vezanih za invazivnu gljivičnu infekciju (IGI) je rano prepoznavanje i postavljanje dijagnoze, pa je u fokusu kliničkih istraživanja je formiranje prediktivnih modela rizika za razvoj glivične infekcije. Nezavisni faktori rizika za razvoj kandidemije u JIL su totalna parenteralna ishrana, akutna bubrežna insuficijencija, srčana oboljenja, septični šok, broj primenjenih antibiotika pre razvoja kandidemije (naročito aminoglikozida). Veći APACHE II skor, abdominalna hirurgija, dijabetes, opekotine, nekrotični pankreatitis takođe su povezani sa učestalijom IGI.

Primena profilaktičke i preemptivne antimikotične terapije se ne preporučuje rutinski kod kritično oblelih. Prvi izbor empirijske i ciljane terapije u septičnom šoku su ehinokandini (Kaspofungin, Mikafungin, Anidulafungin), drugi izbor su Flukonazol i lipidna formulacija Amfotericina B. Obavezna je kontrola izvora infekcije (hirurška, drenaža, uklanjanje katetera). Dužina terapije i step down terapija (prelazak sa ehinokandina na azole) predmet je novih kliničkih istraživanja, a prema zvaničnim preporukama terapija za kandidemiju iznosi dve nedelje nakon prvog negativnog rezultata hemokulture.

Sve veća upotreba antibiotika širokog spektra i COVID-19 infekcija povezuju se sa pojavom novog, multirezistentnog soja *Candida auris*. Ohrabrujući rezultati očekuju se od novih antimikotika u drugoj (Ibrexafungerp) i trećoj fazi kliničkih ispitivanja (Rezafungin, Fosfanogepix), uz strožiju politiku upravljanja antimikorbnim lekovima.

Ključne reči: invazivna kandidijaza, gljivični biomarkeri, faktori rizika, empirijska i ciljana terapija

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Centar za anesteziologiju sa reanimatologijom, Univerzitetski klinički centar Srbije, Beograd

NUTRITIVNA TERAPIJA KOD KRITIČNO OBOLELIH BOLESNIKA

Ivan Palibrk, Marija Đukanović, Jovan Perić, Dona Stefanović, Maja Maksimović

Ko je kritično oboleli bolesnik? Kako ga definisati? Na ova pitanja je teško dati odgovor. Kritično obolelog možemo definisati kao bolesnika sa prisutnom akutnom organskom disfunkcijom i potrbom za stalnim monitoringom vitalnih parametara i stalnom suportivnom terapijom.

Kada započeti ishranu kritično obolelog? Ishrana je neophodan deo terapije kritično obolelog bolesnika. Današnja medicinska literatura obiluje tekstovima o značaju ishrane u lečenju kritično obolelog ali i tekstovima kada uključiti ishranu, koji način ishrane koristiti i koliki energetski tj. gradivni unos treba da bude u pojedinim danima, tj. fazama bolesti. Opšte je prihvaćeno da glavni put ishrane bude enteralni unos hrane, tj. peroralni unos ako je moguće. Preporuka je da enteralni put ishrane treba da se započne unutar 72 sata od početka kritične bolesti (rani unos). Možemo reći da je to akutna faza bolesti, faza sa veoma veoma izraženim katabolizmom. Rano započinjanje enteralnog, tj. oralnog unosa neki put nije moguće. Kod bolesnika koji su u izraženoj malnutriciji se tada započinje parenteralna ishrana. Počinjemo ranu i „progresivnu„ parenteralnu ishranu. Bolesnik ne sme biti bez ishrane. Proteini i energija se postepeno uvode i povećavaju u ishrani kritično obolelelih bolesnika. Unos ne sme biti nagao i količinski veliki. U slučaju da se to pravilo nepoštuje postoji opasnost od elektrolitnih i metaboličkih poremećaja koji mogu da ugroze život bolesnika. Pored toga izvestan stepen „gladovanja“ , stimuliše imunološku odbranu bolesnika.

Nutritivna terapije je neophodna u svim fazama kritične bolesti. Potreban je individualan pristup svakom pacijentu uz praćenje datih smernica.

Ključne reči: kritično oboleli, nutritivna terapija, imuni odgovor

MINI SIMPOZIJUM

100 GODINA KATEDRE ZA HIGIJENU NA MEDICINSKOM FAKULTETU - OD NARODNOG ZDRAVLJA DO MEDICINSKE EKOLOGIJE

Institut za higijenu i medicinsku ekologiju, Medicinski fakultet, Univerzitet u Beogradu

MILAN JOVANOVIĆ BATUT - ISTORIJSKI ASPEKT NARODNOG ZDRAVLJA I PREVENTIVNA MEDICINA

Sanja Milenković

Milan Jovanović Batut (1847-1940) bio je veliki lekar, naučnik, pisac, predsednik Srpskog i Jugoslovenskog lekarskog društva, Društva za čuvanje narodnog zdravlja, predsednik Glavnog sanitetskog saveta i jedan od osnivača Medicinskog fakulteta u Beogradu.U našem narodu je upamćen kao najveći zdravstveni prosvetitelj. Batut je bio izdavač lista „Zdravlje“ - „List za lekarsku pouku narodu“. Batut je znao da kaže: „Znanje treba da prodre u narod. Kako? Knjiga se mora narodu dodvoriti, a koja to ne ume, ili neće, slabo će se primiti, ma bila najčistija i najjasnija istina.“ Napisao je 57 knjiga i brošura i na stotine članaka. Proučavao je i podučavao srpski narod, pronikavši duboko u njegovu dušu da bi govorom i pisanom reči, jednostavnim i razumljivim jezikom mogao objasniti svako medicinsko pitanje.Po sopstvenom priznanju,svoj život je posvetio narodu. Njegovo životno delo je i „Građa za medicinsku terminologiju“. Prepoznao je značaj higijene ishrane, propagirao značaj fizičke aktivnosti, ukazivao na higijenu vode, zemljišta, odevanja, stanovanja, ukazivao na štetnost duvana i alkohola, značaj vakcinacije dece odnosno tada „kalemljenja“. Pisao je da se rak primećen na vreme može operisati i da ne mora uvek biti smrtonosan, uz opise kako ga običan narod može prepoznati na vreme i apel da se ljudi javljaju lekaru i redovno kontrolišu. Batutova uloga u razvijanju svesti ljudi o preventivnoj medicini u Srbiji je ogromna. Preventivna medicinaimala je svoje začetke u praistorijsko vreme, ali u današnjem smislu reči upravo se počela razvijati u XIX veku kao naučna disciplina. Da bi se taj razvoj kontinuirano odvijao potrebno je bilo oduvek kao i danas poboljšanje ekonomskih uslova , obrazovanja i kulture. Batut, kao zdravstveni prosvetitelj našeg naroda upravo je živeo u vreme Petenkofera i drugih velikana kao što su Paster, Koh, Galton, Mečnikov i dr. Oni su mu bili uzori, učitelji i sa njima je održavao prepiske.

Ključne reči: Batut, lekar, prosvetitelj

Institut za higijenu i medicinsku ekologiju, Medicinski fakultet, Univerzitet u Beogradu

PERSPEKTIVA MEDICINSKE EKOLOGIJE I NASTAVNE AKTIVNOSTI KATEDRE ZA HIGIJENU

Dušan Backović

Medicinska ekologija kao segment higijene kao šire preventivne medicinske discipline, predsavlja nauku o odnosima čoveka i sredine koja ga okružuje i ima cilj da svede na najmanju meru dejstvo štetnih faktora i spreči nastanak bolesti. Procenjuje se da činioci životne sredine danas doprinose oko 25% ukupnom opterećenju bolestiu odraslih i oko trećine bolesti kod dece.

Predmet Higijena sa medicinskom ekologijom ima cilj da osposobi buduće lekare na kraju studija osnovnim veštinama preventivne medicine: kako da prepoznaju i procene osnovne faktore rizika iz životnog okruženja (zagađenje vazduha, zemljišta, vode, hrane, štetne faktore socijalnog okruženja) koji mogu dovesti do oštećenja fizičkog i mentalnog zdravlja. Pored toga, studentu se daju osnovna znanja i veštine da proceni prisustvo i uticaj negativnih ponašanja koja su u osnovi najčešćih masovnih hroničnih nezaraznih bolesti, i da preventivnim i terapijskim nefarmakološkim merama deluje pravovremeno na njihovo sprečavanje.

Katedra higijene je na Medicinskom fakultetu Univerziteta u Beogradu osnovana 1923. godine, a nastava sa 88 studenata prve generacije na predmetu Higijena održavana je prvi put na petoj godini studija kao dvosemestralni predmet sa fondomod 120 časova, kojima su bile pokrivene oblasti: opšta i vojna higijena, industrijska, školska higijena i higijena ishrane. Osnivač našeg Fakulteta i naše Katedre Prof. Dr Milan Jovanović Batut je predhodno na studijskom boravku po univerzitetskim centrima Evrope, posetio velikane medicine profesora Pastera i Koha i postao i saradnik Jozefa Petenkofera, rodonačelnika Higijene kao medicinske naučne discipline.

Po novom kurikulumu Higijena sa medicinskom ekologijom u okviru Integrisanih studija Medicine je jednosemestralni predmet sa 75 časova na šestoj godini . Osim toga naša katedra organizuje budućim lekarima i četiri izborna predmeta sa po 30 časova: Životna sredina i zdravlje, Komunalne otpadne materije i zdravstveni problemi, Ishrana u prevenciji i terapiji hroničnih nezaraznih bolesti i Zaštita i unapređenje zdravlja dece i adolescenata, koji opredeljenim studentima nude šira znanja i veštine iz navedenih oblasti.

Ključne reči: medicnska ekologija, higijena, preventivna medicina

Institut za higijenu i medicinsku ekologiju, Medicinski fakultet, Univerzitet u Beogradu

POSLEDIPLOMSKA EDUKACIJA NA KATEDRI HIGIJENE

Branko Jakovljević

Poslediplomska edukacija na Katedri higijene ima dugu tradicije. Nastava je započela pre više od 50 godina organizovanjem magistarskih studija pod nazivom „Higijena". Školske 1971/72. godine upisana su dva magistranta. Školske 1975/76, 77/78. i 78/79. organizuje se preventivna medicina kao Osnovna katedra za poslediplomsku nastavu iz Zaštite čovekove životne i radne sredine, radioaktivne zaštite i komunalne higijene. Školske 1979/80. godine, kada su Medicinskom fakultetu dodeljeni i administrativni poslovi vezani za specijalizaciju i polaganje specijalističkog ispita, formirane su tri interdisciplinarne katedre:

Katedra za higijenu i medicinsku ekologiju sa specijalizacijom iz Higijene i magisterijumom iz Higijene i medicinske ekologije.

Katedra za ishranu zdravih i bolesnih ljudi sa užom specijalizacijom iz Higijene ishrane sa bolesničkom ishranom i magisterijumom iz Ishrane.

Katedra za školsku higijenu, fiziologiju rasta i razvoja sa magisterijumom iz Školske higijene i fiziologije rasta i razvoja.

Godine 1988. formirana je Katedra za specijalističku nastavu iz Higijene koja je nastala grupisanjem tri interdisciplinarne katedre. U okviru nje organizovana je nastava koju su činile:

- Osnovna zdravstvena specijalizacija iz Higijene i
- Uža specijalizacija iz ishrane zdravih i bolesnih ljudi.

Katedra je organizovala i magistarske studije iz Ishrane, Higijene sa medicinskom ekologijom i Školske higijene i fiziologije rasta i razvoja.

Decembra 1993. godine Ministarstvo zdravlja odobrava još dve uže specijalizacije iz Komunalne higijene sa patologijom naselja i Ekotoksikologije. Umesto uže specijalizacije iz Komunalne higijene sa patologijom naselja 2009. godine ustanovljena je uža specijalizacija iz Medicinske ekologije, a uža specijalizacija Ishrana zdravih i bolesnih ljudi promenila je naziv u Dijetoterapija. Godine 2015. akreditovan je modul doktorskih akademskih studija Ekološki i nutritivni faktori i zdravlje.

Danas, Katedra za poslediplomsku nastavu iz higijene sa medicinskom ekologijom organizuje sledeće oblike nastave: osnovnu zdravstvenu specijalizaciju iz Higijene, uže specijalizacije Dijetoterapije i Medicinske ekologije i modul doktorskih akademskih studija Ekološki i nutritivni faktori i zdravlje.

Ključne reči: higijena, medicinska ekologija, poslediplomska edukacija, specijalizacija

Institut za higijenu i medicinsku ekologiju, Medicinski fakultet, Univerzitet u Beogradu

MEDICINSKA DIJETETIKA I ZDRAVSTVENE AKTIVNOSTI INSTITUTA ZA HIGIJENU

Nađa Vasiljević

Institut za higijenu se još od osnivanja Medicinskog fakulteta bavi proučavanjem faktora koji unapređuju ljudsko zdravlje i utvrđivanjem različitih činilaca koji imaju morbogeni učinak. Na povezanost njihovog delovanja i rizičnih efekata na zdravlje ljudi ukazao je još prof. dr Milan Jovanović Batut, prvi profesor Higijene, čime je utemeljio zdravstveno-preventivnu strategiju našeg predmeta. Razvojem Instituta, konstantno se odvijala preventivna aktivnost usmerena kako na edukaciju studenata tako i na istraživanja u oblasti medicinske ekologije. Kompleksnost interakcije činilaca iz životne sredine uticala je na porast prevalencije pre svega hroničnih nezaraznih bolesti, koje opterećuju društvo i zdravstveni sistem. To je prepoznao i naš vrsni profesor dr Božidar Simić, ekspert Svetske zdravstvene organizacije. Zaslužan je za osnivanje Savetovališta za dijetetiku, koje postoji i radi već pune 52. godine. Ova jedinstvena institucija u našoj zemlji je očuvala ideju i viziju prof. Simića neumornim, daljim angažovanjem lekara sa našeg Instituta, specijalista Higijene i subspecijalista Dijetoterapije. Prof. dr Miodrag Milošević, veliki stručnjak iz oblasti Higijene rada, postepeno započinje procenu hazardnih činilaca na zdravlje radno aktivnog stanovništva od 1985 -1995. godine. Sa svojim timom, obilazio je postrojenja u Osijeku, Apatinu, Lazarevcu, Kragujevcu, Rakovici i dr., gde je ispitivao hemijske agense, uslove rada zaposlenih, osvetljenost i mikroklimu. Sem toga, posebno je ispitivao činioce rizika za oboljevanje u ruralnoj sredini. Izloženost prašini hroma i magnezita u okolini fabrike Magnohrom u Kraljevu i uticaj na poljoprivredne kulture, takođe je bio deo radnog opusa tima prof. Miloševića. Prof. dr Mihailo Nikolić je poseban interes pokazao za merenje buke u komunalnoj sredini i uticaj na zdravlje izloženog stanovništva. Početkom 90-ih godina prošlog veka, prof. Nikolić, sa timom specijalista higijene i kolega sa mikrobiologije, započinje zdravstvenu delatnost u okviru Sanitarne mikrobiologije. Ispitivanja su bila orijentisana na procenu bioloških štetnosti u bolničkoj sredini i kontrolu sanitarno-higijenskih karakteristika u zdravstvenim ustanovama.

Ključne reči: dijetetika, zdravstvena delatnost, higijena, medicinska ekologija

Institut za higijenu i medicinsku ekologiju, Medicinski fakultet, Univerzitet u Beogradu

EKOLOŠKI I HUMANI BIOMONITORING

Katarina Paunović

Proučavanje izloženosti ljudi štetnim materijama iz životne sredine i procena njihovog uticaja na zdravlje, odnosno humani biomonitoring, jedan je od najvećih izazova u sa-

vremenoj medicinskoj ekologiji. Da bi biomonitoring bio efikasan u proceni izloženosti ljudi hemijskim supstancama neophodno je da se bazira na pouzdanim i validnim metodama merenja nivoa materije od interesa u životnoj sredini i metodama procene rizika po zdravlje izloženih osoba, kao i da uzima u obzir sve izvore zagađujućih materija, vremenske i prostorne promene u ekspoziciji i distribuciji materija, te specifičnost izložene populacije, a naročito vunerabilnih grupa stanovništva. Istovremeno je poželjno da biomonitoring bude dostupan uz minimalno korišćenje ljudskih i finansijskih resursa.

Humani biomonitoring se bazira na određivanju tri vrste bioloških markera, i to markera izloženosti, uticaja i osetljivosti. Biomarkeri ekspozicije služe za procenu izloženosti populacije materiji od interesa i najčešće uključuju merenje koncentracije hemijskih materija ili njihovih metabolita u krvi, urinu, kosi ili tkivima izloženih osoba. Biomarkeri ekspozicije imaju veliku primenu u proceni izloženosti mnogim teškim metalima ili metaloidima u životnoj sredini (olovo u vazduhu, arsen u vodi), dok su mogućnosti određivanja specifičnih markera ekspozicije gasovima i suspendovanim česticama iz zagađenog vazduha ograničene.

Biomarkeri uticaja odnose se na merenje ranih bioloških efekata prouzrokovanih hemijskom supstancom ili fizičkim agensom i najviše se primenjuju u medicinskom ekologiji. Tako se, na primer, mogu meriti nivoi hormona stresa (adrenalin i kortizol u krvi i salivi) kao markeri efekta buke, te parametari oksidativnog stresa i inflamacije (produkti oksidacije proteina, interleukini, faktor tumorske nekroze u krvi ili urinu) kao markeri efekta buke ili zagađujućih materija u vazduhu. Nova istraživanja koriste ove biomarkere za procenu povezanosti između izloženosti buci ili suspendovanim česticama na razvoj kardiovaskularnih bolesti, imajući u vidu njihovu potencijalnu ulogu u procesu ateroskleroze.

Budući izazov u biomonitoringu je određivanje biomarkera osetljivosti ili podložnosti koji bi trebalo da ukažu na specifičnu sposobnost pojedinca da reaguje na određene faktore iz životne sredine i time doprinesu individualiziciji procene rizika po zdravlje.

Ključne reči: biomonitoring; biološki marker; izloženost; medicinska ekologija

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BUKA I MENTALNO ZDRAVLJE STUDENATA MEDICINE

Jelena Ilić Živojinović

Mnogobrojni negativni faktori okruženja i psihosocijalni stresori doprinose nastansku sve češćih poremećaja psihofizičkog zdravlja univerzitetskih studenata. Većina aktivnosti u kojima mladi danas uživaju povezani su sa izlaganjem visokim nivoima buke. Poslednjih godina veoma je u porastu izloženost buci preko PLD uređaja (personal listening device), naročito zbog široke upotrebe mobilnih telefona. Nedavna istraživanja su pokazala povećanu stopu anksioznosti i depresija kod korisnika ovih uređaja.

Istraživanje je obuhvatilo dve studije preseka sprovedene na Medicinskom fakultetu u Beogradu među studentima šeste godine. Umerene ili teže simptome anksioznosti je prijavilo 16,9% studenata, gde je razlika po polu bila visoko statistički značajna, za razliku od simptome depresije gde nije bilo značajne razlike u odnosu na pol (37,1% studentkinje i 34,0% studenata). Istraživanje je pokazalo da su četrvrtina studenata pušači (25,3%), od kojih 15,5% studenata koristi elektronske cigarete. Većina studenata konzumira alkohol (81,2%) i kafu (84,2%), a njih 19,3% redovno konzumira energetska pića. U multivarijantnoj analizi značajnu pozitivnu povezanost sa depresijom pokazali su faktori: kontinuirana upotreba PLD-a duže od 1 sata dnevno, tinitus, glavobolje i poteškoće sa koncentracijom u bučnom okruženju. Što se tiče anksioznosti, ona je bila značajno i pozitivno povezana sa glavoboljama i upotrebom PLD tokom šetnje. Oko 46% studenata je podložno sindromu izgaranja po kategoriji emocionalne iscrpljenosti i oko 80% po kategoriji depersonalizacije, pri čemu je razlika po polu visoko statistički značajna za skalu emocionalne iscrpljenosti gde je mnogo više devojka u grupi sa visokim skorovima.

Ovim istraživanjem uočena je pozitivna povezanost između učestale dnevne upotrebe PLD-a i depresije, dok je negativan odnos studenata prema buci povezan i sa depresijom i anksioznošću. Studentkinje su podložnije kategoriji emocionalne iscrpljenosti u odnosu na studente.

Ključne reči: buka, anksioznost, depresija, student, sindrom izgaranja

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POVEZANOST ODREĐENIH ASPEKATA PONAŠANJA ADOLESCENATA IZ URBANE SREDINE I POJAVE TINITUSA

Milena Tomanić

Tinitus je svesna percepcija zvuka bez spoljašnjeg zvučnog stimulusa. Na latinskom tinnire znači zvoniti, a iskustvo zujanja ili zvonjenja u uhu svaki čovek doživi bar jednom u životu. U trećini slučajeva javlja se povremeno, kratkotrajno i prolazno zujanje, dok se kod oko 15% odraslih ovaj neprijatni simptom ispoljava kao trajan, teško rešiv ili nerešiv problem. Iako se tinnitus ranije gotovo isključivo povezivao sa oštećenjem sluha kod starijih, danas se prepoznaje u svim uzrastima, kako kod bolesnih tako i zdravih pojedinaca. Sve veći broj studija sagledava tinitus upravo u populaciji dece i adolescenata, naročito u urbanoj sredini. Ovo okuženje obiluje velikim brojem faktora koji mogu potencijalno da indukuju ovaj nejasni zvučni fenomen. Faktori rizika koji se dovode u vezu sa nastankom tinitusa svrstani su u više kategorija:

socio-ekonomski, ekološki, bihejvioralni, hereditarni, dijetetski i drugi. Tinitus kod mladih često može da bude neprepoznat ili da mu se ne pridaje adekvatna medicinska pažnja. Razlog neprepoznavanja tinitusa u ovom uzrastu najčešće je, sa jedne strane, tendenciozno izlaganje bučnim sadržajima uz gotovo potpuno odsustvo tišine tokom dana i maskiranje zujanja u uhu, a sa druge, neupućenost da ovaj fantomski zvuk ukazuje na prisustvo patološkog proces u organizmu i da ne sme biti zanemaren. Adolescenti uprkos zujanju često nastavljaju sa rizičnim ponašanjem što vodi progresiji tegoba sa posledičnim narušavanjem kvaliteta života. Tinitus se dovodi u vezu sa različitim psihičkim i fizičkim poremećajima (nesanica, razdražljivost, problemi sa koncentracijom, oštećen sluh, hipertenzija, gojaznost). Primećena je i sklonost ka somatizaciji tinitusa, tj. da pacijenti pažnju sa zujanja u uhu preusmeravaju na različite druge tegobe koje je klinički nemoguće verifikovati. S obzirom na nezadovoljavajući terapijski učinak svih do sada poznatih procedura lečenja tinitusa, naročito je važno prepoznati što veći broj faktora rizika i na temelju stečenih znanja izraditi neophodne programe u cilju prevencije tinitusa kod mladih.

Ključne teči: tinitus, adolescenti, faktori rizika

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DEZINFEKCIONE METODE U SAVREMENOJ KLINIČKOJ PRAKSI

Ana Jovanović

Rad u zdravstvenim ustanovama zahteva svakodnevno korišćenje velikog broja različitih instrumenata koji dolaze u kontakt sa pacijentima i osobljem. Adekvatna, redovna i kontrolisana dezinfekcija i sterilizacija instrumenata i površina je imperativ u sprečavanju infekcija povezanih sa zdravstvenom zaštitom. Uprkos činjenici da nekritični instrumenti i površine, odnosno predmeti koji dolaze u kontakt sa intaktnom kožom, nose mali rizik od infekcija, značaj dezinfekcije ovih predmeta poslednjih godina raste. Veliki broj istraživanja iz oblasti kontrole infekcija u zdravstvenim ustanovama je pokazao da nekritični predmeti mogu biti rezervoar patogenih mikroorganizama, sa posebnim akcentom na prisustvo multirezistentnih sojeva. Adekvatna dezinfekcija u savremenoj kliničkoj praksi može predstavljati izazov usled čestog nedostatka vremena, naročito ukoliko su u pitanju lični instrumenti poput stetoskopa, čiju dezinfekciju vrši lekar.

U poslednjem istraživanju sprovedenom u Republici Srbiji o navikama dezinfekcije stetoskopa i njihovoj bakterijskoj kontaminaciji je pokazano da svega 21,3% lekara svoj stetoskop dezinfikuje nakon svakog pacijenta, što je u skladu sa preporukama proizvođača. Rastvor 70% etanola se pokazao kao dezinficijens izbora, zbog svoje široke dostupnosti i visoke efikasnosti kojom obezbeđuje redukciju rasta bakterija od 97,3%. Tehnika dezinfekcije koja je pokazala najbolju efikasnost je brisanje površine stetoskopa gazom natopljenom alkoholom. Na ovaj način, pored dezinfekcije, vrši se i fizičko uklanjanje nečistoća, čime se obezbeđuje bolji baktericidni efekat alkohola. Osim upotrebe alkohola u dezinfekciji stetoskopa, istraživanja su fokusirana i na razvijanje savremenih metoda dezinfekcije poput upotrebe ultravioletnog zračenja ili izrade membrana stetoskopa od materijala sa bakteriostatskim efektom.

Bez obzira na opterećenje lekara, koje je u našim uslovima često veliko, dezinfekcija nekritičnih predmeta ne sme biti zanemarena. Izradom jasnih, vidljivih i nedvosmislenih smernica o dezinfekciji stetoskopa, ali i drugih nekritičnih predmeta, obezbeđuje se podizanje svesti o mogućnostima širenjapatogena, sa krajnjim ciljem osiguravanja bezbednije zdravstvene sredine.

Ključne reči: dezinfekcija, infekcije povezane sa zdravstvenom zaštitom, stetoskop

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METABOLIČKI SINDROM I ZDRAVSTVENE NAVIKE

Miloš Maksimović

Interesovanje za faktore rizika za aterosklerotsku bolest počelo je početkom prošlog veka kada su bili prepoznati kao udruženost hiperglikemije, hipertenzije i hiperurikemije, da bi se kasnijim istraživanjima prihvatio termin metabolički sindrom (MSy) od 1998. godine kada je počela da ga koristi Svetska zdravstvena organizacija. Obuhvatao je intoleranciju na glukozu ili **šećernu** bolest tipa 2, plus bilo koji od dva sledeća faktora rizika kao **što** su hipertenzija, hipertrigliceridemija, snižen nivo HDL holesterola, gojaznost izražena preko Indeksa telesne mase ili odnos struk/kuk i mikroalbuminurija ili odnos albumin/kreatinin. Ekspertska grupa za otkrivanje, evaluaciju i lečenje visokog nivoa holesterola kod odraslih (ATP III) je 2001. godine dala novu definiciju MSy koja se smatra najviše korišćenom u kliničkoj praksi, gde se prisustvo MSy utvrđuje postojanjem tri od sledećih pet faktora rizika: abdominalna gojaznost, hipertenzija, hipertrigliceridemija, snižen nivo HDL-a i hiperglikemija. Ova definicija je doživela **čitav** niz korekcija od kojih treba izdvojiti definiciju IDF (International Diabetes Federation) koja je pooštrila kriterijume za abdominalnu gojaznost koja je postala obavezni faktor rizika i hiperglikemiju. Procenjuje se da je učestalost MSy u starijoj populaciji od 20 godina iznad 25%, a da se povećava sa starijom **životnom** dobi i osoba sa aterosklerotskom bolešću.

Zdravstvene navike takođe mogu doprineti riziku za nastanak aterosklerotskih oboljenja, pre svega fizička neaktivnost, konzumiranje alkohola, pušenje i navike u ishrani.

Novija istraživanja su pušenje svrstala u nezavisni faktor rizika za nastanak MSy. Ekscesivno konzumiranje alkohola predstavlja faktor rizika za MSy. Procenjuje se da je sedentaran način **života** odgovoran za smrtni ishod

uzrokovan aterosklerotskom bolešću u 30% slučajeva. Nepravilna ishrana u kojoj dominiraju zasićene masne kiseline, prosti **šećeri**, povećan kalorijski unos može dovesti do aterosklerotske bolesti preko faktora rizika za MSy.

Prevencija faktora rizika za metabolički sindrom, rano otkrivanje i terapija svakog faktora rizika posebno i edukacija o zdravim **životnim** navikama mogu prevenirati aterosklerotska oboljenja i komplikacije.

Ključne reči: metabolički sindrom, zdravstve navike, aterosklerotska bolest

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HRANA – NOVI PRAVCI I IZAZOVI

Dragana Davidović

Porast broja ljudi na planeti, uticaj proizvodnje hrane na klimatske promene i sve češća pojava bolesti koje su povezane sa nepravilnom ishranom jesu izazovi i problemi sa kojima se suočava savremeno društvo. Da li je moguće nahraniti buduću populaciju od 10 milijardi ljudi hranom koja će omogućiti dugoročno dobro zdravlje i sprečiti pojavu najčešćih hroničnih bolesti i pri tome očuvati našu planetu? U cilju uspešnog rešavanja ovih zadataka neophodno je unaprediti procese prozvodnje hrane uz smanjenje količine odbačene hrane kao i promeniti dosadašnje navike u ishrani.

Jedan od načina unapređene, održive poljoprivredne proizvodnje je organska proizvodnja koja podrazumeva proizvodnju hrane bez upotrebe sintetičkih pesticida, veštačkih đubriva i genetski modifikovanih organizama.

Važno je istaći da hrana ne obezbeđuje samo energiju već i različite bioaktivne komponente koje kompleksnim metaboličkim putevima ostvaruju uticaj na dugoročno zdravlje. U tom cilju savremena prehrambena industrija razvija nove funkcionalne proizvode.

Ishrana u skladu sa dobrim zdravljem je ona koja će obezbediti adekvatan kalorijski unos uz visok unos hrane bogate biaktivnim komponentama – povrće, voće, jezgrasto voće, leguminoze, biljna ulja, integralne žitarice, probiotski fermentisani mlečni proizvodi, riba, nizak unos crvenog mesa i minimalan unos rafinisanih žitarica, šećera i visoko prerađene hrane.

Očigledno je da prelazak na ishranu u skladu sa dobrim zdravljem podrazumeva značajne promene uobičajenih navika. Posmatrano na globalnom nivou potrebno je ostvariti dvostruko veći unos povrća, voća, leguminoza, jezgrastog voća i redukciju od preko 50% dodatih šećera i crvenog mesa.

Postoji mnoštvo kombinacija kojima se mogu ostvariti ovi ciljevi. Da bi svaki pojedinac mogao da napravi izbor hrane u skadu sa preporukama neophodno je postojanje tačnih i svebuhvatnih nutritivnih deklaracija na svim prehrambenim proizvodima. Deklaracija sa nutritivnom informacijom bitna je komponenta strategije za borbu protiv nepravilne ishrane i bolesti povezanih sa njom.

Ključne reči: hrana, pravilna ishrana, nutritivna deklaracija

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AKTUELNOSTI U VEZI SA SUPLEMENTACIJOM MAGNEZIJUMOM U HIPERTENZIJI

Nikolina Banjanin

Preporučeni unos magnezijuma je važan za adekvatno obavljanje mnogobrojnih funkcija u organizmu čoveka. U hipertenziji osnovni hemodinamski poremećaj je povećan periferni otpor zbog promena u vaskularnoj strukturi i funkciji. Pored drugih značajnih uloga koje ima u organizmu čoveka magnezijum deluje zaštitno protiv hipertenzije. Magnezijum je važan za prevenciju i tretman hipertenzije i različitim mehanizmima uključujući antagonizam sa kalcijumom, produkciju medijatora vazodilatacije i menjanje vaskularnog odgovora na endotelin I, angiotenzin II i kateholamine ima značajno mesto u regulaciji krvnog pritiska. Između unosa magnezijuma hranom i rizika za hipertenziju postoji negativna povezanost, rizik od hipertenzije se smanjuje za 5 % povećanjem unosa magnezijuma od 100 mg dnevno, a indeks rada levog srca se smanjuje za 0,2 kg*m/m^2 na svakih 100 mg unetog magnezijuma hranom i vodom za piće. Takođe, suplementacija magnezijumom dovodi do smanjenja sistemske vaskularne rezistence. Imajući u vidu da je nizak serumski magnezijum prediktor kardiovaskularnog mortaliteta tretman deficita magnezijuma je važan jer pomaže u prevenciji kardiovaskularnih bolesti. Kod pacijenata obolelih od esencijalne hipertenzije nutritivni unos magnezijuma je ispod preporučenih vrednosti i očekuje se sinergistički efekat magnezijuma sa antihipertenzivnom terapijom. Suplementacija magnezijumom je veoma aktuelna u hipertenziji jer se preporučeni unos magnezijuma teško postiže zbog procesuiranja hrane.

Ključne reči: hipertenzija, magnezijum, suplementacija

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FAKTORI RIZIKA I PREVENCIJA ATEROSKLEROTSKE BOLESTI

Danka Vukašinović

Aterosklerotska bolest je vodeći uzrok morbiditeta i mortaliteta u svetu. Ova bolest pogađa više od 500 miliona ljudi širom sveta i dovodi do oko 19 miliona smtnih slučajeva godišnje. Ishemijska bolest srca, jedna od glavnih manifestacija aterosklerotske bolesti, vodeći je uzrok smrti i u našoj zemlji. Ateroskleroza predstavlja hroničnu inflamaciju arterijskih krvnih sudova izazvanu interakcijom genetskih i faktora životne sredine. Opštepoznati, tradicionalni faktori rizika za aterosklerotsku bolest su hipertenzija, hipeholesterolemija, dijabetes melitus, goja-

znost i pušenje. Uprkos velikim naporima da se kontrolom tradicionalnih faktora rizika smanji globalno opterećenje aterosklerotskom bolešću, značajan rezidualni rizik ipak ostaje. Približno 90% rizika za aterosklerotsku bolest može se pripisati modifikujućim faktorima rizika. Među ovim promenljivim faktorima rizika, netradicionalni, „novi", faktori rizika povezani sa stilom života čine više od polovine kardiovaskularnog rizika i sve su više predmet interesovanja naučne zajednice. Nepravilna ishrana i sedentarizam utiču na brojne netradicionalne determinante kardiometaboličkog zdravlja, kao što su sastav tela, kardiorespiratorna kondicija, mišićna snaga, crevna mikrobiota itd. Redukcija faktora rizika povezanih sa životnim stilom dovodi do niza fenotipskih adaptacija koje, prevodeći proinflamatorni milje u antiaterogeni, posledično smanjuju kardiovaskularni rizik. Najvažnija mera u prevenciji aterosklerotske bolesti je promocija zdravog stila života od najranijeg doba. Primordijalnom prevencijom, odnosno prevencijom razvoja faktora rizika, kardiovaskularni događaji aterosklerotske bolesti mogu se izbeći. Kontrola već postojećih tradicionalnih faktora rizika, kao i promena stila života unapređenjem ishrane i fizičke aktivnosti, ključni su kako u primarnoj tako i u sekundarnoj prevenciji aterosklerotske bolesti. Saradnja lekara i pacijenta je od suštinske važnosti u ovom procesu. Lekar treba da proceni spremnost pacijenta za unapređenje životnog stila, identifikuje potencijalne prepreke, ohrabri ga na tom putu i kontinuirano nadzire njegov napredak.

Ključne reči: aterosklerotska bolest, faktori rizika, stil života, prevencija

MINI SIMPOZIJUM

NOVI TRENDOVI U DOKTORSKOJ EDUKACIJI U OBLASTI BIOMEDICINSKIH NAUKA U EVROPI

Izvršni komitet ORPHEUSa

Katedra interne medicine, Medicinski fakultet, Univerzitet u Beogradu,

Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Univerzitetski klinički centar Srbije

INTERNACIONALIZACIJA DOKTORSKIH AKADEMSKIH STUDIJA I COTUTELLE DOKTORATI

Nebojša Lalić

Nastavljajući tradiciju otvorenosti prema svetu i sledeći strategiju internacionalizacije Univerziteta u Beogradu, Medicinski fakultet se uključuje u savremene trendove internacionalne međuuniverzitetske saradnje u oblasti doktorskih akademskih studija (DAS), u cilju jačanja konstruktivnih akademskih veza i formiranja doktoranada sposobnih za rad u dinamičnom društvu, koje se permanentno menja i unapređuje. U vezi s tim, ulažu se napori za razvijanje institucionalnih i organizacionih okvira za internacionalizaciju, od podizanja svesti o njenom značaju u akademskoj i široj javnosti, do internacionalizacije studijskih programa.

Jedan od pravaca internacionalizacije DAS su *cotutelle* doktorati. To je format kada se doktorski kandidat upisuje na dva partnerska univerziteta tokom punog trajanja ugovora (obično 3-4 godine). Lokacija istraživačkih i studijskih aktivnosti se smenjuje između oba univerziteta. Ovo omogućava kontinuiranu razmenu i trajno korišćenje resursa oba partnera u saradnji. Akademska postignuća koja kandidat treba da ostvari da bi postao doktor nauka podležu pravilima i propisima koji važe na svakom partnerskom univerzitetu. Student DAS na Univerzitetu u Beogradu može realizovati doktorsku disertaciju kroz zajedničko mentorstvo - *cotutelle*, pod uslovom da postoji Međunarodni sporazum o zajedničkom mentorstvu pri izradi doktorske disertacije, koji se zaključuje između Univerziteta u Beogradu i partnerskog univerziteta. To je osnovni pravni akt kojim se preciziraju uslovi i postupak sticanja naučnog naziva doktora nauka za svakog pojedinačnog kandidata. Osim toga, DAS na Univerzitetu u Beogradu, mogu biti realizovane kroz studijski program doktorskih studija za sticanje zajedničke diplome (*joint degree*) ili dvostruke diplome (*double degree*), koje se stiču na doktorskim studijama koje Univerzitet, odnosno fakultet, organizuje sa drugom visokoškolskom ustanovom u inostranstvu, koja je akreditovana za izvođenje studijskih programa DAS.

Ključne reči: doktorske akademske studije, *joint* doktorati, *cotutelle* doktorati

Institut za epidemiologiju, Medicinski fakultet, Univerzitet u Beogradu

OTVORENA NAUKA I DOKTORSKE AKADEMSKE STUDIJE

Tatjana Pekmezović

Otvorena nauka predstavlja novi pristup naučnom procesu zasnovan na kooperativnom radu i novim načinima širenja znanja korišćenjem digitalnih tehnologija i novih kolaborativnih alata (Evropska komisija, 2016). Obavezne prakse otvorene nauke uključuju (1) otvoreni pristup svim naučnim publikacijama, (2) odgovorno upravljanje podacima iz istraživanja korišćenjem FAIR principa (engl. *Findability, Accessibility, Interoperability, and Reuse*), (3) pružanje otvorenog pristupa svim informacijama potrebnim za validaciju naučnih zaključaka (npr. istraživački alati i rezultati, skupovi podataka i kodovi, resursi za intervenciju, strategije skaliranja) i (4) digitalni pristup rezultatima istraživanja. Važno je napomenuti da se otvorena nauka ne razlikuje od tradicionalne nauke; to samo znači da se istraživanja sprovode na transparentniji i kolaborativniji način. Otvorena nauka se primenjuje u svim istraživačkim disciplinama.

U visokom obrazovanju i istraživanju, teme upravljanja otvorenim naučnim i istraživačkim podacima dobijaju sve veći značaj, a stimuliše se i korišćenje otvorenih istraživačkih podataka u nastavi i sticanje pismenosti za upravljanje istraživačkim podacima, čime se unapređuje koncept otvorene nauke. Otvorena nauka, obuhvata ogroman broj potencijalnih strukturnih promena akademske prakse, čija kultura često može biti hijerarhijska i konzervativna. I u situaciji kada istraživači poznaju ciljeve otvorene nauke, možda još uvek ne vide vrednost u njihovoj primeni, pošto postojeći mehanizmi podsticaja još uvek ne odražavaju ovu novu kulturu otvorenosti i saradnje u širem istraživačkom ekosistemu.

Otvorena nauka stvara mogućnost za visoko kvalitetna istraživanja, jer čitav istraživački proces čini transparentnijim i dostupnijim drugim naučnicima i društvu.

Ključne reči: doktorske akademske studije, otvorena nauka, razmena podataka

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MODELI RAZVOJA TRANSLACIONIH ISTRAŽIVANJA I UKLJUČIVANJE STUDENATA DOKTORSKIH STUDIJA

Ivanka Marković

Medicinski fakultet Univerziteta u Beogradu, zajedno sa svojim nastavnim bazama, je po kadrovskim, infrastrukturnim i naučnim resursima najveća i najuspešnija naučno-istraživačka organizacija u oblasti medicinskih nauka u Republici Srbiji. Medicinski fakultet ima i najveći kapacitet za integrisanje predkliničkih i kliničkih istraživanja, čime se stvaraju optimalni uslovi za primenu znanja stečenih u okviru bazičnih istraživanja na istraživanja patoloških procesa u humanoj populaciji (engl. *translational research*). Integracija bazičnih i kliničkih istraživanja, koja se sprovode u nastavnim bazama MFUB, omogućava sistematizovano prikupljanje i analizu uzoraka i podataka vezanih za kardiovaskularne, metaboličke, neurodegenerativne, maligne i bolesti povezane sa starenjem, neuroinflamatorne i autoimunske bolesti, uticaj stila života i činilaca životne sredine na pokazatelje zdravlja i bolesti. Uz to, definisanje novih biomarkera prethodno pomenutih poremećaja u cilju prevencije i rane dijagnostike, kao i praćenja toka bolesti i predviđanja ishoda bi značajno doprineli unapređenju zdravstvenog sistema u Srbiji, i omogućilo strateški pristup preciznoj (personalizovanoj) medicini, čiji su postulati prevencija, predikcija, personalizacija i participacija.

Medicinski fakultet je ujedno u najveća i najproduktivnija naučna i obrazovna baza za kandidate koji upisuju 29 modula doktorskih studija, a koje imaju akreditaciju ORPHEUS-a. Razmena i mobilnost studenata doktorskih studija, njihovo aktivno učešće u radu različitih laboratorija, istraživačkih centara i nastavnih baza, je neophodni preduslov za formiranje nukleusa visoko kompetentnih istraživača, sposobnih za produkciju najrelevantnijih naučnih rezultata i primenu utvrđenih naučnih principa u razvoju tehnologije, dijagnostici i terapiji. Na taj način se omogućava i poboljšanje perspektive visokog obrazovanja, istraživanja (naučnih i primenjenih) i zapošljavanja mladih istraživača.

Ključne reči: translaciona istraživanja, doktorske akademske studije, doktorske disertacije

Institut za anatomiju, Medicinski fakultet, Univerzitet u Beogradu

DOKTORSKE AKADEMSKE STUDIJE NA MEDICINSKOM FAKULTETU UNIVERZITETA U BEOGRADU: PRAVCI ZA UNAPREĐENJE

Petar Milovanović

Medicinski fakultet Univerziteta u Beogradu (MFUB) posvećuje veliku pažnju doktorskim akademskim studijama (DAS), čiji kvalitet je jedan od važnih temelja naučnog rada kao i međunarodne naučne reputacije MFUB. U skladu sa ORPHEUS akreditacijom, Fakultet posebnu pažnju posvećuje ORPHEUS standardima kao i praćenju ključnih načela i trendova u doktorskoj edukaciji u Evropi. Ovi trendovi zahtevaju kontinuiranu analizu kvaliteta i dinamično prilagođavanje kurikuluma DAS u cilju pripreme kandidata za karijeru u dinamičnom društvu. U prethodne dve godine je sprovedena reforma nastave metodologije naučnoistraživačkog rada i istraživačke etike radi bolje pripreme kandidata za vrste istraživanja kojima će se baviti na odgovarajućoj grupaciji modula, odnosno, radi praktičnog upoznavanja sa etičkim principima i dilemama koji su u vezi sa takvim vrstama istraživanja. Ustanovljena je redovna praksa održavanja kurseva za poboljšanje mentorskih veština, a u planu je dodatna edukacija doktoranada u oblasti relevantnih veština (npr. *soft skills*). U cilju daljeg unapređenja i razvoja DAS na MFUB, ohrabruje se pisanje i odbrana doktorskih disertacija na engleskom jeziku, internacionalizacija DAS kroz *cotutelle* i *joint* doktorate sa evropskim univerzitetima, mobilnost kandidata i mentora, kao i povećanje saradnje sa industrijom i partnerima van akademske zajednice. U planu je i dodatna promocija koncepta otvorene nauke kao i međunarodne i interdisciplinarne naučne saradnje. Planirane analize i aktivnosti su važni radi povećanja kompetitivnosti MFUB u evropskom akademskom prostoru.

Ključne reči: unapređenje kurikuluma, trendovi, ORPHEUS standardi

MINI SIMPOZIJUM

100 GODINA INSTITUTA ZA HEMIJU U MEDICINI „PROF. DR PETAR MATAVULJ“ MEDICINSKOG FAKULTETA U BEOGRADU

Institut za hemiju u medicini „Prof. dr Petar Matavulj“, Medicinski fakultet, Univerzitet u Beogradu

100 GODINA INSTITUTA ZA HEMIJU U MEDICINI „PROF. DR PETAR MATAVULJ“ MEDICINSKOG FAKULTETA U BEOGRADU

Danijela Krstić, Kristina Gopčević, Lidija Izrael Živković

Institut za hemiju u medicini Medicinskog fakulteta Univerziteta u Beogradu je jedan od najstarijih instituta i od osnivanja do danas je istraživačko-obrazovna celina u kojoj se obavlja nastavna i naučna delatnost.

Institut za hemiju u medicini „Prof. dr Petar Matavulj“ je osnovan 1923. godine, godinu dana nakon što je prof. dr Petar Matavulj pozvan iz Lozane gde je radio kao asistent na Hemijskom institutu, da drži nastavu iz hemije na Medicinskom fakultetu u Beogradu.

Hemijski institut se više puta selio, a od 1950. godine je preseljen u tada novu zgradu Histofiziološkog instituta, gde se i danas nalazi.

Katedra Hemije je formirana kao zasebna katedra 1978. godine. a do tada je nastava iz hemije bila u okviru zajedničke Katedre koju su činili hemija, biologija i fizika. Fond časova Hemije se menjao od osnivanja do danas. Do 1977. godine nastava je bila dvosemestralna, a od tada jednosemestralna. Do školske 2003/04. nastava se odvijala jednosemestralno u prvoj godini studija sa ukupnim fondom od 135 časova. U skladu sa izmenjenim planom i programom nastave od školske 2004/05. nastava iz hemije se realizuje u okviru zajedničkog predmeta Medicinska biohemija i hemija za studente druge godine integrisanih akademskih studija medicine (na srpskom i na engleskom jeziku). U okviru ovog predmeta nastava iz hemije traje 9 nedelja (III semestar) sa ukupno 72 časa teorijske i praktične nastave. Nastavnici i saradnici Katedre za hemiju u medicini realizuju i 13 izbornih predmeta, učestvuju u realizaciji poslediplomske nastave.

Nastavnici i saradnici Instituta za hemiju imaju veoma plodnu naučnu delatnost iz oblasti proteinske biohemije, toksikološke hemije, mikrobne i humane enzimologije, analitike biomolekula i bioneorganske hemije. Naučna delatnost se odvija kroz domaće i međunarodne istraživačke projekte, u saradnji sa brojnim institutima i klinikama našeg fakulteta kao i renomiranim ustanovama u zemlji i inostranstvu i ogleda u objavljivanju značajnih naučnih publikacija.

Ključne reči: istorijat instituta, redovna nastava, izborna nastava, naučna delatnost

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REMINISCENCIJE

Ivanka Karadžić

Znanja hemije na početku XX veka: prve tri Nobelove nagrade za hemiju: van ’t Hoff- osmotski pritisak, Fischer-ugljeni hidrati i Arrhenius- terija kiselina i baza, duboko srastaju u tkivo medicine dajući osnov daljem zajedničkom razvoju ove dve oblasti, do danas. Snažna sinergija hemije i medicine je daleke 1923.g jasno prepoznata osnivanjem Instituta za hemiju na Medicinskom fakultetu u Beogradu. Na žalost, novim *Curiculum*-om od 2005. godine Hemija u medicini gubi svojstvo samostalnog predmeta i biva asimilovana u tzv. zajednički predmet, čime je izgubila identitet, a njeni nastavnici većinu atributa profesora univerziteta. Ne mogu a da se ne zapitam- da li je na početku III milenijuma hemija potrebna studentima medicine? Poslednje tri Nobelove nagrade za hemiju koje već imaju svoju aplikaciju u medicini: editovanje gena, organokatalizatori u sintezi lekova, biortogonalne reakcije za *in vivo* praćenje bioprocesa i sintezu lekova, jasno pokazuju da su hemija i medicina bliže danas, više nego ikada. Sve oblasti medicine koje percipiraju probleme bolesti i zdravlja na molekulskom nivou, nužno integrišu znanja savremene hemije.

Medicinski fakultet u Beogradu jedan od retkih, ako ne i jedini evropski, na kome hemija nije samostalni predmet, iako Katedra za hemiju ovog fakulteta imam pun kapacitet za njegovu relizaciju, kako po broju nastavnika i saradnika i njihovom viskom naučno- stručnom kvalitetu, tako i prostorno i opremom. Hemija u medicini, 2023. predstavlja predmet, pažljivo koncipiran tako da odgovara potrebama studenata medicine, u kome je ostvarena sinergija između klasične hemije (razumevanje osnovnih hemijskih principa i njihova primena na strukturu, interakcije i funkciju biomolekula) i najnovijih prustupa proučavanju medicinskih važnih biomolekula, uključujući i cutting edge bioanalitičke platforme. U tom smislu, zaista ne postoji ni jedan objektivni i opravdani razlog da Hemija u medicini ne bude samostalni predmet na Medicinskom fakultetu u Beogradu.

Ključne reči: hemija u medicini

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EXPLORING POLYOXOMETALATES AS NON-DESTRUCTIVE STAINING AGENTS FOR CONTRAST-ENHANCED COMPUTED TOMOGRAPHY

Tatjana N. Parac-Vogt , Nada Savic, Greet Kerckhofs, *Marko Stojanović, Čolović Mirjana, Danijela Krstić*

Due to the high complexity and heterogeneous structure of biological tissues, imaging techniques that allow for precise and quantitative structural analyses of such materials are of high importance. These techniques could also advance clinical translation of regenerative medicine by providing better insights in tissue development and disease. The standard techniques for evaluating biological tissues such as histological sectioning and staining, have a high discriminative power, but allow assessment of the tissue distribution only in two dimensions. Due to restricted sectioning orientation and limited depth resolution, this leads to loss of information in three dimensions (3D), and therefore, more precise imaging of the 3D microstructure and spatial interrelationships of the different tissues within organs is crucial. MicroCT can provide full 3D structural information of mineralized tissues and dense biomaterials. However, the intrinsic low X-ray absorption of soft tissues requires contrast-enhancing staining agents (CESAs) to be used. We have shown that a range of polyoxometalate clusters (POMs) can be excellent non-destructive staining agents for high-resolution contrast-enhanced microCT (CE-CT) visualization of various tissues, of bone and its marrow vascularization and adiposity. A range of Wells-Dawson POMs, differing in structure and overall charge, has been synthesized and evaluated for their potential as soft tissue CESAs. We have shown that hafnium-substituted POM (Hf-POM) allows for simultaneous contrast-enhanced microCT (CE-CT) visualization of bone and its marrow vascularization and adiposity. Monolacunary Wells-Dawson POM (Mono-WD POM) showed similar soft tissue enhancement as Hf-WD POM and phosphotungstic acid (PTA), a frequently used but destructive CESA. However, compared to PTA, the POMs are much less destructive and show a better diffusion. The solubility of Mono-WD POM can be improved by simple addition of lithium chloride to the staining solution, leading to further enhancement of the soft tissue contrast. *In vivo* toxicity of Wells-Dawson POM has been also evaluated according to standard toxicological protocols using *Wistar albino* rats, which is the first and important step in evaluating side effects of these polyoxometalate nanoclusters that show large potential as therapeutics and contrast agents.

Key words: polyoxometalates, contrast agents, computed tomography

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MOLECULAR BASIS OF VASCULAR AGEING AND CIRCULATING BIOMARKERS ASSOCIATED WITH VASCULAR AGEING: REPORT FROM VACSAGENET

Eugenia Gkaliagkousi

Aging is a natural physiological process characterized by the progressive loss of tissue and organ function and represents the main risk factor for cardiovascular disease (CVD). The basic manifestation of aging, the gradual decrease in the adaptive abilities of the organism can play a significant role in the development of several other pathologies including malignant diseases, neurodegenerative processes, reduced resistance to infection and diabetes mellitus.

Cardiovascular and cerebrovascular diseases are due to alterations in vascular function or are exacerbated by vascular functional and structural changes. The fundamental cellular and molecular mechanisms of aging include: oxidative stress, chronic low-grade inflammation, cell matrix injury, epigenetic alterations, telomere length, cellular senescence and autophagy, considering in vitro and in vivo preclinical research and clinical studies. Oxidative stress, which is a consequence of imbalance between production and detoxification of reactive oxygen and nitrogen species, is one of the underlying factors in several diseases as well as one of the hallmarks of aging. Chronic low-grade inflammation is considered as one of the main mechanisms underlying biology of vascular aging through multiple mechanisms including endothelial dysfunction, atherosclerosis, increased vascular stiffness and vascular calcification. Disruption of ECM integrity by MMPs greatly changes its composition and substantially impacts vascular homeostasis during aging through structural and functional changes of the vessel wall.

DNA methylation, histone modification and non-coding RNAs are epigenetic regulation processes which strongly influence vascular ageing. To assess the changes leading to biological aging, molecular and cellular biomarkers, as well as non-invasive imaging techniques, can be applied. The term biomarker refers to a broad range of biological measures that can be objectively measured and evaluated as indicators of normal biological and pathogenic processes

or pharmacologic responses to a therapeutic intervention. Several molecules demonstrate the potential to be introduced as valuable circulating biomarkers of vascular aging.

Key words: vascular aging, oxidative stress, inflamaging, epigenetics, cellular senescence

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ASSOCIATING AIR POLLUTION WITH GENOME DAMAGE IN LYMPHOCYTES OF THE GENERAL POPULATION IN CROATIA

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Air pollution is one of the most serious public health issues worldwide and isacknowledged to be a leading environmental cause of cancer deaths. Simultaneously, both the micronucleus and comet assays serve as cancer-predictive methods that are broadly used in human biomonitoring for populations exposed to environmental contamination. In the retrospective part of the study, we evaluated genomic instability in healthy residents from Zagreb (Croatia; N>120) and related them to air pollution levels in the period from 2011 to 2015. Our results showed that measured air pollution parameters were largely below the regulatory limits for the designated period, except for benzo(a)pyrene (B[a]P), and as such, they do not affect genomic instability. In the prospective part of the study, we investigated the possible effects of air pollution and BTEX (benzene, toluene, ethylbenzene, and xylene) exposure on genomic instability using the comet assay on blood cells of the general population (N=60) living in Zagreb (Croatia) during colder and warmer periods of the years 2021/2022. Measured outdoor air pollutants agreed with previously reported results and were below the regulatory limit, except for PM_{10} particles and B[a]P bound to PM_{10}, which exceeded those levels. Here we also did not observe a notable impact of air pollutants on tested parameters.Nevertheless, since air pollution is recognized as a major health threat, it is necessary to conduct prospective studies investigating the effect of air pollution on genome integrity and consequently human health.Moreover, given the transboundary nature of air pollution, all stakeholders have a responsibility to protect the atmosphere and to ensure healthy air on a global scale.

Key words: air pollution, biomonitoring, micronucleus and comet assays

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ENZYMES AS A PLATFORM FOR DRUG DEVELOPMENT

M.B. Čolović, N. Savić, T. Parac-Vogt, D.Z. Krstić

Polyoxometalates are negatively charged polyanions containing early transition metal ions in their high oxidation state surrounded by bridged oxygen. Firstly, these metal-based clusters were used as promising agents in electron-dense imaging, separations, catalysis, and analysis. In recent years, numerous studies *in vitro* and *in vivo* found that these nanocomplexes possess a variety of biological effects including antidiabetic, anticancer, and antibiotic actions. Despite these observed properties, the mechanism of their biological activities has not been completely elucidated so far. On the other hand, the results of enzymatic studies revealed their inhibiting influence on physiologically important extracellular enzymes such as phosphatases, esterases, and ecto-nucleotidases, which are considered target enzymes for the approved biological actions. Accordingly, the overview of the *in vitro* influence of selected polyoxo-vanadates, -tungstates, and – palladates on cholinesterase, ATPase, and phosphatase activities will be given in this presentation. Cholinesterases, enzymes located on the postsynaptic plasma membrane, have a key role in nerve impulse transmission and were confirmed as the targets of drugs for neurological diseases, which are regularly used in clinical practice. Moreover, ATPases and phosphatases were found to be included in the proliferation and migration of tumor cells, thus the inhibition of these enzymes was found as the mechanism of some anticancer drug action.

Key words: antitumor drugs, ATPases, cholinesterase, inhibition, neurological diseases, phosphatases

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METABOLOMIČKO ISPITIVANJE SERUMA OBOLELIH OD SHIZOFRENIJE I BIPOLARNOG POREMEĆAJA NMR SPEKTROSKOPIJOM

Nataša Avramović, Katarina Simić, Nina Todorović, Dejan Gođevac, Zoran Miladinović, Snežana Trifunović, Ljubodrag Vujisić, Vele Tešević, Boris Mandić, Aleksandra Gavrilović, Silvana Jovanović, Ljubica Tasic

Shizofrenija (SCZ) i bipolarni poremećaj (BD) su mentalne bolesti sa nekim zajedničkim simptomima (zablude i halucinacije), što često predstavlja problem u njihovoj dijagnostici. Patofiziologija oba poremećaja je veoma heterogena i složena jer uključuje različite genetske, ekološke i biohemijske faktore. Dijagnoze su uglavnom zasnovane na subjektivnom prepoznavanju simptoma bez kliničkog testa identifikacije biomarkera. Metabolomičkim ispitivanjem uzoraka seruma srpskih pacijenata (51) sa SCZ i zdravih ljudi-kontrola (39), kao i uzoraka seruma pacijenata sa bipolarnim poremećajem (33) i zdravih kontrola (39), primenom ^{1}H NMR spektroskopije i hemometrijskih analiza, odredili smo 26 potencijalnih biomarkera za SCZ i 22 za BD. U svim uzorcima seruma pacijenata sa SCZ iz tri zemlje različitog etničkog i geografskog porekla (Srbija, Brazil i Kina) utvrđeno je 13 istih metabolita (laktat/mlečna kiselina, treonin, leucin, izoleucin, valin, glutamin, asparagin, alanin, γ-aminobuterna kiselina, holin, glukoza, glicin i tirozin), dok je 9 istih potencijalnih biomarkera (laktat, alanin, valin, leucin, izoleucin, glutamin, glutamat, glukoza i holin) određeno u svim uzorcima seruma pacijenata sa BD.

Ključne reči**:** shizofrenija; bipolarni poremećaj; NMR spektroskopija; hemometrijska analiza

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MULTIOMIKS PRISTUPI U ANALIZI BIODEGRADACIJE TOKSIČNIH ORGANSKIH JEDINJENJA

Ana Medić

Zagađenje životne sredine organskim jedinjenjima predstavlja opasan rizik, kako za ekosistem u celini, tako i za zdravlje ljudi. Zbog svoje sveprisutnosti, interakcije sa biotičkim i abiotičkim komponentama životne sredine kao i razgranatih metaboličkih aktivnosti, mikroorganizmi imaju ključnu ulogu u bioremedijaciji životne sredine. Dobro razumevanje metaboličkih puteva može dovesti do razvoja novih strategija za tretiranje zagađivača u poljoprivrednom i ekološkom okruženju. Novorazvijene omiks metode omogućavaju proučavanje ranog molekulskog odgovora organizma na izvore zagađenja, i kao takve mogu se koristiti za identifikaciju specifičnog metaboličkog odgovora na toksičnu supstancu, otkrivanje novih biomarkera, kao i za predviđanje uticaja zagađujuće supstance na biološke vrste i životnu sredinu.

Ekološki izolat poliekstremofilne, hidrokarbonoklastične bakterije *Pseudomonas aeruginosa* san ai pokazao je značajan potencijal za razgradnju različitih zagađujućih supstanci, kao što su 2,6-di-*terc*-butilfenol, fluoren, fenantren, natrijum-benzoat i frakcija nafte. Proteomska analiza *P. aeruginosa* san ai eksponiranog na organske zagađujuće supstance, uporedo sa analizom genoma i ciljanog metaboloma, je potvrdila postojanje ključnih enzima i metabolita β-ketoadipatne putanje za degradaciju aromatičnih jedinjenja. Analiza metaboloma je implicirala postojanje i kateholne i protokatehuatne grane β-ketoadipatnog puta. Razumevanje mehanizama pomoću kojih *Pseudomonas* razgrađuje zagađujuće supstance je od suštinskog značaja za razvoj novih strategija sanacije životne sredine, u čemu ključnu ulogu imaju novorazvijene analitičke omiks platforme.

Ključne reči: biodegradacija, pseudomonas, organska jedinjenja, metabolom, ekotoksikoproteomiks

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TOKSIKOLOŠKA ISPITIVANJA TOKOM RAZVIJANJA NOVIH LEKOVA I MEDICINSKIH SREDSTAVA

Marko Stojanović, Mirjana Čolović, Danijela Krstić

Toksikološka ispitivanja predstavljaju osnovu preklinič-kih ispitivanja novih lekova i medicinskih sredstava. Ovaj tip studija može predstavljati presudni korak u procesu procene daljeg razvoja lekova i medicinskih sredstava. Svedoci smo sve većeg interesovanja industrije i šire akademske zajednice za ovaj tip istraživanja, pa je upoznavanje sa vodičima za sprovođenje ovih studija, potpomognuto primerima iz prakse od ključnog značaja za njihovu edukaciju. Uzimajući sve ovo u obzir neophodno je da šira akademska zajednica bude bolje upoznata sa značajem ovih studija i pravilnim načinom njihovog sprovođenja. Od velikog je značaja upoznati zainteresovane strane o načinu sprovođenja toksikoloških studija, pre svega sa zakonskim okvirima koji se moraju poštovati u toku njihovog sprovođenja. Neophodno je zainteresovanim stranama iz šire akademske zajednice i industrije ukazati na etičke izazove na koje se nailazi tokom sprovođenja jedne toksikološke studije, pogotovu ako se u obzir uzme koliko su ovakva ispitivanja sa etičkog aspekta osetljiva. Postoji

i potreba da se razjasni kako ispitivanje različitih toksikoloških aspekata zahteva i različite pristupe u sprovođenju ovih studija. Tako se npr. međusobno se razlikuju studije koje ispituju akutnu ili hroničnu toksičnost, studije u kojima se ispituju različiti načini primene (*per os,* intravenski,...) lekova i medicinskih sredstva i sl. Sve ove informacije bi trebalo da razjasne zainteresovanim stranama kako pravilno sprovesti toksikološke studije i kako iz njih dobiti relevantne rezultate, a da se pri tome ne krše zakonske odredbe niti etičke norme.

Ključne reči: toksikološka ispitivanja, lekovi i medicinska sredstva

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PUTOVANJE KROZ BIOLOŠKU HEMIJU

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Oksidativni stres nastaje kada se veliki broj generisanih reaktivnih vrsta kiseonika ne može ukloniti ćelijskim antioksidativnim mehanizamima. Ovo stanje dovodi do oštećenja biomakromolekula: lipida, proteina i dezoksiribonukleinskih kiselina, kompromitujući normalno ćelijsko funkcionisanje i opstanak. Ćelije raka su metabolički reprogramirane sa bioenergetskom i signalnom disregulacijom što se u velikoj meri može povezati sa oksidativnim stresom. Onkogeni potencijal oksidativnog stresa baziran je na štetnom efekti povećanog nivoa reaktivnih vrsta kiseonika. Nivo oksidativnog stresa procenjuje se kvantifikacijom biomarkera: malondialdehida - markera lipidne peroksidacije, proizvoda napredne oksidacije protein- markeri oksidativnih oštećenja proteina, 8-okso-2′-deoksiguanozina - markera oksidativnih oštećenja dezoksiribonukleinskih kiselina. Antioksidativni zaštitni mehanizam obuhvata enzimske i neenzimske komponente. Aktivnost superoksid dismutaze, katalaze, glutation peroksidaze, glutation reduktaze i glutation-S-transferaze predstavlja glavnu enzimsku osu antioksidativne odbrane. Neenzimski antioksidansi uključuju različita jedinjenja među kojima su redukovani glutation, vitamini C i E, karotenoidi, bilirubin i dr. Za aktivnost antioksidativnih enzima neophodno je prisustvo u aktivnom centru jona metala: selena, bakra, cinka i mangana.

Određivanje aktivnosti antioksidativnih enzima kao i kvantifikacija neenzimskih jedinjenja kao što su glutation, ukupni tioli, vitamin C i E su parametri koji se koriste za evaluaciju kapaciteta antioksidativne odbrane.

Imajući u vidu da je oksidativni stres u osnovi mnogobrojnih oboljenja, njegov nivo je određivan u sledećim patološkim stanjima: kolorektalnom karcinomu, akutnom infarktu miokarda, karcinomu dojke, benignim i malignim bolestima štitne žlezde. Papilarni karcinom štitaste žlezde i koloidna struma predstavljaju najčešću malignu i benignu bolest štitne žlezda. Smatra se da oksidativni stres ima važnu ulogu u patogenezi ovih bolesti. Rezultati su pokazali da je oksidativni stres prisutan u papilarnom karcinomu štitaste žlezde a nije u koloidnoj strumi, koja je predstavljala kontrolu za određivanje svih parametara oksidativnog stresa. Određivanje parametara oksidativnog stresa u navedenim bolestima može se koristiti kao pouzdan parameter za procenu bolesti , status preživljavnja kao i za klinički tok bolesti.

Ključne reči: oksidativni stress, aktivnost antioksidativnih enzima, kancer

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RAZVOJ NOVIH ANTIDIJABETIČKIH LEKOVA NA BAZI POLIOKSOMETALATNIH NANOKLASTERA

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Zahvaljujući brojnim istraživanjima koja su ispitivala biološka svojstva strukturno različitih polioksometalata, došlo se do zapažanja da ova kompleksna neorganska jedinjenja, pored antimikrobnog i antitumorskog delovanja, mogu biti delotvorna u snižavanju hiperglikemije kod pacova sa eksperimentalno izazvanim dijabetesom. Stoga, cilj ove studije je bio da se ispitaju antidijabetički potencijal i mogući toksični efekti dva polioksovolframata:$(NH_4)_{14}[NaP_5W_{30}O_{110}]\cdot 31H_2O$, $\{\mathbf{NaP_5W_{30}}\}$ i $K_{14}[AgP_5W_{30}O_{110}]\cdot 22H_2O\cdot 6KCl$, $\{\mathbf{AgP_5W_{30}}\}$. U cilju realizacije postavljenog cilja, korišćena su tri eksperimentalna modela: (1) antihiperglikemijska *screening* studija u kojoj je ispitivan uticaj jednokratne intraperitonealne primene $\{\mathbf{NaP_5W_{30}}\}$ i $\{\mathbf{AgP_5W_{30}}\}$ (5, 10 i 20 mg/kg) na snižavanje hiperglikemije kod dijabetičkih pacova, (2) akutna peroralna toksikološka studija koja je istraživala hepato- i nefrotoksične efekte odabranih heteropolivolframata kod zdravih pacova i (3) studija posvećena rasvetljavanju mogućih mehanizama antidijabetičkog delovanja heteropolivolframata. Rezultati *screening* studije su pokazali da su oba ispitivana heteropolivolframata efikasna u snižavanju hiperglikemije, s tim što se $\{\mathbf{NaP_5W_{30}}\}$, u odnosu na $\{\mathbf{AgP_5W_{30}}\}$, pokazao kao moćniji antihiperglikemijski agens. Rezultati biohemijskih parametara funkcije i patohistološka analiza jetre i bubrege korišćenjem konvencionalne svetlosne i transmisione elektronske mikroskopije pokazuju da dvonedeljna primena $\{\mathbf{NaP_5W_{30}}\}$ i $\{\mathbf{AgP_5W_{30}}\}$ (20 mg/kg) izaziva blagi do umereni stepen hepato- i

nefrotoksičnosti kod zdravih životinja. U poslednjem eksperimentalnom protokolu, pokazano je da tronedeljna peroralna primena {$\mathbf{NaP_5W_{30}}$} (20 mg/kg) povećava koncentraciju insulina u serumu dijabetičkih pacova, što može biti jedan od mehanizama njegovog antidijabetičkog delovanja. Takođe, pokazano je da {$\mathbf{NaP_5W_{30}}$} ispoljava hepato-, nefro-, kardio- i neuroprotektivno dejstvo kod dijabetičkih pacova, što je procenjeno na osnovu analize: (1) relativne mase organa, (2) biohemijskih parametara funkcije, (3) parametara oksidativnog stresa u homogenatu tkiva, (4) aktivnosti acetilholinesteraze, Na^+/K^+-ATPaze i *ecto*-ATPaza u sinaptozomima i (5) patohistoloških promena u tkivima korišćenjem konvencionalne svetlosne i transmisione elektronske mikroskopije. Stoga, {$\mathbf{NaP_5W_{30}}$} i {$\mathbf{AgP_5W_{30}}$} mogu se smatrati mogućim neinsulinskim lekovima-kandidatima u terapiji dijabetesa tipa 2, koji bi se podvrgli daljim pretkliničkim istraživanjima.

Ključne reči: heteropolivolframati, antidijabetici, nefrotoksičnost, hepatotoksičnost, mehanizam antihiperglikemijskog delovanja

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MINI SIMPOZIJUM

MAGNETNA REZONANCA SRCA: DA LI JE BUDUĆNOST SVETLA?

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MAGNETNA REZONANCA U BOLESTIMA MIOKARDA I PERIKARDA

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Magnetna rezonanca srca (MRS) je neinvazivna i suverena metoda za tkivnu karakterizaciju miokarda i za diferenciranje i sagledavanje srčane ovojnice tj. listova perikarda.

Miokarditis je zapaljenje srčanog mišića uzrokovano inflamatornim ili neinflamatornim agensima. MRS se pokazala kao efikasan dijagnostički metod kojim se mogu proceniti i kvantifikovati srčana oštećenja nastala posle preležane virusne infekcije u vidu miokarditisa. Na osnovu obrasca deponovanja kontrastnog sredstva koje se obavezno primenjuje tokom snimanja, Gadolinijuma, mogu da se razlikuju ishemijska od neishemijskih oštećenja miokarda. Na mestu gde postoji oštećenje kardiomiocita vidi se LGE fenomen (late Gadolinium enhancement) Utvrđivanje etiologije srčanog oštećenja je od izuzetnog značaja za kliničara u cilju efikasne terapije i lečenja bolesnika.

MRS je neinvazivna, izuzetno pouzdana metoda za postavljanje dijagnoze miokarditisa kojom se može otkriti edem miokarda, hiperemija i fibroza uz izračunavanje volumena leve i desne komore i sistolnih funkcija obe komore. Ali se definitivna dijagnoza miokarditisa postavlja samo endomiokardnom biopsijom.

Miokarditis kao i svaki drugi inflamatorni proces dovodi do pojave edema, hiperemije, nekroze sa mogućom posledičnom fibrozom miokarda. MRS može da otkrije svaki od pomenutih procesa koristeći posebne sekvence za detekciju edema u miokardu, za otkrivanje hiperemije (early gadolinium enhancement) i LGE za pokazivanje fibroze. Od 2009. godine postoje Lake Louise kriterijumi za postavljanje dijagnoze miokarditisa koji se i danas koriste, sa tim da ukoliko postoje napredne tehnike MRS kao mapping i njih treba uključiti u evaluaciju kod ovih pacijenata.

Na magnetnoj rezonanci srca perikard se vidi kao linija niskog intenziteta signala, debljine do 3 mm, koja je okružena epikardnom i medijastinalnom mašću koja je visokog intenziteta signala. Važna je za razlikovanje konstriktivnog peikarditisa od restriktivne kardiomiopatije. Korišćenjem MRS može da se detektuje i minimalni perikardni izliv uz karakterizaciju sadržaja perikardnog izliva.

MRS je važna neinvazivna dijagnostička procedura kojom se dobijaju korisne informacije za procenu i lečenje ovih pacijenata.

Ključne reči: magnetna rezonanca srca, miokard, perikard

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AKUTNI KORONARNI SINDROM: ULOGA MAGNETNE REZONANCE SRCA – KADA, KOME I ZAŠTO?

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U svakodnevnoj kliničkoj praksi se izdvajaju 3 glavne uloge magnetne rezonance srca (MRS) u akutnom koronarnom sindromu (AKS). Prva uloga MRS je u postavljanju prave i pravovremene dijagnoze, druga uloga je njen prognostički značaj u proceni rizika bolesnika, dok je treća uloga vizualizacija komplikacija i procena njihove ozbiljnosti.

MRS je važna u proceni anatomije, funkcije i tkivnih karakteristika miokarda u AKS. Globalna I regionalna funkcija leve komore (LK), funkcija desne komore, veličina infarkta miokarda (IM)/ožiljka, mikrovaskularna opstrukcija (MVO), intramiokardna hemoragija (IMH), miokardna perfuzija, vijabilnost su dileme koje se mogu rešiti uz pomoć MRS u različitim fazama bolesti kod bolesnika sa AKS.

Poseban izazov je doći do etiopatogenetskog procesa bolesti kada se postavi radna dijagnoza infarkta miokarda bez opstrukcije koronarnih arterija (MINOCA). MRS je vrlo često presudna dijagnostička metoda kod ovih bolesnika. Sa pojavom naprednih tehnika kao što su T1 i T2 "mapping", uz pomoć kojih možemo da analiziramo i kvantifikujemo i difuzne promene na tkivu miokarda dosta je olakšana diferencijalna dijagnoza kod bolesnika sa ishemijskom u odnosu na neishemijska kardiološka oboljenja gde spadaju neishemijske kardiomiopatije, miokarditisi, ali i u odnosu na druga u osnovi nekardiološka oboljenja.

Prednost MRS nad drugim dijagnostičkim metodama je njena visoka senzitivnost I specifičnost. Poseban značaj je u slučaju lošeg ehokardiografskog prozora kada je ehokardiografski nalaz inkonkluzivan, a takođe i to što nema primene jonizujućeg zračenja. Mane ove metode su manja dostupnost, pogotovo kod nepokretnih bolesnika, dužina trajanja pregleda.

Ključne reči: magnetna rezonanca srca, akutni koronarni sindrom

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ZNAČAJ MAGNETNE REZONANCE SRCA U DIJAGNOSTIČKOJ EVALUACIJI BOLESNIKA SA HRONIČNOM KORONARNOM BOLEŠĆU

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Precizna identifikacija bolesnika sa hroničnom koronarnom bolešću (HKB) uzrokovanom opstruktivnom koronarnom bolešću (OKB), ostaje stalni klinički izazov, a smernice o dijagnostici i terapiji kao i procene o prognostičkom značaju stabilne OKB se stalno usavršavaju. Kada postoji velika verovatnoća za OKB savetuje se invazivna koronarografija, optimalno sa merenjem frakcije rezerve protoka (FFR). U pacijenata koji imaju srednji rizik za OKB, prvo se koriste neinvazivni dijagnostički testovi, uključujući i perfuziju miokarda magnetnom rezonancom srca (MRS)

Stres MRS koristi dinamičku akviziciju slike prodora kontrastnog agensa kroz vaskulaturu miokarda u stanju maksimalne vasodilatacije koja se postiže intravenskom apikacijiom vazodilatatora. Obično se koriste adenozin ili specifičniji agonist A2 receptora regadenozon. Metoda je semikvantitativna, bezbedna, reproducibilna, sa odličnom senzitivnošću (87-90%) i specifičnošću (87-94%) u detekciji OKB kada se poredi sa invazivnom koronarografijom.

Rezultati nedavno objavljene studije MR-INFORM razjasnili su neke aspekte terapijskih zagonetki kod pacijenata sa srednjom do velikom sumnjom za OKB. Studija je ispitala mogućnost stres MRS da direktno vidi revaskularizaciju miokarda, u poređenju sa standardom primenom FFR. Revaskularizacija je preporučena za pacijente u MRS grupi sa ishemijom najmanje 6% miokarda, a u FFR grupi sa FFR od ≤0,8. Ispitivanje je dizajnirano da proceni neinferiornost neinvazivnog ishemijskog testa u odnosu na FFR u smislu jednogodišnjeg kompozitnog ishoda (smrt, nefatalni IM ili revaskularizacija). Najvažniji rezultat je bila neznačajna razlika u velikim neželjenim događajima između dva modaliteta vođenja revaskularizacije miokarda (p=0,21). Štaviše, ugrupi stres MRS bila je niža incidence koronarne revaskularizacije u odnosu na FFR grupu što je dovelo do značajnog smanjenja nepotrebne invazivne angiografije. Ovi nalazi podržavaju perfuzionu MRS kao pristup prve linije u identifikaciji pacijenata koji bi imali koristi od lečenja revaskularizacijom.

Najnoviji modalitet perfuzione MRS je kvantitativna perfuzija koja omogućava merenje perfuzije miokarda u ml/g/min u miru i tokom maksimalne vazodilatacije, čime se može odrediti rezerva miokardne perfizuje koja ima veliki prognostički značaj.

Ključne reči: stres magnetna rezonanca srca, koronarna bolest, regadenozon

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MAGNETNA REZONANCA U BOLESTIMA DEPONOVANJA

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Magnetna rezonanca srca (MRS) je neinvazivna metoda kojom se bez primene jonizujućeg zračenje može sagledati tkivo miokarda u smislu detekcije edema ili fibroze uz procenu volumena i funkcije obe komore. MRS je zlatni standard u dijagnostici kardiomiopatija (CMP) gde postoji miokardna disfunkcija u odsustvu sekundarnih uzroka kardiovaskularnih oboljenja kao što su koronarna bolest, hipertenzija, bolesti srčanih zalistaka i urođene srčane mane. U najčešće infiltrativne CMP ubrajaju se amiloidoza, hemohromatoza, Anderson-Fabry bolest.

Amiloidoza je bolest kod koje dolazi do ekstracelularnog deponovanja amiloida usled čega se javlja koncentrično zadebljanje zidova leve i desne komore, interatrijalnog septuma, uvećavaju se obe pretkomore, uz moguće perikardne i pleuralne izlive. Na osnovu vrste proteina koji se deponuje u srčanom mišiću postoje amiloidoza lakih lanaca - AL i transtiretinska amiloidoza - ATTR. Tipično deponovanje kontrastnog sredstva gadolinijuma (late gadolinium enhancement - LGE) kod amiloidoze je cirkumferentno subendokardno ili patchy transmuralno. Na osnovu raspodele LGE u miokardu predložen je QALE skor za razlikovanje AL amiloidoze od ATTR. Kod ATTR je obično transmuralno LGE raspoređen i češće je zahvaćena desna komora nego kod AL amiloidoze gde je deponovanje kontrasta cirkumferentno subendokardno. ATTR amiloidoza ima bolju prognozu uprkos većoj masi leve komore i većem nakupljanju LGE.

Anderson-Fabry bolest je genetska bolest koja se prenosi preko X hromozoma, a vezana je za poremećaj metabolizma glikosfingolipida čiji se metaboliti nagomilavaju u endotelijalnim ćelijama dovodeći do ishemije ili infarkta određenog tkiva i organa. Na MRS se obično vidi zadebljanje miokarda sa subepikardnim nagomilavanjem kontrasta u vidu LGE u inferolateralnom zidu.

Hemohromatoza je bolest patološkog nakupljanja gvožđa u organizmu i može zahvatiti jetru i srčani mišić. MRS može da identifikuje i kvantifikuje stepen nagomilanog gvožđa koristeći T2*w sekvencu čije su vrednosti progresivno manje što je stepen gvožđa u tkivu veći. Vrednosti ispod 10 ms ukazuju na teška oštećenja srčanog mišića.

Ključne reči: magnetna rezonanca srca, infiltrativne kardiomiopatije, amiloidoza, hemohromatoza, Fabrijeva bolest

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MAGNETNA REZONANCA U DIJAGNOSTICI UROĐENIH SRČANIH MANA

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Magnetno rezonantni (MR) imidžing urođenih srčanih mana omogućava procenu hemodinamskog efekta anomalije, uključujući detekciju fibroze ili ožiljka, funkcionalnu anatomiju srca, kvantifikaciju parametara funkcije komora, evaluaciju anomalija velikih krvnih sudova i odstupa koronarnih arterija, kao i kvantifikaciju šantova merenjem protoka nad aortom i plućnom arterijom.

Neinvazivnost i odsustvo jonizujućeg zračenja je dovelo do sve veće primene ove metode kao suplementarne imidžing dijagnostike ehokardiografiji kada postoji klinička indikacija za daljom evaluacijom urođene mane, radi incijalne procene ili odluke o daljem kliničkom algoritmu i menadžmentu.

Detekcija manjih šantova je sa druge strane ipak preciznija ehokardiografijom, a procena valvularne patologije transezofagealnom ehokardiografijom. Limiti MR dijagnostike pored dobro poznatih kontraindikacija za magnentno rezonantni imidžing podrazumevaju i neophodnost supervizije i monitoringa same procedure od strane eksperta koji razume kliničko pitanje i može da odabere adekvatan protokol pregleda, poznavanje patološke anatomije i funkcije anomalije, različitih hirurških tehnika lečenja i očekivanog efekta sprovedene korekcije.

Pored inicijalne dijagnostike, procene težine anomalije i njenog hemodinamskog efekta, kako u preoperativnoj evaluaciji, uloga MR imidžinga je u postoperativnoj evaluaciji rezidualne bolesti, hirurških komplikacija i eventualnih intervalnih stečenih oboljenja srca, kao i u praćenju strukturalnih i funkcionalnih promena. Unapređenje dijagnostike i lečenja urođenih srčanih mana je dovelo do redukcije mortaliteta ovih bolesnika, te danas imamo sve veći broj pacijenata sa urođenim srčanim manama i u adultnoj populaciji, koji zahtevaju periodičnu dodatnu imidžing evaluaciju u sklopu kliničkog i ehokardiografskog praćenja, gde je MR idealna metoda izbora.

Ključne reči: magnetno rezonantni imidžing srca, urođene srčane mane, kardiovaskularni imidžing

MINI SIMPOZIJUM

STO GODINA KLINIKE ZA NEUROLOGIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE - EVOLUCIJA NEUROLOŠKE MISLI TOKOM JEDNOG VEKA

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PRISTUP PROGRESIVNOJ MIOKLONIČKOJ EPILEPSIJI: KONTINUITET NEUROLOŠKE MISLI UZ NAPREDAK TEHNOLOGIJE

Dragoslav Sokić

Progresivne miokloničke epilepsije (PME) obuhvataju klinički i genetski heterogenu grupu retkih oboljenja koje odlikuje postojanje spontanih ili stimulusom izazvanih miokloničkih trzaja, drugih tipova epileptičkih napada i progresivnih neurodegenerativnih poremećaja (ataksija, kognitivno oštećenje ili demencija). EEG beleži epileptiformna pražnjenja šiljak-spor ili polišiljak-spor talas kompleksa različite frekvencije, a vremenom se osnovna aktivnost usporava. U početku je neurološki razvoj normalan, koji se usporava sa pojavom epilepsije i mioklonusa.

Većina PME su opisane na prelazu 19. i 20. veka na osnovu karakteristične kliničke slike, i histoloških promena, što ohrabruje da se i danas inicijalna diferencijalna dijagnoza postavi minucioznom kliničkom analizom, što je rađeno decenijama na Klinici za neurologiju. Spojna tačka za raznorodne entitete PME je mioklonus, koji se interponuje u motornu radnju, i remeti korišćenja ruku, hoda ili stajanja. Prepoznavanje vrste PME nije lako zbog genetske raznolikosti, fenotipske sličnosti, a preklapanja sa kliničkom slikom drugih epilepsija i neurodegenerativnih bolesti. Nasleđivanje PME je obično autozomno-recesivno i za najveći broj tipova je utvrđen mutirani gen, kao i patogeneza poremećaja, što otvara mogućnost proizvodnje specifičnog leka za taj poremećaj. Za sada osnov terapije predstavljaju antimiokloničkiantiepileptički lekovi, poput valproata, klonazepama, levetiracetama, brivaracetama, zonisamida, topiramata i perempanela, čije je dejstvo samo delimično.

Najčešće PME su **Unvericht-Lumborgova** bolest (mutacija EPM1 gena; produkt je cistatin B). **Laforina** bolest (mutacija EPM2A i EPM2B gena sa produktima laforin i melin, čija disfunkcija dovodi do nagomilavanja nerastvorljivih partikula glikogena u neuronima, a primena metformina to može da uspori), **neuralna ceroidna lipofuscinoza** (13 različitih genskih tipova, od kojih za kasnu infantilnu formu NCL2 postoji lek Ceriliponaza alfa (Brinecura)), **Gaucherova** bolest (mutacija GBA1; u tipu 2 koja je neurološka forma, enzimska supstituciona terapija nije efikasna). Ređe forme PME su **miokloničk epilepsija sa ragged-red fibers (MERRF)**, **sindrom akcionog mioklonusa sa bubrežnom insuficijencijom**, **PRICKLE-1 PME sa ataksijom**, **PME severnog mora**, i **sijalidoza** tipa 1 i 2.

Ključne reči: progresivne miokloničke epilepsije, mioklonus, ataksija, demencija, EEG, genetska etiologija

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PARKINSONOVA BOLEST NA KLINICI ZA NEUROLOGIJU

Marina Svetel

Od prvog opisa bolesti u Eseju o drhtećoj paralizi koji je 1817 objavio James Parkinson dogodila su se mnoga važna otkrića vezana za ovo degenerativno oboljenje, a Klinika za neurologiju bila je svedok važnih dogadjanja poslednjih 100 godina.

U ovom periodu definisane su u svetu kliničke i patološke karakteristike bolesti, njena anatomija i neurohemija, načinjeni su eksperimentalni modeli bolesti, analizirani sredinski i genetski faktori koji je uslovljavaju. Napravljen je značajan napredak u dijagnostičkoj tehnologiji i terapijskim pristupima bolesti.

Godine 1973. godine Klinika dobija usko specijalizovane neurološke odseke koji su odražavali stručno i naučno interesovanje tadašnjih lekarskih ekipa kada se i formira odsek za sistematska progresivna neurološka oboljenja.

Klinika za neurologiju je pokušala da prati ritam dogadjanja u parkinsonologiji u svetu kroz klinički rad, rad Genetske laboratorije, primenu dopunskih dijagnostički procedura u saradnji sa Centrom za nuklearnu medicinu KC, istraživanja imidžinga, neurofiziološke studije u okviru Laboratorija za eksperimentalnu fiziologiju, epidemiološka istraživanja, primenu fizikalne terapije kao metode poboljšanja stanja bolesnika, praćenje kognitivnih funkcija u Centru za poremećaje pamćenja i primenu hirurških metoda lečenja u saradnji sa Klinikom za neurohirurgiju KC Srbije.

Veliki klinički rad upotpunjen je istraživačkim koji je ostvarivan u saradnji sa epidemiolozima, genetičarima, biohemičarima u zemlji i inostranstvu. U poslednjih 40 godina publikovano je reko 100 publikacija posvećenih Parkinsonovoj bolesti.

Stručna usavršavanja u inostransvu trasirala put za dalju saradnju u ovoj oblasti čiji će značaj postati sve veći

sa starenjem populacije ali i sa činjenicom da se degenerativnim bolestima mozga grupa lekara sa Klinike za neurologiju bavi stručno i posvećeno.

Ključne reči: parkinsonizam, genetske mutacije, neurodegeneracija, neurofiziologija

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EVOLUCIJA PRISTUPA OSOBAMA SA MULTIPLOM SKLEROZOM

Jelena Drulović

Evolucija pristupa osobama sa multiplom sklerozom (MS) je uslovljena stalno dostupnim novim naučnim saznanjima, pre svega iz oblasti epidemiologije i patofiziologije ovog neurološkog oboljenja koje predstavlja vodeći uzrok netraumatske onesposobljenosti kod mladih odraslih osoba.

Porast broja ljudi sa MS je stalan, pa je tako 2023. godine pokazano da od MS boluje 2,9 miliona osoba u svetu. Do nedavno je smatrano da je MS hronično inflamatorno oboljenje centralnog nervnog sistema (CNS), koje nastaje prodorom autoreaktivnih T-limfocita, aktivisanih na periferiji, koji prolaze kroz krvno-moždanu barijeru i reaktivišu se ponovnim susretom sa antigenom. Posledica te reaktivacije je produkcija inflamatornih medijatora, sledstvena inflamacija, demijelinizacija i neurodegeneracija. Danas se, međutim, razmatra mogućnost da je MS "tinjajuće" oboljenje koje karakteriše primarna neurodegeneracija i oštećenje aksona, dok se autoimunski odgovor i inflamacija uključuju naknadno ili istovremeno, difuzno zahvatajući moždano tkivo.

Od 1995. godine do danas je kontinuirano dostupan sve veći broj lekova koji modifikuju prirodni tok bolesti (DMT). Oni različitim mehanizmima smanjuju zapaljenjske promene u CNS i time redukuju broj relapsa i lezija na magnetnoj rezonanciji mozga i kičmene moždine, i blago usporavaju progresiju onesposobljenosti. Međutim, do sada nije dokazano ni za jedan lek da utiče na prevenciju procesa neurodegeneracije i da dovodi do oporavka oštećenog tkiva. Stoga je u cilju očuvanja moždanog tkiva, neophodno se kod svih osoba sa MS, što pre započne sa primenom lekova iz grupe DMT. U tom smislu, potrebno je da se što pre postavi tačna dijagnoza, pa se stoga dijagnostički kriterijumi redovno unapređuju. Dijagnoza se danas postavlja 10 puta brže nego 1980-ih, a brojni dokazi sada pokazuju da je rana primena adekvatnih intervencija (DMT, odgovarajući način života) znatno efikasnija u usporavanju progresije bolesti u odnosu na kasno započinjanje tretmana.

Ključne reči: multipla skleroza, dijagnoza, terapija, epidemiologija, patofiziologija

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EVOLUCIJA U PRISTUPU I LEČENJU BOLESTI MOTONEURONA

Zorica Stević

Amiotrofična lateralna skleroza (ALS), najčešća bolest motoneurona, danas se označava kao fatalni progresivni neurodegenerativni mulfaktorijalni sindrom i multisistemska bolest sa postojanjem, osim motornih, i ekstramotornih promena u frontotemporalnom korteksu, hipokampusu, talamusu, supstanciji nigri, spinocerebelarnom traktu, dorzalnim kolumnama i perifernim nervima. Bazirajući se na primarnim kliničkim manifestacijama kao okosnici dijagnoze, u Srbiji je prvi pacijent sa ALS dijagnostikovan na Klinici za neurologiju početkom dvadesetog veka. Za razliku od dijagnostike, terapija ALS ima dugu istoriju neuspeha. Razlozi za ovo su višefaktorijalni, uključujući i raskorak između efikasnosti primenjenih lekova na životinjskom modelu u odnosu na ALS pacijente, odsustvo stratifikacije pacijenata po fenotipu i genotipu, dužini trajanja bolesti, nedostatak biomarkera progresije bolesti i mnogi drugi. Ipak, u ovom trenutku svedoci smo značajnog napretka u terapijskom pristupu kod ALS pacijenata. Najveći iskorak postignut je napuštanjem koncepcije da se jednom patogenetskom kaskadom mogu objasniti kliničke i patološke karakteristike ove heterogene bolesti. Poslednjih godina u toku su brojne kliničke studije koje se zasnivaju na primeni oligonukleotidne antisens terapije ili na ispitivanju novih supstanci koje su usmerene ka modifikovanju patogenetskih procesa, kao što su oksidativni stres, apoptoza, neuroinflamacija, mitohondrijalna disfunkcija, poremećaj aksonskog transporta, autofagije i dr. Rezultati pozitivnog terapijskog efekta novi lekova u kombinaciji sa simptomatskom terapijom otvaraju novo poglavlje u lečenju ALS kao izuzetno kompleksne i heterogene bolesti.

Ključne reči: amiotrofična lateralna skleroza, dijagnostika, terapija, antisens terapija, oksidativni stres

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NEUROEPIDEMIOLOGIJA OD VON EKONOMA DO KOVIDA-19

Tatjana Pekmezović

Neuroepidemiologija je translaciona nauka zasnovana na korišćenju epidemioloških podataka i metoda u domenu neuroloških bolesti. Epidemiološki podaci imaju za cilj razumevanje uzroka bolesti, kao i procenu posledica i bremena bolesti. Objedinjujući koncept u epidemiologiji podrazumeva korišćenje odgovarajućih analitičkih metoda za razradu heterogenih i složenih informacija kojima se procenjuju odnosi i veze između različitih determinanti neuroloških poremećaja.

Rane neuroepidemiološke studije naglašavale su deskriptivne aspekte neuroloških bolesti, kao što su obrasci incidencije, prevalencije i mortaliteta. Tako je Konstantin von Ekonomo 1917. godine publikovao opis sedam slučajeva nove bolesti koju je nazvao encefalitis letargika i koja je, prema njegovim pretpostavkama bila povezana sa istovremenom epidemijom španskog gripa u Evropi. Sto godina kasnije, bez obzira na napredak medicine u svim njenim oblastima, sposobnost detekcije i lečenja kovid-19 bolesti nije bila dovoljna da se u XXI veku izbegne "višak smrtnosti" od zarazne bolesti. Udruženost kovid-19 bolesti i neuroloških poremećaja tokom pandemije je dobro dokumentovana. Pokazano je da kovid-19 infekcija može da modifikuje klinički spektar manifestovanih neuroloških poremećaja, i da takođe igra ulogu u nastanku budućih neuroloških bolesti kao dugoročnih posledica. Poseban izazov u pandemiji kovid-19 bolesti bilo je lečenje neuroloških pacijenata sa imunosupresivnim stanjima, bilo da su ona izazvana imunološkim poremećajima zbog osnovne bolesti ili lekovima, što je značajno uticalo na promenu imunokompetencije koja modifikuje sposobnost efikasnog imunskog odgovora na infekciju i/ili vakcinaciju.

Neuroepidemiologija naglašava pristup prevenciji neuroloških poremećaja zasnovan na dobrom poznavanju faktora rizika, prirodnog toka bolesti, učestalosti i distribucije u populaciji, kliničkih ishoda i efektivnih terapijskih strategija.

Ključne reči: neuroepidemiologija, KOVID-19, imunski posredovana neurološka oboljenja

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PROGRES U RAZUMEVANJU DEMENCIJA - ZNAČAJ RAZLIČITIH BIOMARKERA IZ KRVI I LIKVORA U SAVREMENOJ DIJAGNOZI

Elka Stefanova

Brzi napredak u određivanju biomarkera iz cerebro-spinalne tečnosti (CST) , kao i nove vizualizacione tehnike sa obeleživa̧ima patoloških dogadjaja, doprineli su razumevanje dinamičkih veza između patofizioloških procesa povezanih sa Alchajmerovom bolešću (AB) u živom ljudskom mozgu. I pored toga, ostalo je nerešeno pitanje za razumevanje patofiziologije u AB: zašto značajan procenat amiloid-b (Ab)-pozitivnih i kognitivno neoštećenih pojedinaca ne razvija uočljivu nizvodnu tau patologiju i posledično, kliničko pogoršanje. U skorije vreme pokazano je da da povećana reaktivnost astrocita, na šta ukazuje povišeni glijalni fibrilarni kiseli (acidofilini) protein GFAP u plazmi, igra ulogu u povezanosti Ab sa ranom tau fosforilacijom u pretkliničkoj AB.

Klasična patofiziološka obeležja AB (beta amiloid Aβ (A), tau (T) koji podrazumeva Fosfo tau (F-Tau) i Totalni tau (T-tau) i neurodegeneracija (N) se mogu određivati u CST; ali se njihovo prisustvo može prikazati i različitim tehnikama snimanja poput Pozitrone emisione tomografije (PET), bilo sa amiloidnim obeleživa̧em ili sa tau- ligandom kao zlatnim standardima amiloidne i tau patologije. Međutim, obe metode su ili invazivne i/ili veoma skupe u isto vreme, tako da su napravljeni veliki napori da se osnovni i specifičniji biomarkeri određuju u krvi kao manje invazivna i dostupnija procedura, ali trenutno ne postoje široko prihvaćeni testovi u krvi za neuroinflamaciju, astrocitnu, mikroglijalnu aktivaciju u AB.

Relativno je lako zamisliti testiranje na Aβ i tau patologiju koristeći odnos Aβ42/Aβ40 u plazmi i F-tau u plazmi kao alate za skrining. Dok je razlika u odnosu Aβ42/Aβ40 u plazmi između Aβ-pozitivnih i negativnih pojedinaca prilično skromna (smanjenje od 14-20%), povećanje koncentracije F-tau u plazmi je oko 3 puta, što daje veoma veoma visoku dijagnostičku tačnost za AB (85-95%), što sugeriše da bi plazmin F -tau mogao da posluži kao skrining test u krvi, uz uključivanje biomarkera reaktivnosti astrocita u modeliranju biomarkera i biološke definicije AB.

Ključne reči: Alchajmerova bolest, beta amiloid, tau protein, neurodegeneracija

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TERAPIJA AKUTNOG ISHEMIJSKOG MOŽDANOG UDARA: KAKO JE STAVLJENA TAČKA NA NIHILIZAM

Dejana R. Jovanović

Pravovremena rekanalizacija okludirane arterije je jedina efikasna terapija za akutni ishemijski moždani udar. Uspešno uvođenje intravenske trombolize u lečenje akutnog ishemijskog moždanog udara pre skoro 3 decenije ukinulo je vekovni nihilizam lečenja koji je pratio ovu tešku bolest. Danas se intravenska tromboliza smatra standardom u terapiji ishemijskog moždanog udara. Međutim, tromboliza je daleko od idealne zbog kratkog terapijskog prozora i značajno ograničenog potencijala rekanalizacije kod okluzije velikih i srednjih cerebralnih krvnih sudova. Značajno veći efekat rekanalizacije sa manje neželjenih efekata postignut je primenom mehaničke trombektomije. Metaanaliza koja je obuhvatila pet randomizovanih kliničkih studija pokazala je statistički značajno bolji oporavak u interventnoj grupi ispitanika nakon 90 dana (mRS 0-2 kod 46% ispitanika u interventnoj grupi naspram 26,5% u kontrolnoj grupi). Ovo je i jedna od najefikasnijih metoda lečenja u medicini uopšte, jer je potrebno lečiti samo 2,6 pacijenta da bi jedan bio bez invaliditeta. Zahvaljujući ovim rezultatima, mehanička trombektomija je sada preporučen metod lečenja pacijenata koji se javljaju u prvih 7 sati nakon pojave akutnog

ishemijskog moždanog udara sa okluzijom terminalnog dela karotidne arterije ili proksimalnog segmenta srednje moždane arterije. Naknadne studije su potvrdile da se reperfuziona terapija može primeniti i u produženom terapijskom prozoru. Tako, se intravenska tromboliza može primeniti i do 9 sati od nastanka simptoma, a mehanička trombektomija može koristiti kod odabranih pacijenata do 24 sata nakon pojave moždanog udara kada moderne neuroimidžing metode potvrde postojanje perfuzionog deficita. Sprovođenje reperfuzione terapije je praćeno je nizom nepoznatih situacija i zahteva značajnu reorganizaciju prehospitalne hitne službe i prijemno bolničkih službi, kako neuroloških tako i neuroradioloških.

Ključne reči: akutni ishemijski moždani udar, terapija, intravenska tromboliza, mehanička trombektomija

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NEUROMIJELITIS OPTICA SPEKTAR BOLEST (NMOSD)

Šarlota Mesaroš

Neuromijelitis optica spektar bolest (NMOSD) je retka autoimunska bolest centralnog nervnog sistema (CNS) koja je izdvojena iz spektra multiple skleroze (MS) 1999.g, ali je jasniju distinkciju u odnosu na MS dobila 2006.g, nakon otkrića, da patogentski proces pokreću autoantitela koja su usmerena na akvaporin-4 (AQP-4), protein vodenih kanala u astrocitima, optičkim nervima, kičmenoj moždini ali i drugim delovima CNS kao što su area postrema, periependimalne strukture oko komornog sistema, strukture diencefalona, pre svega hipotalamus, zatim kortikospinalni trakt i korpus kalozum. U skladu sa ovim lokalizacijama pogođenim autoimunskim procesom, prisutna je i vrlo heterogena klinička slika koja je danas, ipak dobro definisana, naročito od 2015.g kada je objavljeno 6 ključnih kliničkih karakteristika bolesti. Osim toga, treba napomenuti da postoji i deo NMOSD koji je AQP-4 negativan, ali je tada dijagnostički algoritam komplikovaniji i veoma je važno da se poznaju „crvene zastavice" koje su sada dorbo definisane od 2015.g. Za potvrdu dijagnoze je najvećez značaja detekcija antitela na AQP-4 ali je i veoma važno poznavanej neuroradioloških karaktreistika promena na magnetnoj rezonanci mozga i kičmene moždine koje se u potpunosti razlikuju od MS.

Ova retka bolest ima, najčešće relapsno remitentan tok, ali u manjem procentu bolesnika može da ima i monofazni tok. Dovodi do značajnog stepena invaliditeta zbog težeg stepena destrukcije tkiva u CNS (B ćelijski posredovana oštećenje astrocita, antitelom indikovana aktivacija komplementa, depoziti imunoglobulina, značajna infiltracija makrofaga i mikroglije) koji rezultiraju u teškom oštećenju astrocita a sekundarmo i mijelina. Višestruko češće se javlja kod žena nego kod muškaraca, najčešće počinje posle 40 godine bolesti i kod skoro trećine bolesti je udružena sa drugim autoimunskim bolestima.

Poslednjih nekoliko godina je napravljen značajan napredak u terapiji ove bolesti, koja se osim imunosupresivnim lekovima, sada može lečiti i novim pristupom, odnosno monoklonskim antitelima koja su visoko efikasna u prevenciji novih ataka bolesti.

Ključne reči: NMOSD, akvaporin-4, dijagnoza, klinička slika, terapija

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ISTORIJA NEUROLOGIJE KROZ ISTORIJU NEUROLUESA: DA LI JOŠ UVEK UČIMO?

Nikola Vojvodić

Razvoj moderne neurologije u poslednjih 150 godina je bio tesno povezan sa otkrićem i saznanjima o neurosifilisu (*neuroluesu*), naročito krajem 19. i u prvoj polovini 20. veka. Prvi poznati uzrok demencije bila je progresivna paraliza (*dementia paralytica*). Hienrich Erb je opisao spastičnu paraparezu 1892. godine kod bolesnika sa spinalinim sifilisom (tabes dorsalis). Lumbalna punkcija koju je kao dijagnostičku metodu uveo Quincke, prvi put je izvedena kod bolesnika sa neurosifilisom 1891. godine i kasnije se koristila za dijagnozu infekcije čak i kod asimptomatskih osoba. Jedan od pionira neurologije u 19. veku Moritz Heinrich Romberg, opisao je svoj čuveni test kod bolesnika koji je imao tabes dorsalis.

Infektivni uzročnik neurosifilisa (*treponema pallidum*) je otkriven 1913. godine, a prvu efiksanu terapiju je otkrio Julius Wagner-Jauregg 1918. godine kada je uočio da povišena temperatura dovodi do ublažavanja kliničke slike ili čak izlečenja kod osoba sa paralitičkom demencijom. On je posle toga kod obolelih od sifilisa izazivao prolongirana febrilna stanja inokulacijom malaričnog parazita i za tu metodu je 1927. godine dobio Nobelovu nagradu. Jedan od utemeljivača srpske neuropsihoijatrijske škole profesor Vladimir Vujuć je dve godine (1924-1925) proveo na dodatnom usavršavanju u Beču kod profesora Wagnera-Jauregga.

Tokom poslednjih osamdeset godina, dva bitna faktora su uticala na učestalost javljanja i kliničku prezentaciju neurosifilisa, a to su uvođenje penicilina u svakodnevnu praksu i pojava HIV infekcije. Pored smanjenja učestalosti neurosifilisa u neuropsihijatrijskim bolnicama, klasične kliničke prezentacije kao što su meningovaskularni sifilis, progresivna paraliza i tabes dorsalis bile su zamenjene manje tipičnim ispoljavanjima kao što su glavobolje, poremećaji vida, konfuzna stanja i epileptički napadi. Na Klinici za neurologiju je kao posebno retka, ali vrlo dramatična klinička prezentacija neurosifilisa opisan nekonvulzivni epileptični status (ES) uz preporuku da se na ovo oboljenje misli kod bolesnika sa ponavljanim epizodama ES, naročito ukoliko postoji i progresivna kognitivna deterioracija.

Ključne reči: istorija neurologije, neurosifilis, epileptični status

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CEREBELARNE ATAKSIJE – OD VUJIĆEVE PROBE DO SEKVENCIRANJA GENOMA

Nataša Dragašević Mišković

Cerebelarne ataksije predstavljaju grupu neuroloških poremećaja koja se karakteriše velikom fenotipskom i genetskom heterogenošću. U osnovi je cerebelarni sindrom, ali često udružen i sa drugim neurološkim i sistemskim znacima. Poslednjih 30 godina naša saznanja u oblasti stečenih i naslednih ataksija su se značajno promenila što je rezultiralo u boljem razumevanju njihove patofiziološke osnove. Napravljeni su značajni iskoraci u molekularnom i genetskom istraživanju koji su dovela do otkrića više od 40 genskih mutacija koje uzrokuju autozomno dominantne cerebelarne ataksije, i preko 80 genetskih varijanti koje se povezuju sa autozomno recesivnim ataksijama, a koje mogu biti primarne ili predstavljaju kompleksna metaboličko nasledna oboljenja u kojima je ataksija značajan deo kliničke slike. Razvoj genetskog testiranja je išao od korišćenja PCR metode za utvrđivanje prisustva ekspanzija trinukleotidnih ponovaka do metoda sekvenciranja novije generacije koje otkrivaju niz različitih konvencionalnih varijanti mutacija. Ovo genetsko testiranje je omogućilo da se uoči značajna genska pleiotropija u ovim bolestima, koja dovodi do različitog fenotipskog ispoljavanja, a postali smo svedoci i značajnog preklapanja sa drugim grupama neurodegenerativnih bolesti,nhereditarnim spastičnim paraplegijama i hereditarnim neuropatijama što ima značajne dijagnostičke implikacije. Pored naslednih, u degenerativne ataksije spadaju i sporadične ataksije koje su se ranije nazivale idiopatskim tj. nepoznatog uzroka. Poslednjih godina se i u ovoj grupi bolesti otkrivaju genetske varijante, pre svega ekspanzije u intronskom delu gena, pa je utvrđeno da značajan broj osoba sa kasno nastalom atakijom ima mutaciju u genu za subjedinicu replikacionog faktora C (*RFC1*) kao i u genu za fibroblastni faktor rasta (*FGF14*). U oblasti stečenih ataksija, pre svega imunski posredovanih ataksija (paraneoplastičnih i čisto imunski posredovanih) su takođe napravljeni značajni koraci sa otkrićem velikog broja autoimunih antitela što je rezultiralo u rasvetljavanju etiologije do sada potpuno nepoznatih bolesti. Sva ova otkrića imaju sve veće implikacije kako na dijagnostički protokol tako i terapijske strategije koje primenjujemo kod ovih bolesnika.

Ključne reči: cerebelum, ataksija, neurodegeneracija, genetske mutacije

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NEUROGENETIKA U PROTEKLIH 100 GODINA: OD GENA DO GENOMA I DALJE

Ivana Novaković

Genetičke osnove različitih neuroloških poremećaja zapazili su još velikani neurologije XIX i prve polovine XX veka, opisujući obrasce nasleđivanja ili porodičnu sklonost ka oboljevanju kod pacijenata. Svoju punu afirmaciju neurogenetika je stekla u poslednje četiri decenije, koje su obeležene identifikacijom gena odgovornih za veliki broj monogenskih bolesti, proučavanjem faktora podložnosti za multifaktorske bolesti, i postavljanjem osnova za farmakogenetički pristup i personalizovanu terapiju u neurologiji. Nove metode omogućile su integrativni pristup u analizi nasledne osove (gen, genom) i različitih nivoa genske ekspresije. Za bazičnu nauku je značajno otkriće novih i bolje razumevanje poznatih etiopatogenetskih mehanizama, a krajnji efekat u kliničkoj praksi je efikasnija dijagnostika, racionalnije praćenje i lečenje, kao i adekvatno genetičko savetovanje obolelog i njegove porodice. Prateći savremena saznanja i trendove, u genetičkoj laboratoriji Neurološke klinike UKCS, Beograd, već 15 godina rade se dijagnostički testovi za veliki broj neuroloških oboljenja, od dobro poznate Hantingtonove bolesti i Fridrajhove ataksije, pa do vrlo retkih entiteta. Istraživanja realizovana u ovoj laboratoriji osvetlila su spektar genskih mutacija koji postoji u našoj sredini, ukazala na izvesne specifičnosti naše populacije, i dala značajan doprinos savremenoj neurogenetici uopšte. Vreme koje dolazi biće obeleženo integrativnim sagledavanjem rezultata analize gena (genomika) i različitih nivoa genske ekspresije (transkriptom, metabolom, interaktom, epigenetičke modifikacije...), za šta je potrebna vrhunska kompetentnost ne samo iz oblasti različitih biomedicinskih nauka već i bioinformatičke obrade podataka.

Ključ**ne reči:** neurogenetika, geni, genom, mutacije

MINI SIMPOZIJUM

100 GODINA KLINIKE ZA PSIHIJATRIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE

Medicinski fakultet, Univerzitet u Beogradu,
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PROŠLOST I BUDUĆNOST KLINIKE ZA PSIHIJATRIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE

Srđan D. Milovanović, Nikola Lalović, Ana Opanković

Nakon Prvog svetskog rata, 1922. godine osniva se nakon višegodišnje težnje i Medicinski fakultet u Beogradu. Naredne godine se formira i Neuropsihijatrijska klinika Medicinskog fakulteta (MF) u Beogradu. Sedište je bilo u prostorijama Duševne bolnice u Beogradu, koja postaje nastavna baza. Katedra za psihijatriju i neurologiju osnovana je dolaskom profesora dr Laze Stanojevića 10. oktobra 1923. godine, kada je održao i predavanje na novoformiranom fakultetu. Od nastavnika Medicinskog fakulteta Univerziteta u Beogradu, nakon prof Stanojevića poseban doprinos su pružili srpskoj psihijatriji tokom XX veka, prof. dr Vladimir Vujić, akademik prof. dr Jovan Ristić, prof. dr Srboljub Stojiljković, prof. dr Slavka Morić Petrović, prof. dr Predrag Kaličanin, prof. dr Josif Vesel, prof. dr Dimitrije Milovanović, prof. dr Vladimir Paunović.

Neposredno nakon otkrića prvog antipsihotika hlorpromazina, u Francuskoj, u Srbiji se počinje sa njegovom primenom. Tokom narednog vremena se prate i uspešno usvajaju znanja iz domena psihofarmakoterapije a formira se i beogradska psihofarmakološka škola. Uporedo sa razvojem kliničke psihijatrije i biološki orijentisanih terapija razvija se i psihoanaliza i dinamička psihijatrija. Godine 1938. osniva se Beogradsko psihoanalitičko društvo. Prvo odeljenje na kome su se lečili bolesnici od neurotskih poremećaja i gde se primenjivala psihoanaliza osnovano je 1952. godine u okviru tadašnje Neuropsihijatrijske klinike, inicijator i rukovodilac je bio dr Mirko Švrakić.

U ovoj godini jubileja 100 godina od osnivanja, Klinika za psihijatriju je postavila kao jedan od prioriteta nabavku opreme i obuku kadrova za primenu neuromodulacione terapije ponavljajuće magnetne stimulacije (rTMS). Osim toga, u saradanji sa Institutom za medicinska istraživanja, Univerziteta u Beogradu, tim istraživača Klinike za psihijatriju UKC Srbije sprovešće kliničko istraživanje pod nazivom „Praćenje terapijskih efekata neinvazivne neuromodulacije na kognitivne i afektivne funkcije kod osoba sa psihijatrijskim poremećajima“. Od svog osnivanja do danas, a verujemo i u vremenu koje sledi, Klinika za psihijatriju Univerzitetskog kliničkog centra Srbije ostaje nacionalni predvodnik u domenu psihijatrijskih terapija i edukacije.

Ključne reči: psihijatrija, klinika, razvoj, fakultet

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ANKSIOZNI POREMEĆAJI I SOMATSKE BOLESTI – TEORIJSKI I TERAPIJSKI KONCEPTI I DOSTIGNUĆA

Milan Latas

Anksiozni poremećaji su jedna od najčešćih grupa mentalnih poremećaja u odrasloj populaciji, sa globalnom prevalencom od 7,3%. Oni se javljaju zajedno sa drugim mentalnim poremećajima kod većine pacijenata ali se javljaju udruženo i sa medicinskim bolestima. Cilj ovog pregleda je da predstavi nedavne nalaze o različitim aspektima odnosa između anksioznih poremećaja i somatskih bolesti. Rad se fokusira na prevalenci, kliničkoj relevantnosti i implikacije na lečenje koegzistirajućih anksioznih poremećaja i različitih medicinskih bolesti.

Mnogi raniji radovi ukazuju na visoku stopu simptoma anksioznosti i poremećaja anksioznosti kod pacijenata sa različitim medicinskim oboljenjima i obrnuto – visoku stopu medicinskih bolesti u uzorcima pacijenata sa anksioznim poremećajima i veću težinu i anksioznih poremećaja i medicinskih bolesti, ako postoje istovremeno kod istog pacijenta.

Stope prevalencije anksioznih poremećaja kod pacijenata sa medicinskim oboljenjima su visoke, sa procentom do 29% kod pacijenata sa epilepsijom, 48,9% kod pacijenata sa multiplom sklerozom, 30,1% kod pacijenata sa Parkinsonovom bolešću, 30% kod pacijenata sa kardiovaskularnim oboljenjima, 47,0% kod pacijenata sa šećernom bolešću i dr. Najčešći anksiozni poremećaji kod osoba sa somatskim oboljenjima su generalizovani anksiozni poremećaj i panični poremećaj. Pored toga, različite medicinske bolesti su veoma rasprostranjene u uzorcima pacijenata sa anksioznim poremećajimaa. Komorbiditet anksioznih poremećaja sa medicinskim bolestima ima brojne kliničke implikacije, uključujući veću težinu i negativan uticaj na ishod lečenja i medicinskih bolesti i anksioznih poremećaja.

Za kliničare je važno da ispitaju moguće postojanje anksioznih poremećaja kod pacijenata sa medicinskim bolestima. Dalja istraživanja moraju da utvrde kako naj-

bolje lečiti pojedince koji pate i od AD i od medicinskih bolesti i da se usredsrede na pitanje uzročnosti kada se ova stanja istovremeno javljaju.

Ključne reči: anksiozni poremećaji, somatske bolesti, generalizovani anksiozni poremećaj i panični poremećaj

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INOVATIVNI PRAVCI U PSIHOFARMAKOTERAPIJI

Maja Ivković

Poslednje decenije donele su intenzivni razvoj neuronauka kao i napredniji uvid u procese u centralnom nervnom sistemu. Napredak u ovom polju je rezultat razvoja tehnologija iz oblasti neurovizuelizacije, epigenomike, kliničke genetike i imunologije. Primena sofisticiranih metodologija zasnovana na biološkim mehanizmima dovela je do porasta broja potencijalnih meta za dejstvo lekova koji poboljšali tretman poremećaja, uključujući i mentalne bolesti. Međutim, psihijatrija nije do sada uspela da efikasno iskoristi napredak neuronauka. Jedan od razloga za to je nejasna povezanost mnogih novotkrivenih „meta" i kliničke ekspresije mentalnih poremećaja. Shodno tome, broj odobrenih novih lekova u oblasti psihijatrije poslednje decenije je u značajnom zaostatku u poređenju sa drugim medicinskim disciplinama.

Razlozi za složenost razvoja psihijatrijskih lekova su među ostalima farmakološka restrikcija vezana za krvno-moždanu barijeru, razlika u definisanju bioloških procesa u animalnim i humanim modelima, klinička heterogenost psihijatrijskih poremećaja, kao klasifikacije zasnovane na fenomenima čiji su etiopatogenetski mehanizmi uglavnom nepoznati.

Neki od načina za brži razvoj inovativnih psihofarmaka su primena takozvane precizne psihijatrije, zasnovane na biološkim fenotipovima odnosno biotipovima, utvrđivanje biomarkera za psihijatrijske poremećaja, primena digitalne tehnologije, kao i intenzivnije učešće samih pacijenata.

Ključne reči: neuronauka, mentalne bolesti, psihijatrijski lekovi, klasifikacija

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POREMEĆAJI SPECIFIČNO POVEZANI SA STRESOM: PROMENE U MKB 11

Zorana Pavlović, Sreten Vićentić, Milena Stevanović

Poremećaji specifično povezani sa stresom su grupa oboljenja čija je pojava direktno povezana sa izlaganjem jednom ili više stresogenih događaja. Prema dosadašnjoj Međunarodnoj klasifikaciji bolesti - 10 revizija (MKB-10) ova oboljenja su svrstana u grupu Reakcija na težak stres i poremećaji prilagođavanja. Naredna klasifikacija MKB-11 uvodi određene promene u vidu novih dijagnostičkih kategorija i rekonceptualizacije postojećih poremećaja. Akutna stresna reakcija je reklasifikovana u poglavlje Faktori koji utiču na zdravstveni status i kontakt sa službama.

Poremećaj produženog tugovanja je nova dijagnostička kategorija, dok je kategorija Trajne promene ličnosti posle katastrofičnog iskustva zamenjena novim poremećajem pod nazivom Kompleksni posttraumatski stresni poremećaj. Izvršene su i rekonceptualizacije dijagnostičkih kriterijuma za Posttraumatski stresni poremećaj i Poremećaj prilagođavanja. U kategorije posttraumatskog stresnog poremećaja, kompleksnog posttraumatskog stresnog poremećaja, poremećaja produženog tugovanja i poremećaja prilagođavanja uključene su sve starosne grupe. Reaktivni poremećaj vezivanja u detinjstvu i Dezinhibisani poremećaj vezivanja izmešteni su iz klastera Poremećaji ponašanja i poremećaji emocija sa početkom u detinjstvu i adolescenciji i svrstani u Poremećaje specifično povezane sa stresom, čime se naglašava relevantnost stresa u etiologiji poremećaja.

Tako, MKB – 11 obuhvata sledeće kategorije: Posttraumatski stresni poremećaj, Kompleksni posttraumatski stresni poremećaj, Poremećaj produženog tugovanja, Poremećaj prilagođavanja, Reaktivni poremećaj vezivanja u detinjstvu, Dezinhibisani poremećaj vezivanja, Drugi specifikovani poremećaji specifično povezani sa stresom, Poremećaji specifično povezani sa stresom, nespecifikovani.

Ključne reči: stres, poremećaji, klasifikacija, MKB -11

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PSIHIJATRIJSKI POREMEĆAJI KOD RETKIH BOLESTI KRVNIH SUDOVA MOZGA

Maja Lačković

CADASIL (cerebralna autozomno dominantna arteriopatija sa subkortikalnim infarktima i leukoencefalopatijom) je retko, nasledno cerebrovaskularno oboljenje, prouzrokovano mutacijom Notch3 gena, koje se klinički karakteriše pojavom migrena, rekurentnih tranzitornih ishemijskih ataka (TIA) ili lakunarnih infarkta, poremećajima raspoloženja i padom kognitivnih funkcija. MELAS (mitohondrijalna encefalopatija sa laktatnom acidozom i epizodama sličnim moždanim udarima) je retko, nasledno neurodegenerativno oboljenje prouzrokovano disfunkcijom mitohondrija i gubitkom ATP. Psihijatrijski poremećaji kod ovih oboljenja do sada nisu sistematski ispitivani. Ovaj rad ima za cilj da ukaže na važnost dijagnostikovanja psihijatrijskih manifestacija ovih retkih bolesti u kliničkoj praksi. U istraživanja psihijatrijskih poremećaja uključeno je 25 bolesnika sa definitivnim di-

jagnozama CADASIL-a (15) i MELAS-a (9) uspostavljenim elektronskom mikroskopijom kože (nalaz GOM-a oko glatkih mišićnih ćelija arteriola), odnosno skeletnog mišića (nalaz subsarkolemalne akumulacije abnormalnih mitohondrija). Rezultati na Hamiltonovoj skali za procenu depresivnosti pokazali su da je različite stepene depresivnosti kod CADASIL-a pokazivalo 11 bolesnika (73,3%), a kod MELAS-a 3 bolesnika (42,8%). Rezultati na Hamiltonovoj skali za procenu anksioznost pokazali su da je 12 (80%) pacijenata sa CADASIL-om, odnosno 6 (85,8%) sa MELAS-om imalo različite stepene anksioznosti. Ispitivanja kognitivnih funkcija pomoću mini mental testa (MMT) su pokazala da je kognitivni pad postojao kod 6 bolesnika (37,5%) sa CADASIL-om i kod 3 pacijenta (33,3%) sa MELAS-om. Na osnovu rezultata ove studije može se zaključiti, kao prvo, da je pri dijagnostikovanju CADASIL-a ili MELAS-a važno obratiti pažnju na psihijatrijske poremećaje pošto njihov adekvatan tretman može unaprediti kvalitet života pacijenata, i kao drugo, da psihijatrijski simptomi mogu predstavljati početne kliničke manifestacije CADASIL-a ili MELAS-a, te kao takvi mogu biti od značaja da se dijagnoza ovih bolesti ne propusti, što može imati reperkusije na njihovu prognozu i terapiju.

Ključne reči: CADASIL, cerebrovaskularno oboljenje, migrena, poremećaj raspoloženja i pad kognitivnih funkcija.

MINI SIMPOZIJUM

LAJMSKA BOLEST: NOVA SAZNANJA I AKTUELNE DILEME

Gradski zavod za javno zdravlje Beograd

EPIDEMIOLOŠKI PODACI O LAJMSKOJ BOLESTI - STALNI IZAZOV

Nevenka Pavlović

Lajmska bolest je zoonoza bakterijske etiologije, čiji je uzročnik spiroheta *Borrelia burgdorferi* sensu lato. Infekcija se na čoveka prenosi ubodom krpelja roda *Ixodes*. U Evropi i u našoj zemlji, osnovni vektor su krpelji vrste *Ixodes ricinus*. Najrasprostranjenija je i najčešće registrovana vektorski prenosiva infekcija na evropskom kontinentu. Rizik za obolevanje ljudi postoji na svim područjima na kojima se nalaze krpelji zaraženi *B. burgdorferi*. On je povezan sa prostornom distribucijom i brojnošću krpelja vrste *Ixodes ricinus*, njihovom stopom inficiranosti uzročnikom lajmske bolesti i ponašanjem ljudi koje doprinosi izloženosti ubodu krpelja. Publikovani podaci o učestalosti obolevanja ukazuju na neujednačenost i značajne razlike između regiona i zemalja Evrope, kao i između manjih ili većih područja unutar iste zemlje. Međutim, ovi podaci ne omogućavaju poređenje među zemljama, pa samim tim ni sagledavanje ukupne slike o epidemiologiji lajmske bolesti u Evropi iz nekoliko razloga. Prisutna je heterogenost u odnosu na sisteme nadzora, obavezu prijavljivanja bolesti, definiciju slučaja i metode testiranja. Dodatni izazov su nedoslednosti u izveštavanju utvrđenih slučajeva, podregistracija i preterano dijagnostikovanje kod slučajeva koji nisu lajmska bolest.

Obzirom na višestruki značaj tačnosti epidemioloških podataka, kako za potrebe planiranja i preventivne prakse tako i za dalju obradu u okviru naučnih istraživanja, pokrenute su aktivnosti za prevazilaženje navedenih nedostataka. One su usmerene na sprovođenje standardizovanog sistema nadzora nad lajmskom bolesti, uspostavljanje jedinstvene definicije slučaja i sistema izveštavanja. Predviđeno je da se nadzor sprovodi nad promenama nastalim usled zahvatanja nervnog sistema - lajmskom neuroborelioza (Evropska komisija i Evropski centar za kontrolu i prevenciju bolesti). Istovremeno, aktuelna saznanja iz domena eko-epidemiologije lajmske bolesti ukazuju na potrebu daljih istraživanja uzročnika i vektora, i razvijanje modela za procenu rizika od infekcije *B. burgdorferi* na našem geografskom području.

Ključne reči: lajmska bolest, epidemiološki podaci, nadzor, procena rizika

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UZROČNICI LAJMSKE BORELIOZE U KRPELJIMA I REZERVOARIMA U SRBIJI

Snežana Tomanović

Uzročnici lajmske borelioze, spirohete iz kompleksa *Borrelia burgdorferi* sensu lato, u prirodi se održavaju kruženjem između krpelja kao vektora i različitih vrsta kičmenjaka kao rezervoara. Poznavanje prisustva i geografske distribucije patogena, kako na specijskom tako i na subspecijskom nivou je od izuzetnog značaja za razumevanje ekologije i epidemiologije bolesti, adekvatnu procenu rizika, uspostavljanje mera prevencije, kao i efikasnu dijagnostiku i terapiju. U Evropi, geografska distribucija vrsta iz kompleksa *B. burgdorferi* s.l., pokazuje značajnu vremensku i prostornu dinamiku. Kao dominantna vektorska vrsta javlja se krpelj *Ixodes ricinus* dok se po pitanju kičmenjačkih rezervoara uočavaju različite preferencije. Diverzitet i prevalencija vrsta borelija u krpeljima najbolje oslikavaju kompleksnost fakune rezervoara u određenom regionu. U Srbiji, *B. burgdorferi* s.l. je prvi put detektovana 1993. u okolini Beograda u krpelju *I. ricinus*, a iste godine je i izolovana iz miševa vrste *Apodemus flavicolis*. Od tada, *B. burgdorferi* s.l. se u krpeljima detektuje sa prevalencijom od 10,8% do 42,5%, u zavisnosti od regiona, sezone i metode detekcije. Studije sprovedene do 2008. navode prisustvo dve vrste borelija (*B. burgdorferi* sensu stricto i *B. afzelii*), dok je danas poznato da u prirodi na ovim prostorima cirkulišu i *B. garinii*, *B. valaisiana*, *B. lusitaniae* i *B bavariensis*. Novije studije su pokazale znatan diverzitet na specijskom i subspecijskom nivou, neočekivanu dominaciju vrste *B. lusitaniae*, kao i znatno prisustvo koinfekcija različitih vrsta borelija u krpeljima. Borelije su detektovane sa različitom prevalencijom i diverzitetom i kod kičmenjačkih domaćina – 5,4% kod lisica (*B. burgdorferi* s.s., *B. garinii* i *B. lusitaniae*); 8,1% do 26,1% kod pasa; 1,9% kod sitnih glodara (*B. afzelii*). U krpeljima prikupljenim od zlatnih šakala, identifikovana je vrsta *B. garinii*.

Ključne reči: lajmska borelioza, *Ixodes ricinus, Borrelia burgdorferi*

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LABORATORIJSKA DIJAGNOSTIKA LAJMSKE BORELIOZE

Ivana Kelić

Lajmska borelioza (LB) je najčešće oboljenje koje prenose krpelji na severnoj hemisferi. To je multisistemsko

oboljenje koje zahvata kožu, nervni sistem, zglobove i srce. Simptomi i tok bolesti variraju među pacijentima, **što delimično zavisi od vrste genospecijesa koji je izazvao infekciju. D**ijagnostika LB podrazumeva primenu indirektnih serooloških metoda (ELISA i Line blot testovi), tzv. dvostepeno testiranje. Direktne metode, kao što su kultivacija *B.burgdorferi sesu lato* iz uzorka bioptata kože i PCR metoda iz krvi, cerebrospinalne i sinovijalne tečnosti se ne koriste u rutinskom radu, zbog niske senzitivnosti metoda.

Lajmska borelioza je diferencijalna dijagnoza velikog broja oboljenja, za koja se rutinski izvode skrining testovi na prisustvo antitela na *B.burgdorferi sesu lato.* Stoga, česta lažna pozitivnost serooloških testova u antitelima IgM klase nije iznenađenje, ali može dovesti do grešaka u tumačenju rezultata. Lažno pozitivni nalazi se javljaju iz mnogobrojnih razloga, od poliklonskog imunskog odgovora kod infektivne mononukleoze i herpes virusnih infekcija do autoimunskih i reumatoloških oboljenja. U mnogim slučajevima poreklo antitela IgM klase ostaje nepoznato.

Laboratorijska dijagnostika neuroborelioze podrazumeva određivanje vrednosti indeksa intratekalne sinteze antitela, citobiohemjski nalaz i izvođenje potvrdnog Line blot testa. Nedavna istarživanja ukazuju da prisustvo biomarkera hemokin -13 (CXCL-13) u cerebrospinalnoj tečnosti govori u prilog rane neuroborelioze.

Zbog velike antigenske raznovrsnosti *B.burgdorferi sensu lato* do danas nije napravljen test koji bi zamenio tzv. dvostepeno testiranje i na taj način učinio ovu dijagnostiku jednostavnijom i jeftinijom.

Iz navedenih razloga se zaključuje da je u dijagnostici lajmske bolesti najvažnija klinička slika pacijenta, dok su laboratorijski nalazi samo pomoćno sredstvo i na njih se ne možemo oslanjati u potpunosti.

Ključne reči: lajmska borelioza, neuroborelioza, indeks intrateklane sinteze antitela

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KLINIČKI ASPEKTI LAJMSKE BOLESTI

Jasmina Poluga

Infektivne bolesti prenosive krpeljima, uključujući i lajmsku bolest/lajmsku boreliozu (LB) postaju sve veći problem u Evropi. LB je multisistemska bakterijska infekcija koju izaziva *Borrelia burgdorferi* i prenosi se na ljude ubodom zaraženog krpelja genus *Ixodes*. LB se naziva velikim imitatorom, jer njeni simptomi podsećaju na oko 350 različitih oboljenja, te je samim tim diferencijalna dijagnoza kompleksna. Lajmska bolest se odvija u tri stadijuma: 1. rana lokalizovana bolest, 2. rana diseminovana bolest, i 3. kasna diseminovana bolest (pozna-hronična infekcija). U prvom stadijumu javlja se karakteristična promena na koži-*Erythema migrans* koja predstavlja jedini tipičan klinički nalaz u toku lajmske bolesti. U prvom stadijumu mogu biti prisutne i tzv. konstitucionalne tegobe: malaksalost, povišena temperatura, glavobolja, ukočenost vrata i uvećanje regionalnih lgl. Drugi stadijum lajmske bolesti se karakteriše neurološkim (serozni meningitis, pareza n. facialisa, radikulitis), reumatološkim (akutni artritis), kardiološkim (poremećaji srčanog ritma, mioperikarditis) i dermatološkim (benigni limfocitom) manifestacijama. U trećem stadijumu se javljaju hronični artritis, neuritis, encefalopatija, encefalomijelitis i karakteristična kožna promena (Acrodermatitis chronica atrophicans). Lajmska neuroborelioza je pojam koji obuhvata sve promene koje se odnose na zahvatanje centralnog i perifernog nervnog sistema i daje potencijalno najteže posledice.

Prošlo je više od 40 godina od otkrića uzročnika LB, i mnogo toga o ovom oboljenju je poznato. Međutim, iako su dijagnoza i lečenje rane LB generalno jasne, pozna faza predstavlja klinički izazov, sa posebnim akcentom na hroničnu LB, termin koji je nedovoljno definisan. Načelno se smatra da je dijagnoza LB "precenjena" što rezultira čestim i nepotrebnim testiranjem, ponekad pogrešnim tumačenjem dobijenih rezultata, što dovodi do prekomernog i nesvrsishodnog tretmana. S druge strane, ne sme se zanemariti činjenica da se radi o hroničnoj bolesti koja znatno narušava kvalitet života. S obzirom na navedeno, potrebna je kontinuirana medicinska edukacija, koja bi omogućila podizanje svesti o postojanju LB, ali i uticala na smanjenje pogrešnih dijagnoza i neadekvatnih tretmana.

Ključne reči: lajmska bolest, Erythema migrans, neuroborelioza

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MODERNI TERAPIJSKI PRISTUPI LAJMSKOJ BOLESTI

Uroš Karić

Najčeća krpeljska vektorska bolest na severno od ekvatora je lajmska bolest. U Evropi se godišnje detektuje nešto manje od 20.000 novih slučajeva lajmske bolesti. Kako bolest nije podložna obaveznom prijavljivanju u svim evropskim zemljama, a i deo inficiranih pacijenata ostaje nedijagnostikovan, pretpostavlja se da je stvarna incidenca lajmske bolesti značajno veća. Na osnovu novih saznanja Američko društvo za infektivne bolesti (eng. Infectious Disease Society of America – IDSA) u saradnji sa Američkom neurološkom akademijom (eng. American Academy of Neurology – AAN) i Američkim reumatološkim koledžom (eng. American College of Rheumatology – ACR) je 2020. godinu objavilo je nove smernice za dijagnostiku, lečenje i prevenciju lajmske bolesti. Doksiciklin, cefurokism, azitromicin, amoksicilin, ceftriakson, cefotaksim i penicilin G su preporučeni antibiotici za lečenje lajmske bolesti. Najvažnije novine u terapiji lajmske

bolesti su skraćenje dužine lečenja lokalizovane forme bolesti, te se preporučuje da se doksiciklin primenjuje 10 dana naspram ranije savetovanih 14 do 21 dan. Takođe, eksperti savetuju primenu azitromicina 5 do 10 dana kao alternativnu terapijsku meru za lokalizovanu lajmsku bolest. Prema aktuelnim saznanjima, najveći broj kliničkih entiteta vezanih za lajmsku bolest (karditis, artritis, neuroborelioza) može se zbrinuti peroralnom terapijom ako bolest nije toliko teška da zahteva hospitalizaciju. Retretman antibioticima se savetuje samo u slučaju lajm artritisa.

Ključne reči: lajmska bolest; terapija, doksiciklin

MINI SIMPOZIJUM

ŠTA, KADA I KAKO – PROCES DONOŠENJA ODLUKE U URGENTNOJ HIRURGIJI, KONTROVERZE I STREMLJENJA

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Centar za Radiologiju, Univerzitetski klinički centar Srbije, Beograd

ABDOMINALNI KOMPARTMENT SINDROM-DILEME I IZAZOVI

Krstina Doklestić Vasiljev, Zlatibor Lončar, Pavle Gregorić, Vladimir Resanović, Dušan Micić, Miljan Ćeranić, Nenad Ivančević, Dragan Vasin

Sindrom abdominalnog kompartmenta (SAK) je dugotrajni intraabdominalni pritisk (IAP) preko 20 mmHg, sa ili bez abdominalnog perfuzionog pritiska ispod 60 mmHg, koji je povezan sa disfunkcijom ili otkazivanjem ostalih organa. Sindrom abdominalnog kompartmenta najčešće je posledica prekomerne reanimacije tečnostima (>5 L za 24 sata) ili masivne transfuzije krvi (>10 jedinica za 24 sata) kod kritično obolelih, septičnih hirurških pacijenta i teško povređenih. Klinički znaci su nespecifični i javljaju se kasno. Klasični nalazi su posledica povećanja abdominalnog intraperitonealnog pritiska sa otokom crevne mukoze, poremećajem enteralne barijere, reperkusijom na vaskularizaciju i hipoperfuzijom organa abdomena, uticajem na sistem vene kave, rad bubrega, pluća, razmenu gasova na nivou disajnih puteva, smanjenim izlučivanjem urina i enormnom distenzijom trbušnoh zida. Dijagnoza zavisi od proaktivnog merenja IAP kod pacijenata sa faktorima rizika.

Značajna prognostička vrednost povišenog intraabdominalnog pritiska podstakla je jedinice intenzivne nege (JIN) da usvoje protokole merenje ovog fiziološkog parametra kao rutinskog vitalnog znaka kod pacijenata sa rizikom. Temeljno razumevanje patofizioloških implikacija povišenog intraabdominalnog pritiska je od suštinskog značaja za prepoznavanje prisustva intraabdominalne hipertenzije i sindroma abdominalnog kompartmenta, efikasno lečenje pacijenata obolelih od ovih potencijalno po život opasnih komplikacija, kao i prevenciju SAK i posledični razvoj multiorganske disfunkcije.

Terapijske opcije uključuju evakuaciju intraluminalnog sadržaja, optimizaciju terapije tečnostima, pravilno pozicioniranje tela, adekvatnu analgeziju i neuromuskularnu blokadu. Definitivni tretman je hirurška dekompresija abdomena koja zahteva specifične hirurške tehnicke i principe *Open abdomen* pristupa. Abdominalni kompartment je teška i poživot opasna komplikacija osnovne bolesti, koja je fatalna bez lečenja. I pored adekvatne terapije, smrtnost je visoka.

Ključne reči: abdominalni kompartment sindrom hirurška dekompresija

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AKUTNI PANKREATITIS – DIJAGNOSTIČKI I TERAPIJSKI IZAZOVI

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Akutni pankreatitis (AP) je često oboljenje i za lečenje predstavlja jedno od najkomplikovanijih bolesti. Na osnovu lokalnih i sistemskih komplikacija akutni pankreatitis varira od blagog samolimitirajućeg inflamatornog procesa do veoma teškog oblika sa razvojem MODS-a i visokim mortalitetom.

Postavljanje dijagnoze akutnog pankreatitisa podrazumeva prisustvo najmanje dva od tri dijagnostička kriterijuma – karakterističan abdominalni bol, povišene vrednosti serumskih alfa amilaza ili lipaza i radiološki znaci pankreatitisa. Povišene vrednosti serumskih lipaza omogućavaju širi dijagnostički prozor od alfa amilaza. Međutim one se ne mogu koristiti kao prognostički parametri u ovom oboljenju.

Radiološke vizualizacione tehnike (EHO, CT i MRi) imaju sve veću i značajniju ulogu u dijagnozi i praćenju lokalnih, peripankreatičnih komplikacija i proceni ozbiljnosti akutnog pankreatitisa. Lokalne ili sistemske komplikacije su glavni faktori za određivanje težine AP koja se rutinski prati uz pomoć CT abdomena i zapaljenskih parametara (Le, CRP i prokalcitonin).

Inicijalno lečenje AP podrazumeva konzervativnu terapiju u slučaju težih oblika to obuhvata agresivnu potporu oštećenih organskih sistema, stalni monitoring i ciljanu AB terapiju u slučaju dokazane inficirane nekroze. Međutim ukoliko se konzervativno lečenje iscrpi indikovana je hirurška intervencija. Najveći izazovi u lečenju AP su upravo povezani za pravim odabirom bolesnika, blagovremenu i adekvatnu intervencija. Rana hirurška intervencija, u okviru prvih 14 dana od početka tegoba, se ne preporučuje osim ako ne postoje specifične indikacije odnosno razvoj akutnog abdomena (peritonitis, intraabdominalno krvarenje i ileus). Ukoliko je indikovana hirurška intervencija usled nekrotičnog pankreatitisa idealno je odložiti operaciju najmanje 4-6 nedelja od po-

četka bolesti kako bi se omogućilo da nekrotični proces postane ograničen.

Trenutno ne postoji idealan biohemijski test koji omogućava postavljanje dijagnoze, identifikaciju etiologije i ranu procenu ozbiljnosti akutnog pankreatitisa. Imajući to u vidu, klinički parametri i radiološke metode zadržavaju značajno mesto u evaluaciji obolelih. Fleksibilni pristup prilagođen svakom bolesniku pojedinačno, trebalo bi da bude zlatni standard u lečenju ove izuzetno teške i opasne bolesti…

Ključne reči: akutni pankreatitis, alfa amilaze, lipase

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INTRAABDOMINALNE INFEKCIJE

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Termin „intraabdominalne infekcije" predstavlja široku raznovrsnost kliničkih stanja koje zahvataju trbušnu duplju. Intraabdominalne infekcije (IAI) su važan uzrok morbiditeta i mortaliteta. Rana klinička dijagnoza, adekvatna kontrola izvora infekcije, odgovarajuća antibiotska AB terapija i brza reanimacija kod kritično obolelih su kamen temeljac u terapiji IAI.

Rana klinička evaluacija je od suštinskog značaja za dijagnozu IAI. Pomaže optimizaciji dijagnostičkog testiranja i može dovesti do ranije primene odgovarajuće terapije.

Jedan od najvažnijih modaliteta u lečenju komplikovanih IAI je hirurška kontrola izvora infekcije. Kod većine pacijenata sa IAI i sepsom/septičkim šokom je indikovana hitna hirurška intervencija u cilju kontrole izvora infekcije. Uzimanja mikrobiološkog uzorka se preporučuje uvek kod kritično obolelih i pacijenata sa nozokomijalnom i/ili komplikovanom IAI. Takođe, mikrobiološko uzorkovanje treba obaviti pri svakoj reintervenciji. Mikrobiološko ispitivanje podrazumeva Gram bojenje, aerobna i anaerobna kultura, kao i testiranja osetljivosti izolovanih mikroba na AB.

Doza i pravilna primena AB treba da obuhvataju: 1) udarnu dozu kada je to indikovano, posebno kod kritično obolelih; 2) produženo intravensko davanje beta-laktamskih AB; 3) peritonealna distribucija. Optimalno davanje AB se zasniva na izvoru infekcije i time da li je došlo do komplikovanog oblika. Na primer u uslovima nekomplikovanog akutnog holecistitisa i akutnog apendicitisa, postoperativna antimikrobna terapija nije neophodna odnosno potrebno je dati samo AB profilaksu. Međutim, kod pacijenata sa komplikovanom IAI koji nisu životno ugroženi i samo kada je adekvatna kontrola izvora infekcije uspostavljena predlaže se kratkotrajno davanje (3-5 dana) postoperativne AB terapije. Naravno, kod pacijenata koji su životno ugroženo AB terapija treba nastaviti do njihovog oporavka i procene kliničara na osnovu njegovog iskustva i drugih dijagnostičkih i laboratorijskih parametara.

Ključno u lečenju teško/kritično obolelih pacijenata u jedinici intenzivnog lečenja u cilju izbagavanja smrtnog ishoda je adekvatna kontrola izvora infekcije i odgovarajuća AB zaštita. Insuficijencija organa sa razvojem sepse je povezana sa povećanim rizikom od smrtnog ishoda.

Ključne reči: intraabdominalne infekcije, sepsa

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DIJAGNOSTIČKI ALGORITAM I ODLUKA O TERAPIJSKOM PRISTUPU U AKUTNOM HOLECISTITISU

Zlatibor Lončar, Dušan Micić, Krstina Doklestić Vasiljev, Miljan Ćeranić, Vladimir Resanović, Nenad Ivančević, Pavle Gregorić, Dragan Vasin

Kalkulozna žučna kesa je veliki zdravstevni problem širom sveta, posebno kod odraslih osoba. Akutni holecistitis zauzima drugo mesto po frekvenci i čini 10-15% svih akutnih hirurških oboljenja, a nakon 50 godina starosti izjednačen je po učestalosti kod žena i muškaraca. Brojni uzroci doprinose razvoju žučnih kamenčića kao što su gojaznost, hiperkalorična ishrana, dijabetes, ciroza jetre, hemolitička bolest, fizička neaktivnost, višestruke trudnoće I dugotrajno lečenje polnim hormonima.

Laparoskopska holecistektomija je postala operacija izbora za simptomatsku holelitijazu. Laparoskopski pristup je i dalje ostao kontraverzan za pacijente sa akutnim holecistitisom, zbog tehničkih problema, koji u poređenju sa otvorenom procedurom, mogu da dovedu do povećanja komplikacija, pre svega do povreda glavnog žučnog voda i infekcija.

Rani laparoskopski pristup je tehnički izvodljiv i siguran kao i otvoreni pristup u operaciji žučne kese. Kod opsežnih upalnih procesa, prisutnih adhezija i perforacija može doći od otežane i teške disekcije Calot-ovog trougla. Konverzija u otvorenu proceduru predstvalja i dalje opciju izbora kako bi se obezbedila sigurnost pacijenta i izbegle moguće komplikacije.

Laparoskopska holecistektomija kod akutnog holecistitisa smanjuje postoperatvni bol, mogućnost infekcija rane, dužinu bolničkog ležanja, morbiditet i mortalitet. Povratak na uobičajene radne aktivnosti je nakon 7-10 dana. Urgentna laparoskopska holecistektomija ima nizak stepen komplikacija, ali postoji mogućnost konverzije u otvorenu proceduru kada se povećava morbiditet i moratlitet u zavisnosti od težine upalnog procesa.

Po međunarodnoj kalsifikaciji donešenoj u tokiju 2013 i revidiranoj 2017 godine u smislu težinebolesti akutni holecistitis je podeljen prema stepenu težine. U više studija je potvrđena veza između težine zapaljenja i dužine hospitalizacije, kao I broja konverzija u otvorenu

holecistektomiju i broja postoperativnih komplikacija. Različite studije su pokazale da je optimalno vreme za operativno lečenje akutnog holecistitisa u prvuih 72 sata od početka simptoma ali da operaciju treba da ivodi iskusan i uvežban hirurški tim kao i da odloženi hirurški tretman ne smanjuje broj konverzija i morbiditet već povećava.

Ključne reći: akutni holecistitis, holelitijaza, laparoskopska holecistektomija

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DIVERTIKULITISI – MODALITETI LEČENJA

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Inicijalnu procenu pacijenata sa divertikulitisom bi trebalo uraditi nakon detaljne anamneze, fizikalnog pregleda i laboratorijskih analiza (najvažniji su CRP, prokalcitonin i fekalni kalprotektin).

MSCT skener abdomena i male karlice je dijagnostička procedura izbora kod pacijenata sa divertikulitisom.

Ultrazvuk i magnetna rezonanca mogu biti korisne alternative pri početnoj proceni pacijenta kada MSCT snimanje nije dostupno ili je kontraindikovano.

Neoperativno lečenje divertikulitisa najčešće uključuje primenu antibiotika.

Perkutana drenaža se preporučuje kod hemodinamski stabilnih pacijenata sa apscesima veličine do 3 cm.

Prestanak unošenja duvana, smanjen unos mesa, pojačanje fizičke aktivnosti i smanjenje indeksa telesne mase se preporučuju kako bi se potencijalno smanjio rizik od divertikulitisa.

Mesalamin, rifaksimin i probiotici se obično ne preporučuju da bi se smanjio rizik od ponavljanja divertikulitisa ali mogu biti efikasni pri smanjenju hroničnih simptoma.

Nakon završetka epizode akutnog komplikovanog divertikulitisa, trebalo bi da se uradi kolonoskopija da bi se potvrdila dijagnoza.

Nakon uspešnog nehirurškog tretmana, potrebno je da se planira resekcija dela kolona i rektuma koji su zapaljenski izmenjeni a naročito ako postoji fistulozna forma, opstrukcija ili suženje, a sama odluka o hirurškom lečenju se donosi konzilijarno i individualno .

Resekcija kolona kod pacijenata mlađih od 50 godina se ne preporučuje jer je značajno manji broj komplikovanih divertikulitisa kod njih.

Kod pacijenata sa difuznim peritonitisom ili kod kojih antibiotska terapija nije delovala, potrebno je da se uradi hitna resekcija inflamiranog dela kolona a nekada i proksimalnog rektuma uz formiranje terminalne kolostome. Ponovno uspostavljanje kontinuiteta digestivnog trakta se sprovodi individualno, shodno godinama, opštem stanju pacijenta i propratnim oboljenjima.

Laparoskopska lavaža se ne preporučuje kod pacijenata sa fekalnim peritonitisom jer je praćena većom procentom reintervencija u odnosu na resekciju inflamiranog dela creva koju treba uraditi pri takvoj situaciji.

Kada je hirurg obučen za bezbedno sprovođenje minimalno invazivnih kolorektalnih procedura, navedena metoda se preporučuje zbog bržeg oporavka, manjeg reza, manjeg gubitka krvi i potrošnje antibiotika i analgetika.

Ključne reči: akutni divertikulitis, laparoskopija, terapija

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INDIVIDUALNI PRISTUP PACIJENTU U USLOVIMA URGENTNE HIRURGIJE - KADA JE TRENUTAK ZA HIRURŠKU INTERVENCIJU?

Vladimir Resanović, Zlatibor Lončar, Krstina Doklestić Vasiljev, Pavle Gregorić, Dušan Micić, Miljan Ćeranić, Nenad Ivančević, Dragan Vasin

Hirurgija lečenja akutnih stanja u modernom društvu pred zdravstvene sisteme stavlja izuzetne izazove u vidu neočekivanih i obimnih intervencija. Pacijenti koji su podvrgnuti urgentnoj hirurškoj intervenciji imaju 8 puta veću verovatnoću neželjenog ishoda lečenja u odnosu na pacijente koji su operisani kao elektivni. Rano prepoznavanje hirurškog akutnog stanja i pravovremeno započinjanje njegovog lečenja predstavljaju ključne faktore u smanjenju morbiditeta, skraćivanju dužine hospitalizacije, ali i umanjenju mortaliteta. Odlaganje postavljanja dijagnoze, neprepoznate indikacije za hirurgijom i kašnjenje same hirurške intervencije utiču na ishod lečenja, posebno kod starijih pacijenata sa komorbiditetima. U donošenju pravovremenih odluka kod kritično obolelih pacijenata najznačajniji je sistem trijaže, koji mora jasno označiti pacijente kojima se na najbrži mogući način mora pružiti adekvatna medicinska nega. Za trijažu pacijenata neizostavna su tri faktora: hemodinamski status, priroda hirurškog oboljenja i težina sepse, odnosno infekcije.

Pandemija Kovid 19 je pokazala da je od ključnog značaja postojanje adekvantog sistema trijaže – donošenje pravovremene odluke kada je u pitanju hirurško lečenje kritično obolelih pacijenata. U okruženju kada su medicinski sistemi širom planete bili dovedeni na ivicu funkcionalnosti zbog preopterećenosti, od izuzetnog značaja se pokazala potreba za identifikacijom pacijenata najvećeg stepena hitnosti kojima je potrebno pružiti hirurško lečenje, zadržavajući dobar kvalitet lečenja, promišljenu upotrebu dostupnih medicinskih sredstava, ali i zaštitu zdravstvenih radnika. Novi trijažni sistem – TACS (Timing in Acute Care Surgery) koji je predložen 2022 godine od strane Svetskog Udruženja Urgentne hirurgije (WSES) koristi 5 boja kojima se (slično bojama na semaforu, crveno – narandžasto - žuto – zeleno - plavo) označava stepen hitnosti pacijenata sa akutnim hirurškim stanjima.

Predloženi sistem trijaže dovodi do skraćenja čekanja na hiruršku intervenciju, značajno umanjuje broj urgentnih operacija tokom noći (sa 27.4 na 23.5%) i uvećava broj najhitnijih intervencija označenih crvenom bojom (sa 45.2 na 62.7%). Predloženi sistem trijaže garantuje skraćenje čekanja na urgentnu hiruršku intervenciju, obezbeđujući pritom da budu sprovedene hemodinamska stabilizacija i izbor adekvatne hirurške procedure.

Ključne reči: trijaža, hirurgija lečenja akutnih stanja

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LAPAROSKOPSKA ILI OTVORENA HIRURGIJA PERFORATIVNOG APENDICITISA

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Apendektomija je hirurško uklanjanje slepog creva (apendiksa). Slepo crevo je crvoliki deo koji se nalazi na cekumu-početnom delu debelog creva. Akutna upala slepog creva je najčešće hitno hirurško oboljenje koje zahteva hitnu hiruršku intervenciju. Akutna upala slepog creva se javlja u oko 7% do 10% populacije tokom. Apendicitis se može javiti u svakoj životnoj dobi, ali je najveća učestalost izmežu 10. do 30. godine života.

Apendektomija se može vršiti klasičnim ili laparoskopskim pristupom. U eri laparoskopske hirurgije, izbor između laparoskopske i otvorene metode još je uvek predmet stalnih rasprava. U pojedinim bolnicama u vreme dežurstva nije uvek na raspolaganju tim hirurga koji je edukovan za laparoskopsku apendektomiju. Izbor između laparoskopske i otvorene apendektomije danas zavisi kako od sposobnosti I utreniranosti hirurga I hirurškog tima, tako I od saamog lokalnog nalaza kod pacijenta, laboratoriskih analiza kao I sprrovedinih dijagnostičkih procedura.

Iako su rezultati lečenja u obe tehnike podjednaki, preferira se laparoskopska apendektomija zbog kraćeg boravka u bolnici, bržeg oporavka, manje učestalosti infekcija, boljeg estetskog rezultata i manjeg broja komplikacija, pacijent ustaje nakon nekoliko sati od operacije I bolnicu napušta isti dan ili sutradan. Laparoskopski pristup ima prednost pri sumnji na alternativne dijagnoze, a u žena u proceni patologije jajnika i alternativnih dijagnoza.U lečenju akutnog apendicitisa smrtnost od operacije iznosi 0,1–0,5%, a morbiditet 5– 7%. U slučaju perforativnog apendicitisa stopa smrtnosti je 5–15%, a morbiditet 15–60%. Odlaganje operacije, kasno prepoznavanje dijagnoze ili neprepoznavanje apendicitisa povećavaju stopu mortaliteta i morbiditeta.

Od kraja XIX veka akutni apendicitis se leči hirurški. Međutim, u poslednje vreme, u eri antibiotika, lečenje apendicitisa postaje dinamičan proces, sa različitim fazama u kojima se može različito reagovati. U celom svetu broj klasičnih apendektomija je sve manji, dok se povećava broja laparoskopskih I sve češći je konzervativni način lečenja, kod blažih formi inflamacije, koji polako zauzima svoje mesto.

Ključne reči: akutni appendicitis, perforativni appendicitis, klasična i laparoskopska apendektomija

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IMIDŽING U ABDOMINALNOM KOMPARTMENT SINDROMU – ULOGA RADIOLOGA

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Abdominalni kompartment sindrom (ACS) je oboljenje koje podrazumeva disfunkciju abdominalnih organa usled povišenog intraabdominalnog pritiska. Radiološka dijagnostika ACS je kompleksna i najčešće se postavlja u slučajevima kada je prisutan određeni broj radioloških znakova u kliničkim stanjima koja ukazuju na kompartment ili ukoliko postoji pogoršanje radioloških nalaza na kontrolnim snimanjima.

Radiološke metode koje se koriste u proceni razvoja ACS su radiografija, ultrasonografija, kompjuterizovana tomografija (CT) i magnetna rezonanca (MR). Najčešće korišćena metoda kojom se postavlja sumnja na razvoj ACS je CT iz razloga što se CT pregledom dobijaju najpreciznije morfološke informacije o stanju intraabdominalnog pritiska. Najpouzdaniji CT znak u ACS je odnos visine peritonealne i abdominalne duplje mereno u nivou duodenuma. Ukoliko je taj odnos veći od 0.52 smatra se da je intraabdominalni pritisak veći od 12mmHg i u odgovarajućem kliničkom kontekstu se može postaviti sumnja na ACS. Drugi važan CT znak je "round belly sign" – odnos maksimalnog anteroposteriornog i transverzalnog dijametra abdomena meren u nivou leve renalne vene koji je normalno manji od 0.8. Ukoliko je odnos veći od 0.8 smatra se da je vrednosti intraabdominalnog pritiska veća od 20mmHg. Na CT pregledu se mogu verifikovati i drugi znaci ACS: elevacija dijafragmi, kolaps donje šuplje i renalnih vena, mozaična perfuzija jetre, distenzija želuca i ingvinalne hernijacije. S obzirom na teško stanje ovih pacijenata ultrasonografski pregled je otežan i ređe se koristi za procenu ali može postaviti sumnju u slučaju da se detektuje spori protok kroz hepatične i renalne vene na dopler pregledu.

Uloga radiologije u ACS je važna i podrazumeva njegovu detekciju, karakterizaciju uzroka i eventualno planiranje tretmana što je veoma važno u poboljšanju prognoze ovih teških pacijenata.

Ključne reči: radiologija, kompartment, kompjuterizovana tomografija.

MINI SIMPOZIJUM

NARKOMANIJA, ASFIKSIJE, SAMOUBISTVO – NOVI PATOFORENZIČKI ASPEKTI

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ZLOUPOTREBA KOKAINA – DA LI SE NEŠTO PROMENILO TOKOM VREMENA?

Danica Đukić

U forenzičkoj kolekciji profesora Milovana Milovanovića (1884–1948) nalazi se zanimljiv eksponat iz 1929. godine koji se sastoji od tri staklena šprica i dve male staklene bočice sa plutanim čepovimama. Nalazili su se u džepu kaputa mladića starog 30 godina, nekadašnjeg studenta medicine, čije je telo nađeno u šupi u dvorištu jedne kafane. Bio je alkoholičar, narkoman i član tzv. četvoročlane kokainske ruske bande. Obdukcija je pokazala klasične znake hronične zloupotrebe kokaina, masno izmenjenu jetru, zapaljenje pluća i malokrvnost. Toksikološka analiza pokazala je kvalitativno prisustvo kokaina u organima pokojnika, kao i prisustvo morfijuma u jednoj od bočica. Naknadna toksikološka analiza materijala iz jednog od špriceva i druge bočice koja svojevremeno nije analizirana, urađena na savremenom aparatu (gasna hromatografija sa masenom spektrometrijom), pokazala je prisustvo narkotika u tragovima, čak devedeset godina posle prve analize urađene daleke 1929. godine u tadašnjoj Državnoj hemijskoj laboratoriji. U vreme kada je rađena obdukcija, kokain se tek pojavio kod nas nelegalna psihoaktivna supstanca i ubrizgavao se potkožno. Kako je dejstvo kokaina relativno kratkotrajno, to treba da se uzima u manjim vremenskim razmacima, pa je obdukcioni nalaz relativno karakterističan: veliki broj uboda po koži karakterističnog rasporeda i izgleda. Danas se kokain unosi u organizam pre svega ušmrkavanjem, tako da je i obdukcioni nalaz drugačiji i manje karakterističan.

Ključne reči: obdukcija, kokain, muzejska zbirka, toksikologija.

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SAMOUBISTVO I AKUTNO PIJANO STANJE

Bojana Radnić

Akutni unos alkohola može izazvati suicidalne ideje i pokušaj samoubistva kod ljudi koji su prethodno već bili pod rizikom. Takođe, samoagresija je intenzivnija nakon konzumiranja alkohola. Cilj ove studije bio je da se proceni odnos između koncentracije alkohola u krvi (engl. *blood alcohol concentration – BAC*) i stepena letalnosti načina izvršenja samoubistva. Posmatrano je više različitih socio-demografskih i kliničkih prediktora koji se odnose na izbor metoda visoke letalnosti. Ova retrospektivna autopsijska studija učinjena je na obdukcionom materijalu Instituta za sudsku medicinu u Beogradu i obuhvatala je 11-godišnji period. Uključuje 308 ispitanika sa potvrđenim *BAC* iznad 0‰ koji su izvršili samoubistvo. Metode samoubistva su bile dihotomizovane u smislu letalnosti – niskoletalne metode (*low lethality method LLM* – predoziranje drogom/trovanje ili upotreba oštrog predmeta, n = 20) i visokoletalne metode (*high lethality method – HLM* – vešanje, vatreno oružje i eksploziv, utapanje, skakanje sa visine i samospaljivanje, n = 288). Naša studija nije otkrila statistički značajnu razliku između koncentracije alkohola i *HLM*. Ipak, očigledan je trend visokoletalnih metoda u rastu *BAC* kategorija, ali bez statističkog značaja. Od svih testiranih prediktora, samo muški pol ima značajnu povezanost sa visokoletalnim metodama (p = 0,036). Kako su literaturni podaci bili u suprotnosti sa rezultatima ove studije može se zaključiti da je povezanost alkoholemije i suicida veoma složena.

Ključne reči: akutni unos alkohola, visokoletalne metode samoubistva, faktori rizika za samoubistvo

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ODREĐIVANJE HEMIJSKE STRUKTURE NOVOSINTETISANIH KANABINOIDA

Vera Lukić

Poslednjih godina na tržištu droga pojavili su se novi molekuli – *nove psihoaktivne supstance*. One predstavljaju raznoliku grupu hemijskih jedinjenja koja izazivaju psihoaktivne efekte, koji su slični ili intenzivniji od efekata koje izazivaju postojeći ilegalni molekuli. Teži se da ove nove psihoaktivne supstance legalna zamena tzv. klasičnim drogama. Sintetički kanabinoidi jesu jedna od najčešćih klasa novosintetisanih jedinjenja. Pokazuju visok afinitet za kanabinoidne receptore CB1 i CB2 u ljudskom organizmu, zbog čega se koriste kao alternativa marihuani. Sintetički se kanabinoidi najčešće uzimaju pušenjem biljnih mešavina koje ih sadrže. Raznovrsnost hemijske strukture novosintetisanih jedinjenja i njihov rastući broj čini ih stalnom pokretnom metom za analitičara. Danas se toksikolozi, odnosno anlitičari u toksikološkim laboratorijama, koji rutinski vrše analize i identifikuju ove nov supstance, utrkuju sa osobama koje ilegalno sintetišu nove psihoaktivne supstance. Državna regulatorna tela obično kasne sa stavljanjem ovih novosintetisaih supstanci pod zakonsku kontrolu, jer procena rizika za svaku od ovih supstanci može da potraje: novije varijante sintetičkih kanabinoida često su već dostupne na tržištu pre nego

što su prethodne identifikovane i zabranjene. Otkrivanje i identifikacija sintetičkih droga prvi su neophodni i ključni koraci kako za širenje informacija radi sprovođenja njihove kontrole na nacionalnom i međunarodnom nivou, tako i za proučavanje njihovih toksičnih efekata ili dovođenja u vezu sa intoksikacijama.

Ključne reči: novosintetisani kanabinoidi, identifikacija, forenzička toksikologija.

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SMRT POSLE UZIMANJA IBOGAINA

Tijana Petrović

Ibogain je prirodna psihoaktivna supstanca nađena u korenu biljke koja pripada familiji svilenica (*Apocynaceae*), poznatoj kao iboga (*Tabernanthe iboga*). Ibogain je halucinogen koji je zabranjen u nekim zemljama, dok se ponegde koristi za lečenje zavisnosti od opijata, metamfetamina i drugih lekova. Njegovo se dejstvo zasniva na izazivanju osećaja euforije, vizualnih i čujnih halucinacija, kao i izazivanju zvučnih, mirisnih i gustatornih sinestezija. Prikazujemo slučaj naprasne smrti heroinskog zavisnika koji je umro nekoliko sati posle ingestije praha *Tabernanthe iboga*, korišćenog u procesu detoksikacije od heroina. Mladić je nađen u svom krevetu, bez povreda. Obdukcijom je ustanovljen teški edem pluća, bez bitnijih patoloških promena na drugim unutrašnjim organima. Koncentracija ibogaina je bila 3,26 mg/L. Na osnovu podataka o okolnostima umiranja, obdukcije i sprovedenih dodatnih analiza (mikroskopskog pregleda organa i hemijsko-toksikološke analize), zaključeno je da je smrt je nastupila usled letalnih efekata unetog ibogaina na kardiovaskularni sistem.

Ključne reči: forenzička toksikologija, ibogain, detoksikacija od opioida.

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POSTURALNA ASFIKSIJA I PSIHOAKTIVNE SUPSTANCE

Vladimir Živković

Posturalna asfiksija nastaje kada su respiratorni pokreti onemogućeni zbog položaja u kojem se nalazi žrtva. Jedan od činilaca koje olakšavaju nastanak posturalne asfiksije, a istovremeno otežava samospasavanje, jeste dejstvo psihoaktivnih supstanci – alkohola, lekova ili droga. Kod osoba pod dejstvom psihoaktivnih supstanci, posturalna asfiksija može biti potcenjeni, doprinoseći, ili čak i glavni uzrok smrti. Razlozi za neprepoznavanje posturalne asfiksije kao mogućeg uzroka smrti, sa jedne strane, leže u neadekvatnim, nedovoljnim ili nepreciznim podacima sa lica mesta u vreme vršenja obdukcije, a sa druge, u odsustvu, slaboj izraženosti ili nespecifičnosti njenih znakova na obdukciji. Dobra istraga često je ključna za postavljanje dijagnoze posturalne asfiksije kao doprinosećeg ili glavnog uzroka smrti. Ona uključuje adekvatan i pažljiv pregled lica mesta, opis mesta i položaja tela, kao i podatke u vezi sa eventualnim njegovim pomeranjem od strane osoba koje su ga pronašle. Znakovi na obdukciji najčešće su nespecifični, a uključuju opšte znake asfiksije. U nekim situacijama, raspored i oblik mrtvačkih mrlja i bledila, mogu ukazati na specifičan položaj tela. Jedan od znakova koji takođe može biti od koristi jeste hiposfagma. Hiposfagma ili subkonjunktivalna hemoragija predstavlja ekstenzivno, konfluentno krvarenje između konjunktive i episklere. Ona nastaje kao rezultat teške venske kongestije glave i vrata, te može biti korisna kao indikator posturalne asfiksije, pogotovo u slučajevima gde se uzrok smrti ne može objasniti samo prisustvom psihoaktivnih supstanci, jer seu detektovane u tragovima ili u niskim koncentracijama. Kod obdukcionog nalaza hiposfagme, treba biti oprezan u zaključivanju, s obzirom na mogućnost njenog postmortalnog nastanka, kao i nastanka u sklopu traume ili Valsavinog manevra.

Ključne reči: posturalna asfiksija; psihoaktivne supstance; hiposfagma; subkonjuktivnalna hemoragija; obdukcija.

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UBISTVO ILI ZADES – ISKRVARENJE IZ BUTNE ARTERIJE

Dragan Ječmenica

Ovo je prikaz slučaja sumnjive smrti žene, dugogodišnjeg uživaoca heroina, koja je zatečena mrtva u krevetu, u ležećem položaju, sa velikom lokvom krvi pored i upadljivom ranom u desnoj preponi: garderoba je bila delimično svučena, a rana je ličila na ubodinu. Pored kreveta pronađen je i šrafciger, kojim je ovakva povreda mogla biti naneta. Međutim, na obdukciji je ustanovljena pseudoaneurizma femoralne arterije, koja je formirala fistulu sa kožom – što je pri njenoj rupturi dovelo do obilnog spoljašnjeg krvarenja, odnosno iskrvarenja. Toksikološkom analizom u telesnim tečnostima nije utvrđeno prisustvo heroina, niti drugih droga. Usled ponavljanog ubrizgavanja heroina u butnu venu i nenamernog povređivanja zida butne arterije postepeno se formira pseudoaneurizma, koja pri pokušaju venepunkcije može da rupturira. U konkretnom slučaju, ne može se isključiti ni spontana ruptura pseudoaneurizme. Poznavanje ove komplikacije ubrizgavanja droge u preponu i dobra saradnja forenzičkih patologa i istražnih organa omogućava pouzdan zaključak o uzroku i poreklu smrti.

Ključne reči: heroin, narkomanija, butna arterija, pseudoaneurizma, iskrvarenje.

Institut za sudsku medicinu, Medicinski fakultet, Univerzitet u Beogradu

PSEUDOANEURIZME BUTNIH ARTERIJA KOD UŽIVALACA HEROINA

Aleksa Leković

U osoba koje heroin unose intravenskim putem na mestu čestog ubrizgavanja nastaju ožiljne promene u potkožnom mekom tkivu. Ožiljavanje može znatno da otežа dalje ubrizgavanje droge u krvni sud na tom mestu. Takođe, ovakve promene na rukama predstavljaju lako uočljivu stigmu. Stoga, pojedini narkomani pribegavaju drugom, lako dostupnom mestu i veni velikog kalibra: butnom trouglu i femoralnoj veni. Ubrizgavanje droge na ovome mestu, međutim, skopčano je sa nekoliko ozbiljnih, potencijalno smrtonosnih komplikacija, među kojima je i nastanak pseudoaneurizme femoralne arterije. Zbog bliskog anatomskog odnosa butne arterije i vene, zid arterije može da se povredi pri pokušaju venepunkcije, a ponavljanjem ovoga i pseudoaneurizma, koja postepeno progredira, te može da rupturira i dovede do obilnog, smrtonosnog krvarenja. Ovakvi slučajevi najčešći su u narkomana koji drogu ubrizgavaju više godina, imaju očigledne stigme takvog ponašanja, a u kojih u trenutku smrti u telesnim tečnostima nema droge: krvarenje verovatno najčešće nastaje pri pokušaju venepunkcije, mada se ni spontana ruptura pseudoaneurizme ne može isključiti. Iskrvarenja su moguća iz malih pseudoaneurizmi, koje više liče na kutano-arterijsku fistulu, ali i kod onih veličinu pesnice, kada se u pseudoaneurizmi uočavaju brojne kavitacije obložene endotelom. Mikroskopskom analizom pseudoaneurizme mogu se uočiti fibrinoidna nekroza, akutni i hronični zapaljenski infiltrat, elementi i svežeg i starog krvarenja. Opšti znaci iskrvarenja su lako uočljivi. Međutim, pošto ovakva naprasna smrt, bez očevidaca i/ili pod sumnjivim okolnostima, može da liči na ubistvo, poznavanje bitnih karakteristika lokalnog obdukcionog nalaza olakšava donošenje zaključka o poreklu smrti.

Ključne reči: heroin, ubrizgavanje u preponu, komplikacije, iskrvarenje, obdukcija.

Institut za sudsku medicinu, Medicinski fakultet, Univerzitet u Beogradu

INFEKTIVNE KOMPLIKACIJE UBRIZGAVANJA NARKOTIKA U BUTNE VENE

Slobodan Nikolić

Usled ubrizgavanja nelegalnih psihoaktivnih supstanci, odnosno narkotika i to najčešće heroina u butnu venu ispod projekcije preponskog ligamenta, mogu, pored ostalog, da se razviju i lokalne gnojne infekcije, koje daljim širenjem postaju fatalne. Od prvih subjektivnih simptoma do smrti može proći i samo nedelju dana, te može da postoji nesrazmera između spoljašnjeg i unutrašnjeg lokalnog nalaza pri obdukciji, a na preponi zahvaćenoj infekcijom. Ponekad se infekcija vidi samo samo kao manji ili veći otok gornjeg dela butine, a nekad je opet cela noga vrlo otečena. Ukoliko se lokalna infekcija duže vreme razvijala, onda se i na koži prepone mogu videti nekroza i i fistule preko kojih se gnoj spontano drenirao. Ove se gnojne infekcije šire duž fascija mišića: neke ne prelaze anatomski granicu preponskog ligamenta, ali neke zahvataju i mišiće prednjeg trbušnog zida, glutealne mišiće i iliopsoase, šireći se preko pudendalnog i obturatornog kanala. Usled svega ovoga može nastati i pelveoperitonitis sa kasnijim razvojem septičnog šoka. I pored uglavnom teškog apsedirajućeg zapaljenja, krvni sudovi u preponi uglavnom nisu zahvaćeni. U pitanju su mlađe osobe, ali koje su obično duži niz godina uživaoci heroina, koji iz različitih razloga koriste butnu venu u preponi za ubrizgavanje droge. Čak i minimalni ubod u preponu, gde na koži već postoji veliki broj najrazličitijih vrsta patogenih bakterija, kod osoba koje su već sa oslabljenim imunskim odgovorom, a uživaoci heroina to jesu, može da provocira razvoj teške i fulminantne infekcije i uzrokuje smrt.

Ključne reči: heroin, ubrizgavanje u preponu, komplikacije, infekcija, obdukcija.

MINI SIMPOZIJUM

DELO PROF. DR ALEKSANDRA Đ. KOSTIĆA – POVODOM 130 GODINA OD ROĐENJA I 40 GODINA OD SMRTI

Institut za histologiju i embriologiju „Prof. dr Aleksandar Đ. Kostić", Medicinski fakultet, Univerzitet u Beogradu

PROF. DR ALEKSANDAR Đ. KOSTIĆ: ISTORIJSKA LIČNOST SRPSKE MEDICINE

Nela Puškaš

Aleksandar Kostić, dobrovoljac i medicinar u Balkanskim i Velikom ratu, mladi lekar i saradnik prof. Pola Buena na Medicinskom fakultetu u Strazburu, na poziv prof. Subbotića vratio se u rodni Beograd, kako bi organizovao rad Histološkog instituta i nastavu histologije i embriologije na novoosnovanom Medicinskom fakultetu. Sticajem specifičnih socio-ekonomskih i istorijskih prilika, taj zadatak je čak nekoliko puta uspešno obavio, a poslednji put neposredno nakon II svetskog rata, pošto je Institut bio porušen u aprilskom bombardovanju. Bio je prvi i skoro tri decenije jedini profesor histologije i embriologije na Medicinskom fakultetu u Beogradu. Izuzetan pedagoški potencijal, o kome postoje brojni zapisi, uticao je da Institut postane stecište ambicioznih i talentovanih mladih saradnika, koji su ostavili značajan trag i istoriji medicine i Medicinskog fakulteta, kao i sam Kostić. Uz njihovu pomoć uspešno je razvijao i naučno-istraživački rad na Institutu. U tri mandata je bio dekan, a učestvovao je i u osnivanju Farmaceutskog i Fakulteta veterinarske medicine. Za svoj doprinos u razvoju medicinske nauke nagrađen je francuskim Ordenom Legije časti.

Međutim, pored rada na Institutu, njegov profesionalni život obeležila su široka interesovanja i brojne aktivnosti, kojima je unapredio i zadužio ne samo Fakultet već i medicinu u Srbiji. Naime, bavio se medicinskom terminologijom i sastavio jedinstven višejezični medicinski rečnik, koji i danas predstavlja kapitalno delo srpske medicine. Bio je utemeljivač medicinske fotografije i filma na našim prostorima, a njegove fotografije zdravstvenih ustanova u okviru Vojne i Opšte državne bolnice vremenom su postali izuzetno vredan arhivski materijal. Kostić je bio i prvi lekar koji se bavio seksologijom. Preveo je i napisao više značajnih dela iz te oblasti. Radio je na seksualnom obrazovanju i zdravstvenom prosvećivaju stanovništva. Bio je urednik više stručnih časopisa i izdanja, bavio se istorijom medicine i ostavio dubok trag u svakoj oblasti kojom se bavio.

Ključne reči: Aleksandar Kostić, Histološki institut, medicinski rečnik, seksologija, medicinska fotografija i film, istorija medicine

Institut za histologiju i embriologiju „Prof. dr Aleksandar Đ. Kostić", Medicinski fakultet, Univerzitet u Beogradu

Srpska akademija nauka i umetnosti, Beograd

ISTRAŽIVANJA PROFESORA KOSTIĆA U OBLASTI HISTOLOGIJE I EMBRIOLOGIJE

Vladimir Bumbaširević

Ime Aleksandra Đ. Kostića vezuje se za osnivanje Instituta za histologiju i embriologiju (koji je nazvan po njemu) i razvoj ovih naučnih disciplina u našoj zemlji, ali i za razvoj medicinske fotografije, medicinske terminologije, seksologije i popularizacije seksualnog vaspitanja u našoj zemlji.

Svoje prve radove, profesor Kostić je objavio 1920. godine, pred kraj studija u Strazburu, uključujući i studiju o epidemiji skorbuta među srpskim vojnicima tokom perioda Prvog svetskog rata. S obzirom da je istraživanja za svoju doktorsku tezu obavljao u histološkoj laboratoriji kod mentora Profesora Pola Buena, to je njegova doktorska disertacija, kao i najveći deo naučnih radova iz oblasti histologije. Među najznačajnije naučne doprinose profesora Kostića spadaju istraživanja štetnog uticaja različitih toksičnih supstanci, posebno alkohola, joda, arsena, olova, sulfonamida i drugih na testise, odnosno na proces spermatogeneze, kao i na jajnike i proces ovogeneze. Izbor ispitivanih supstanci se uvek odnosio na one čija toksičnost može realno da se ispolji među stanovništvom, bilo akcidentalno ili jatrogeno.

Takođe, značajan deo njegovih istraživanja odnosio se i na proučavanje funkcije slezine, organa o kome se u to vreme veoma malo znalo. Ova istraživanja spadaju u pionirska i ukazuju na povezanost imunskog i endokrinog sistema.

Profesor Kostić je uvek težio da poveže strukturu sa funkcijom, baveći se histologijom sa funkcionalnog aspekta, a ne samo faktografskim opisima morfoloških karakteristika ili promena. Na taj način su njegovi radovi i predavanja uticali na razvoj histologije u ovom pravcu. Rezultate istraživanja, profesor Kostić je pretežno publikovao u francuskim časopisima, ali i u nemačkim, i jugoslovenskim.

Na žalost, udaljavanjem sa Medicinskog fakulteta u punoj naučnoj zrelosti, udaljen je i od laboratorijskog rada, tako da smo uskraćeni za njegov još značajniji doprinos nauci.

Ključne reči: Aleksandar Kostić, histologija, alkohol, testis

Srpsko lekarsko društvo, Beograd

RAD ALEKSANDRA KOSTIĆA NA MEDICINSKOJ TERMINOLOGIJI I MEDICINSKOM REČNIKU

Zoran Vacić

Prof. dr Aleksandar Đ. Kostić (1893–1983) bio je naučnik (histolog, embriolog, seksolog), poliglota, najznačajniji srpski medicinski terminolog i leksikograf, kompozitor, pijanista, utemeljivač medicinske fotografije i filma kod nas, književnik – pripovedač i esejista, arheolog, zdravstveni prosvetitelj, predsednik odbora za priređivanje Prve zemaljske higijenske izložbe u Beogradu (1933), muzeolog... drugim rečima poslednja renesansna ličnost srpske medicine.

Osnivač i rukovodilac Terminološkog seminara (1924–1941, 1951–1952) koji je radio u okviru Histološkog instituta. U Srpskom arhivu za celokupno lekarstvo, stručnom časopisu Naš jezik, zbornicima radova Naučnog društva za istoriju zdravstvene kulture Jugoslavije i drugim publikacijama objavio je više članaka iz oblasti medicinske terminologije.

Srpskom medicinskom terminologijom počeo je da se zanima kao student medicine u Nansiju (1913), prikupljanje reči i njihovu obradu nije prekidao ni za vreme Velikog rata. Posle prvog svetskog rata objavio je „Rečnik histoloških izraza“ (1924), bio je urednik „Velike medicinske enciklopedije za narod (narodni zdravstveni učitelj)“ (1930–1932), autor je enciklopedijskog rečnika „Moj lekar“ (1934, 1940, 1961, 1972).

Za vreme Drugog svetskog rata, udaljen iz nastave, nastavlja rad na izradi višejezičnog medicinskog rečnika. U julu 1946. obaveštava Dekana Medicinskog fakulteta da sprema za štampu „Rečnik medicinskih izraza“. Prvo izdanje kapitalnog dela naše medicinske leksikografije „Medicinski rečnik“ objavio je 1956. Rečnik sadrži oko 41.000 reči na latinskom, nemačkom, engleskom, francuskom i srpskom jeziku i eponimni rečnik). Drugo izdanje „Višejezični medicinski rečnik (Lexicon medicum polyglottum)“ objavljeno 1971. ima oko 125.000 reči na 7 jezika (uz pet jezika prvog izdanja rečnika još i ruski i italijanski). Četvrto izmenjeno i dopunjeno izdanje uredio je po Kostićevim instrukcijama prof. dr Slobodan Đorđević. Objavljeno je šest izdanja ovog rečnika (1956, 1971, 1976, 1987, 1996, 2009). Glavni je urednik „Medicinskog leksikona za lekare i studente“ (1957, 1968, 1970, 1981).

Dobitnik je Oktobarske nagrade Beograda za drugo izdanje Rečnika (1971), i mnogobrojnih odlikovanja, između ostalih Ordena zasluga za narod sa zlatnom zvezdom i francuskog Ordena legije časti.

Ključne reči: terminološki seminar, medicinska terminologija, medicinski rečnik

Institut za savremenu istoriju, Beograd

RAD PROFESORA ALEKSANDRA KOSTIĆA NA MEDICINSKOM FAKULTETU OD 1944. DO 1952. GODINE

Dragomir Bondžić

Profesor Aleksandar Kostić je po završetku studija medicine u Strazburu 1921, po pozivu došao na novoosnovani Medicinski fakultet u Beogradu. U međuratnom periodu je održavao nastavu histologije i embriologije, rukovodio Histološkim institutom, bio jedan od najistaknutijih nastavnika na fakultetu. Do kratkog prekida aktivnosti profesora Kostića došlo je tokom Drugog svetskog rata zbog okupacije i neslaganja sa okupatorskim i kolaboracionističkim režimom. Posle oslobođenja Beograda, Kostić je već decembra 1944. vraćen za redovnog profesora na Medicinskom fakultetu, a jula 1945. je ponovo postavljen za upravnika Histološkog instituta. Međutim, njegov rad na fakultetu u posleratnim godinama se odvijao u sasvim novim ideološko-političkim uslovima. Osvojivši vlast revolucionarnim putem Komunistička partija Jugoslavije je počela sa izgradnjom nove države i novog društveno-političkog sistema zasnovanog na političkim postavkama i ideologiji marksizma-lenjinizma. Jedna od odlika novog sistema bila je potpuna kontrola Komunističke partije nad svim oblicima državnog, političkog, društveno-ekonomskog i kulturno-prosvetnog života, pa tako i nad životom i radom na Univerzitetima i fakultetima. U novim uslovima profesor Kostić je nastavio sa predavanjima i vežbama, aktivnostima na fakultetu i van njega. Međutim, već od 1945. bilo je vidljivo njegovo ideološko-političko neslaganje sa osnovnim postavkama novog režima i načinom delovanja komunista na fakultetu, koje je postepeno prerastalo u sve otvoreniji sukob. Nova vlast je s pravom smatrala Kostića svojim ideološkim „neprijateljem“, „buržoaskim“ intelektualcem koji se nije mirio sa revolucionarnim metodama, ukidanjem višepartijskog sistema i rušenjem parlamentarne demokratije i, posebno, sa pokušajima komunista da unesu politiku i ideologiju u naučni i nastavni rad fakulteta i nastavnika preko partijskih organa i studentskih organizacija. Posle nekoliko godina odmeravanja snaga sprovedena je koordinirana akcija partijskih, državnih i fakultetskih organa i studentske organizacije, i profesor Kostić je marta 1952. godine prevremeno penzionisan, a u stvari, iz ideološko-političkih razloga uklonjen sa Medicinskog fakulteta.

Ključne reči: Aleksandar Kostić, Medicinski fakultet u Beogradu, Komunistička partija Jugoslavije, ideologija, progon

Filološko-umetnički fakultet, Kragujevac

ZNAČAJ I ZNAČENJE MUZIKE U ŽIVOTU PROF. DR ALEKSANDRA KOSTIĆA

Snežana Nikolajević

Uz svoj medicinski rad, prof dr Aleksandar Kostić je imao različita interesovanja i bio je jedna od retkih renesansnih ličnosti dvadesetog veka. U domenu muzike njegovo prevashodno usmerenje i njegova preokupacija bio je pijanizam. Još u gimnazijskim danima mnogo je vremena provodio za klavirom, a kada je 1912. godine otišao na studije medicine u Nansi odlučio je da učini veliki napor i da u isto vreme upiše majstorski kurs kod Luja Dimiera, jednog od najčuvenijih francuskih pijanista i pedagoga. Kurs je završio veoma uspešnim resitalom.

Čini se da je od svih njegovih susreta i kontakta sa velikim pijanistima najzanimljivija bila saradnja sa Alfredom Kortoom, čuvenim francuskim pijanistom i pedagogom. Leto 1935. provodi u Parizu na njegovom kursu i specijalizira Šopena, a posle četiri godina sreće se ponovo na kratko sa njim.

U jesen 1969. profesor Kostić je osetio potrebu i sposobnost da izađe na binu i odsvira solistički koncert; saopštio je tu ideju svom sinu, istaknutom kompozitoru Vojislavu Vokiju Kostiću, koji je pokušao da mu organizuje koncert. No, u sklopu podozrenja koje je prof. Kostića pratilo niz godina i u njegovim profesionalnim vodama, uprkos njegovoj erudiciji, njegovom znanju i njegovim izuzetnim sposobnostima, ovaj koncert je bio osujećen. I njegovom sinu nije ostalo ništa drugo nego da angažuje studio i omogući svom ocu da snimi pripremljen resital.

Vojislav Kostić je čuvao snimljene trake preko tri decenije i onda je uspeo da digitalnom tehnikom uradi postprodukciju 50 numerisanih CD primeraka. I ti raritetni snimci najbolje govori o pijanistički sposobnostima profesora Kostića, o izvođaštvu neobičnom i fascinantom za jednog amatera. Ono će biti predmet našeg razmatranja, pa i ocene, uz primenu kriterijuma kojima se vrednuju profesionalne interpretacije.

Ključne reči: Aleksandar Kostić, pijanizam i komponovanje

Institut za srpsku kulturu Priština – Leposavić

KNJIŽEVNA OSTAVŠTINA PROFESORA ALEKSANDRA Đ. KOSTIĆA

Jasmina Ahmetagić

U našem su fokusu dve prozne zbirke koje je za života, u rasponu od 60 godina, objavio lekar i univerzitetski profesor Aleksandar Đ. Kostić (1893–1983), jedan od osnivača Medicinskog fakulteta u Beogradu, pisac histoloških udžbenika i drugih stručnih knjiga, sa ciljem da se ta mala književna zaostavština opiše, sagleda u poetičkim okvirima srpske književnosti i vrednuje. I *Priče iz Strašne Kuće* (1928) i *Vedrine u oluji* (1984) tematski su zaokupljene Prvim svetskim ratom, ali su poetički sasvim različite. U *Vedrinama u oluji* Kostić u nizu epizoda datih u hronološkom poretku predstavlja trogodišnji period Prvog svetskog rata koji je proveo sa srpskom vojskom, kao vojnik i lekar, te se u zbirci spaja anegdotsko, memoarsko i dokumentarno, a hronološki poredak dopisuje nešto od romaneskne zamisli ovom pripovednom vencu. S druge strane *Priče iz Strašne kuće* odlikuje daleko modernija struktura: naturalistički opisi stoje naporedo sa alegorizacijom i simbolizacijom.

Mada nije reč o vrhunskim ostvarenjima umetnosti reči – jer one nastaju gotovo uzgred i na rubu drugih Kostićevih interesovanja i akademskog stvaranja, njihova vrednost, kako ćemo pokazati, nije samo dokumentarna

Ključne reči: Aleksandar Kostić, književna ostavština, *Priče iz Strašne kuće*, *Vedrine u oluji*. Prvi svetski rat

Muzej grada Beograda, Beograd

IMAGINARNI KABINETI ČUDA PROFESORA ALEKSANDRA KOSTIĆA

Miloš Spasić

Kabineti kurioziteta ili sobe čuda (nem. *kunstkammer,* eng. *wonder rooms*) pojavili su se u Evropi tokom 16. veka kao preteče muzeja. Ove zbirke prirodnih i umetničkih predmeta bile su privatne kolekcije koje su sakupljali i izlagali plemići i članovi evropske aristokratije. Osim što su imale za cilj da predstave karakteristike i neobičnosti iz prirodnog sveta i antike, sobe čuda su takođe služile kao moćni mediji za stvaranje i kuriranje odnosa i veza u aristokratskim društvenim sredinama. Aleksandar Kostić, srpski profesor histologije i embriologije, sakupljao je različite predmete sa teritorije Srbije, uključujući fosile i arheološke predmete, kao i dela likovne i primenjene umetnosti. Početkom 1930-ih godina, profesor Kostić je sproveo amatersko arheološko iskopavanje na svom privatnom imanju u selu Dubočaj kod Beograda. Nakon što je otkrio drevno rimsko nalazište sa ostacima grobnica i stambenih građevina, profesor Kostić se zaljubio u arheologiju i postao strastveni sakupljač brojnih i važnih predmeta, stvarajući značajnu kolekciju koja je kasnije donirana opštini Grocka. Fosili praistorijskih životinja i biljaka, neolitski ritualni predmeti, rimsko i srednjovekovno oružje, te svakodnevni predmeti ističu se kao najznačajniji eksponati iz ove kolekcije. Profesor Kostić je napravio korak dalje od pukog antikvarstva i pokušao da prouči i objasni mesto predmeta koje je sakupljao u širem kontekstu prirodne i ljudske evolucije. Ovaj rad ima za cilj da predstavi razloge osnivanja ove kolekcije i njenu važnost za predstavljanje lokalnih istorijskih narativa.

Ključne reči: Aleksandar Kostić, kolekcionarstvo, kabineti čuda, praistorija, Antika, srednji vek

Centar za kulturu Grocka

„KABINET RETKOSTI“: LEGAT DR ALEKSANDRA Đ. KOSTIĆA U GROCKOJ

Zorica Atić

Legat dr Aleksandra Đ. Kostića u Grockoj čuva i izlaže zaostavštinu jedne od najsvestranijih ličnosti i naučnika svoga vremena. Primarni deo zbirke, značajne paleontološke i arheološke predmete, polihistor dr Aleksandar Đ. Kostić (1893–1983), svestrani profesor, pasionirano je decenijama prikupljao na terenu u Grockoj gde je 1931. godine sagradio letnjikovac, da bi nalaze 1978. godine zaveštao Opštini Grocka - kao podsticaj za dalja istraživanja. Zbirka je prvobitno izložena 1982. godine u Rančićevoj kući, pod tematskim nazivom *Zavičajni muzej Grocke*. Nakon njegove smrti 1983. godine, sin Vojislav Voki Kostić, kompozitor, predaje opštini Grocka na čuvanje očeve lične predmete, rukopise, deo biblioteke, čime se formira Legat kao celina. Već 90-tih godina 20. veka, *Zavičajni muzej* je van upotrebe i pod ključem u nebrigom oronuloj kući, deleći istorijski trenutak i društvene okolnosti zemlje, a postavka je 2001/2002. godine povučena.

Zbirka je ponovo izložena 2018. godine u vidu stalne postavke „Legat dr Aleksandra Kostića – GO Grocka“, u namenskoj galeriji formiranoj u holu biblioteke „Ilija Garašanin“ u Grockoj, koja se nalazi u Gročanskoj čaršiji, prostornoj kulturno-istorijskoj celini od velikog značaja za RS. Svojevrsni „kabinet retkosti“ profesora Kostića čini sačuvana zaostavština iz *Zavičajnog muzeja*, dopunjena predmetima koji pripadaju njegovoj supruzi, Smilji Kostić Joksić, kao i predmetima iz letnjikovca u Grockoj, a koji ima, u svojoj raznovrsnosti i ilustrativnosti, pored muzeološkog, i veliki dokumentarni i edukativni značaj, takođe i kao doprinos kulturi sećanja.

Ključne reči: kabinet retkosti, legat dr Aleksandra Đ. Kostića u Grockoj, zaostavština, stalna postavka, biblioteka „Ilija Garašanin“, Rančićeva kuća, Zavičajni muzej

MINI SIMPOZIJUM

SAVREMENI PRISTUP RADIOLOGIJI DOJKE: DIJAGNOSTIČKE I BIOPSIJSKE PROCEDURE

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MODERN APPROACH TO DIAGNOSE BREAST LESIONS WITH ULTRASOUND

Boris Brkljačić

Ultrasound of the breast has to be used in conjunction with mammography and breast MRI. In the lecture basic physics of B-mode, colour Doppler and sonoelastography of the breast will be presented, as well as ultrasound features of benign and malignant masses and BI-RADS categorization of lesions. While the B-mode is most important ultrasound modality, colour Doppler is useful to demonstrate vascularization of lesions and sonoelastography to demonstrate the stiffness of lesions. Differences between strain and shear-wave elastography will be presented. Importance of compound imaging will be emphasized.

Benign lesions are usually horizontally located, with regular borders and soft, while malignant lesions are often vertically oriented, have irregular borders and are stiff. However, there are many exceptions and ultrasound guided biopsy is needed to make the diagnosis. Clinical indications for the use of breast ultrasound will be discussed: the role of ultrasound in the detection and characterization of lesions, as well as the role in the evaluation of neoadjuvant chemotherapy and its role in the immediate postoperative evaluation and long-term surveillance after surgery. Role of ultrasound in screening will be discussed. Bases of ultrasound guided ablations of small breast cancer will be presented. Several clinical examples will be shown.

Keywords: breast, ultrasound, elastography, BI-RADS, breast carcinoma

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CONTEMPORARY APPROACH TOWARDS MAMMOGRAPHY – CONSTRAST-ENHANCED MAMMOGRAPHY

Maja Marolt Mušič

Mammography remains the basic imaging and the most often used modality in detecting breast cancer (BC) in women over 40. However, in women with dense breasts, the sensitivity of this examination is limited. Therefore, imaging methods that add additional functional information due to the enhancement of pathological lesions are appreciated.

Contrast-enhanced mammography (CEM) uses iodinated contrast material to visualize breast neovascularity. Enhancement has been compared to an MRI of the breast, considered the most sensitive examination in detecting malignant and premalignant changes. The sensitivity and accuracy of CEM are reported to be very similar.

The indications for CEM are numerous, and it seems to be an excellent tool for the work-up of recalled women in screening mammography. CEM is a convenient tool for detecting BC in women with dense breasts. It is helpful in the preoperative staging of women with newly diagnosed breast cancer to evaluate additional foci in the same and contralateral breast and to assess response to neoadjuvant therapy.

Due to the increased utilization of CEM, in 2022, a supplement to ACR BI-RADS® Mammography lexicon was published with the descriptors for CEM.

CEM is quickly and easily performed, which allows better accessibility. It is also a good solution for women with claustrophobia and is a viable alternative to breast MRI.

Keywords: contrast-enhanced mammography, breast carcinoma, BI-RADS

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SAVREMENI PRISTUP PERKUTANIM BIOPSIJSKIM PROCEDURAMA U DOJKAMA: BIOPSIJE POD KONTROLOM MR I CESM

Mirjan Nadrljanski

Vakuum asistirane biopsijske procedure u dojkama pod kontrolom MR (magnetne rezonancije) i CESM (kontrastne mamografije), predstavljaju perkutane procedure, koje se realzuju u skladu sa definisanim indikacijama, odnosno u slučaju kada je lezija detektabilna isključivo na MR, odnosno na CESM i nije dostupna vizuelizaciji / biopsiji pod kontrolom drugih imidžing modaliteta.

MR-VAB (Vakuum asistirana biopsija pod kontrolom MR), predstavljaju protokolisanu proceduru, koja pdorazumeva dijagnostički aspekt – vizuelizaciju i karakterizaciju lezije, odnosno pripremni pregled namenskim protokolom sa dojkom komprimovanom u gridu, na

osnovu kog se procenjuje mogućnost tehničke izvodljivosti procedure. Lezije, koje su lokalizovane prepektoralno, retroareolarno ili neposredno u subkutisu nisu pogodne za MR-VAB. Planiranje pregleda podrazumeva izbor pogodne igle (9G), sa različitom širinom akvizicione komore (12mm / 20 mm), odnsno definisanje "target-lezije" i njenih koordinata u trodimenzionalnom sistemu, kao i definisanje pristupnog puta (lateralni / medijalni). Procedura ne podrazumeva vizuelizaciju u realnom vremenu.

VAB pod kontrolom CESM, podrazumeva složenu proceduru, koja je u fazi standardizacije i može da podrazumeva različite pristupe realizaciji, primenom jednog ili dva mamografska aparata. Optimalni način realizacije, podrazumeva vremenski interval (Tmax) do 10 min. nakon aplikacije kontrastnog sredstva na bazi joda, što omogućuje adekvatan dijagnostički pregled – vizuelizaciju zone postkontrastnog povećanja denziteta i definisanje target-lezije za VAB.

Lokalizacija lezije, definisanje optimalnog pristupnog puta igle, kao i razlike u specifičnosti između MRI i CESM, predstavljaju izazove u (re)definisanju indikacija i realizaciji same procedure.

Ključne reči: magnetna rezonancija, kontrastna mamografija, biopsija, perkutana procedura

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KLINIČKO-RADIOLOŠKA KORELACIJA: ZNAČAJ U PROCENI I PLANIRANJU HIRURŠKE INTERVENCIJE U HIRURGIJI KARCINOMA DOJKE

Marko Buta, Nada Santrač

Korelacijom inicijalne radiološke i hirurške evaluacije pacijenta određuje se inicijalni T i N stadijum bolesti, a zatim se planira i način izvođenja biopsije. Na osnovu biološkog profila tumora i stadijuma bolesti, donosi se multidisciplinarna i personalizovana odluka o lečenju pacijenta.

Ukoliko se korelacijom radiološke evaluacije i palpatornog pregleda utvrdi da se radi o solitarnoj leziji u dojci, moguće je učiniti poštednu operaciju dojke. Kada se radi o multiplim tumorima, od izuzetne je važnosti da se utvrdi distribucija lezija, odnosno da se odredi multifokalnost / multicentričnost tumora, na osnovu čega se planira hirurški zahvat. Pacijentkinje koje imaju multifokalni tumor mogu biti kandidati za poštednu hirurgiju ukoliko odnos volumena dojke i ukupnog volumena tumorskih lezija omogućava adekvatno uklanjanje tumora, uz zadovoljavajući estetski ishod, što podrazumeva onkoplastični pristup u hirurgiji karcinoma dojke. Multicentrični tumori su ranije implicirali radikalni hirurški zahvat, mastektomiju sa ili bez očuvanja kože, areole i mamile, što je i danas najčešći pristup lečenju, ali su prema novijim preporukama ovi pacijenti su kandidati za onkoplastičnu hirurgiju, uz dobru preoperativnu pripremu za zahvat.

U sklopu procene N stadijuma bolesti, palpatorni pregled može utvrditi konzistenciju, oblik i mobilnost limfnih nodusa, odnosno njihovu međusobnu slivenost, ali u zavisnosti od konstitutcije pacijenta nije uvek pouzdan, naročito kod gojaznih pacijenata ili naglašenih aksilarnih produžetaka. Od izuzetnog značaja za adekvatno N stažiranje jeste ultrazvučna evaluacija aksilarnih i supraklavikularnih nodusa, dok magnetna rezonancija omogućava i uporednu evaluaciju drenažnih područja i objektivniju procenu nalaza koji nisu jasno patološki.

Ukoliko se korelacijom kliničkog i radiološkog nalaza utvrdi da su pacijenti N0, preporuke su da se izvede biopsija limfnih čvorova stražara - SLNB, bilo u "upfront" hirurškom pristupu ili nakon neoadjuvantne terapije, ukoliko je stadijum i dalje N0. Kod pacijenata kod kojih se klinički i radiološki verifikuju suspektni limfni nodusi, potrebno je načiniti biopsiju dostupnih suspektnih limfnih nodusa u cilju patohistološke verifikacije i plasiranja titanijumskog klipsa kod pacijenata koji su kandidati za ciljanu aksilarnu disekciju.

Ključne reči: karcinom dojke, hirurgija, radiološka evaluacija, klinički pregled, poštedne operacije, radikalna hirurgija

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ZNAČAJ I ULOGA PERKUTANIH PROCEDURA U PLANIRANJU HIRURŠKIH INTERVENCIJA U HIRURGIJI KARCINOMA DOJKE

Milan Žegarac

Perkutane procedure su preduslov u svremenom lečenju karcinoma dojke. Kada su u pitanju suspektne promene u dojci biopsija širokom iglom je svakako standarna procedura za dobijanje tkivnog uzorka. Iz uzorka se određuje priroda promene i u koliko se radi o malignoj leziji molekularna klasifikaciaj karcinoma dojke. Na osnovu molekularne klasifikacije,vrednosti estrogenskih i progesteronskih receptora, HER2 stausa i vrednosti proliferativnog indeksa Ki 67, donosi se odluka o redosledu primena onkoloških modaliteta.

Ako su u pitanju mikrokacifikacije u dojci SVAB procedura je standard radi određivanja histologije lezija i dobijanja smernica za dalji tretman. Punkcija cisticnih lezija omogučava dobijanje uzoraka za citološku analizu koja usmerava daljei tretman.U dijagnostici promena u dojci perkutane procedure su preduslov za planiranje hirurških intervencija.

Ključne reči: perkutane procedure, biopsija, karcinom dojke

MINI SIMPOZIJUM

SINHRONIZOVANO DO CILJA – 20 GODINA KOHLEARNE IMPLANTACIJE NA KLINICI ZA OTORINOLARINGOLOGIJU I MAKSILOFACIJALNU HIRURGIJU UNIVERZITETSKOG KLINIČKOG CENTRA SRBIJE

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20 GODINA KOHLEARNE IMPLANATACIJE U KLINICI ZA ORL I MFH, UKCS

Nenad Arsović, Ljiljana Čvorović, Zoran Dudvarski, Bojana Bukurov, Biljana Krstić

Kohlearna implantacija (KI) je najsavremeniji vid tretmana pacijenata sa teškim senzorinim oštećenjem sluha. Primena ove intervencije kod dece u svetu je započeta 1990, a u Klinici za ORL i MFH, UKCS, koja je najveći centar za implantaciju u Srbiji, radi se od 11. jula 2003. Multidisciplinarni tim za procenu kandidata za KI, od samog početka, sačinjavaju otohirurg, audiolog, anesteziolog, surdolog, logoped i psiholog, UKCS je jedini centar za implantaciju u Srbiji koji ima adekvatnu kadrovsku, prostornu i kompletno opremljenu Kliniku za sve faze u KI koje podrazumevaju preoperativnu pripremu, samu operaciju i dugogodišnji (re)habilitacioni tretman.

Ove godine obeležavamo 20 godina od početka kohlearne implantacije tokom kojih je implantirano 280 implanata (MED-EL 90%, Advance Bionics 10%). Republički fond za zdravstveno osiguranje je pokrivao troškove jednostrane KI kod dece i pacijenata do 26 godine života od 2005, a kod starijih od 26 godina od 2020 (od tada odnos implantirana deca: odrasli 55:36). Kod 83% implantirane dece je uspostavljna adekvatna oralna komunikacija, 80% je obrazovano u redovnim školama.

U početku su se koristile dve tehnike KI- Transkanalni direktni pristup- Veria i Transkanalni direktni pristup sa fiziološkim uglom insercije- naša modifikacija. 10. avgusta 2013. načinjena je prva atraumatska operacija za prezervaciju ostataka sluha i od tada se primenjuju dva pristupa za KI- kroz zadnju timpanotomiju i transkanalni pristup sa fiziološkim uglom insercije. Bilateralna KI je započeta od 2014. i do sada je operisano sedmoro dece. Na Klinici se operišu i komplikovani i kompleksni kandidati za KI kod kojih se očekuje razvoj osifikacije kohlee i oni se operišu po prioritetu (imali smo šestoro pacijenata nakon preležanog meningitisa, troje nakon COVID-19 infekcije i šestoro sa kohlearnom otosklerozom). Operisano je šestoro pacijenata sa kohleo- vestibularnom anomalijom. Pacijenti sa Coganovim sindromom koji imaju progresiju oštećenja vida uz oštećenje sluha takođe imaju prioritet u KI i kod nas ih je operisano troje.

Ključne reči: kohlearna implantacija, senzorni gubitak sluha

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BILATERALNA KOHLEARNA IMPLANTACIJA: AKTUELNI KONCEPTI

Ljiljana Čvorović, Bojana Bukurov, Zoran Dudvarski, Nenad Arsović, Biljana Krstić

Slušanje sa dva uva je odredila priroda jer nudi bolju razumljivost govora, lokalizaciju izvora zvuka, razumevanje govora u bučnom okruženju i dovoljno glasno slušanje. Bilateralnu stimulaciju slušanja treba razmotriti za sve pacijente sa gubitkom sluha.

Većina vodiča za kohlearnu implantaciju (KI) nedvosmisleno preporučuje da se deci sa bilateralnim teškim/dubokim senzornim gubitkom sluha ponudi bilateralna KI, u simultanoj operaciji, pre 9/12 meseci starosti. Kod sekvencijalne bilateralne KI kod dece sa teškim/dubokim gubitkom sluha, preporučuje se smanjenje intervala između dva implantata, poželjno na manje od 12/18 meseci. Preporučuje se i ohrabrivanje dece sa jednostranim KI da nose kontralateralne slušne aparate kada je prisutan rezidualni sluh uz procenu percepcije zvuka testovima slušanja u buci. Preporučuje se da primenjena hirurška tehnika implantacije čuva preostale funkcionalne strukture unutrašnjeg uha što je više moguće. Sve više se diskutuje o neophodnosti ispitivanja funkcije vestibularnog čula u unutrašnjem uvu u preoperativnoj pripremi, njegovom uticaju na odluku koje uvo prvo implantirati i mogućnost bilateralne KI, kao i o uticaju bilateralne KI na vestibularno čulo u ranom motoričkom razvoju deteta. U svim razvijenim zemljama bilateralna KI kod dece je finansirana iz državnog zdravstvenog fonda.

Bilateralna KI kod odraslih sa obostranim teškim/dubokim senzornim gubitkom sluha sa neadekvatnom rehabilitacijom slušnim aparatima se preporučuje nezavisno od starosti pacijanta. U većini razvijenih zemalja zdravstveni fondovi finansiraju bilateralnu implantaciju kod odraslih, izuzev Velike Britanije gde se finansira samo ukoliko pacijent ima i teško oštećenje vida ili postoji oboljenje koji dovodi do obostrane obliteracije labirinta. Kod odraslih sa postlingvalnim teškim/dubokim gubitkom sluha preporučuje se bilateralna sekvencijalna kohlearna

implanatcija do godinu dana od prve. Nekonzistentan je stav o maksimalnom periodu koji može proteći do implanatcije drugog implanta, odnosno koja je maksimalna dužina trajanja gluvoće na neimplantiranom uvu da bi se pacijent bilateralno implantirao. Pojedini vodiči navode pet godina, dok drugi zastupaju stanovište individualnog pristupa svakom pacijentu.

Ključne reči: bilateralna kohlearna implantacija, teško senzorno oštećenje sluha kod dece, teško senzorno oštećenje sluha kod odraslih.

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PRINCIPI HIRURGIJE ČUVANJA OSTATAKA SLUHA KOD KOHLEARNE IMPLANTACIJE

Zoran Dudvarski, Nenad Arsović, Ljiljana Čvorović, Bojana Bukurov

Kohlearna implantacija (KI) predstavlja revolucionarni napredak u lečenju osoba sa urođenom ili stečenom gluvoćom. Indikaciono područje je vremenom značajno prošireno, a principi savremene KI su rezultat napretka u hirurškoj tehnici i tehnološkim dostignućima proizvođača implanata.

Skoro polovina odraslih pacijenata (46%) kojima se radi primarna KI imaju rezidualni sluh na niskim frekvencijama. Zbog toga su indikacije za KI proširene na pacijente koji imaju rezidualni sluh ili normalan sluh u niskofrekventnom regionu i gubitak sluha za srednje i visoke frekvencije tj. imaju audiogram „strmog nagiba" („ski-slope") tako da nemaju koristi od konvencionalnog slušnog aparata. Ova audiološka indikacija se naziva parcijalna gluvoća. Region funkcionalnog sluha na niskim frekvencijama se u okviru ove indikacije proširio sa 500 Hz na 1500 Hz.

Još 1997. godine uveden je koncept kombinovanja električne i akustične stimulacije u lečenju pacijenata sa ne-progresivnom parcijalnom gluvoćom, demonstrirajući izvodljivost KI bez potpunog gubitka rezidualnog sluha. Pokazano je da centralni auditivni sistem može da integriše informacije dobijene električnom stimulacijom pomoću kohlearnog implanta sa akustičnom stimulacijom sluha. Na taj način dobija se kompletnija prezentacija govornih frekvenci i bolje razumevanje govora.

Podaci iz literature govore o kompletnom čuvanju rezidualnog sluha prilikom KI kod 0-68% pacijenata. Gubitak rezidualnog sluha može nastati prilikom KI kao posledica intrakohlearne traume ili posle izvesnog vremena zbog hroničnog inflamatornog procesa. Zbog toga se preporuke za čuvanje rezidualnog sluha odnose na pravilan izbor elektrode, postepenu i sporu inserciju elektrode, širok otvor kohleostome, primenu kortikosteroida, izbegavanje aspiracije perilimfe i sprečavanje kontaminacije unutrašnjeg uva krvlju i koštanom prašinom. Čak i kada se preduzmu sve mere atraumatske hirurgije, rezidualni sluh se gubi kod najmanje 10-20% pacijenata. Takođe, gubitak rezidualnog sluha može nastati posle KI kao rezultat progresije same bolesti.

Aktuelne studije pokazuju da je novi otohirurški softver (Otoplan) pogodan za izračunavanje veličine kohlee, a algoritmi kohlearne segmentacije će dodatno poboljšati kohleometrijske podatke. Za sada ne postoji definitivni zaključak o „idealnoj" dužini elektrode, a čuvanje rezidualnog sluha kod KI zavisi od individualnih anatomskih karakteristika kohlee, nivoa rezidualnog sluha, etiologije gubitka sluha, kao i brojnih drugih faktora pacijenta.

Ključne reči: kohlearna implantacija, rezidualni sluh

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NEONATALNI SKRINING SLUHA - IMPERATIV SADAŠNJOSTI ZA ZDRAVIJU BUDUĆNOST

Snežana Babac

Prevalencija teških kongenitalnih, ili perinatalno stečenih oštećenja sluha iznosi 1 do 3 na 1000 živorođene dece. Obostrana oštećenja sluha veća od 40 dB, onemogućavaju adekvatan razvoj govora, jezika, mentalnih i intelektualnih sposobnosi i dvode do socijalne izolacije praćene emocionalnim poremećajima. Za sada, ne postoji medikamentna niti hiruška terapija koja bi omogućila izlečenje senzorineuralne nagluvosti. Međutim ranom dijagnostikom oštećenja sluha i ranom habilitacijom/rehabilitacijom mogu se izbeći mnogobrojne posledice koje za sobom ostavlja slušni deficit. Uspeh rehabilitacije je u direktnoj zavisnosti od vremena započinjanja. Prevazilaženje problema koje za sobom ostavljaju kongenitalna štećenja sluha postiže se organizovanom ranom detekcijom kroz program unuverzalnog neunatalnog skriniga sluha.

Cilj rada je da se prikažu rezultati univerzalnog neonatalnog skrininga na nivou bolnice i da se ukaže na glavne probleme u implementaciji skrininga i mogućnosti za prevazilaženje istih. Uzorak istraživanja su predstavljala 6645 novorođenčadi u periodu od 2,5 godine.na odeljenju neonatologije KBC "Zvezdara" . Primenjivan je dvofazni skrining protokol sa dve skrining tehnologije, TEOAE i AABR. U slučaju negativnog ishoda retesta, audiološka procena je planirana do trećeg meseca.

Nakon inicijalnog testa (TEOAE) na retest je upućeno 8,5% novorođenčadi a javilo se samo 54%. Na audiološku dijagnostiku upućeno je 0,9% a odziv je bio 64%. Obostrano ošteženje sluha veće od 40 dB je potvrđeno u 1,1 ‰. Prosečna starost pri postavljanu dijagnoze oštežena je bila 3,5 meseca.

Ispitivanje sluha kod svih novorođenčadi kroz unuverzalni neonatalni krining sluha predstavlja imperativ sadašnjosti za zdraviju budućnost. Uvođenje UNSS u porodilišta omogućava započinjanje rane auditivno- verbalne habiliticije primenom slušnog aparata kod nagluve

i kohlearnog implanta kod gluve dece i od presudnog je značaja za integraciju ove dece u društvo.

Ključne reči: univerzalni neonatalni skrining sluha, novorođenčad, dijagnostika, intervencija

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SLUŠNO OŠTEĆENO DETE- DIJAGNOSTIČKI I HABILITACIONI PUT OD PORODILIŠTA DO OSNOVNE ŠKOLE

Biljana Krstić

Univerzalni skrining sluha novorođenčadi (eng.Universal Neonatal Hearing Screening, UNHS) podrazumeva testiranje sluha kod svih beba u prvim danima nakon rođenja. Program ranog otkrivanja sluha i intervencije (eng. Early Hearing Detection and Intervention, EHDI) ima za cilj postizanje referentnih vrednosti EHDI 1–3–6 koje je postavio Zajednički Komitet za dečji sluh (eng. Joint Committee on Infant Hearing, JCIH): pregledajte do 1. meseca života, identifikujte do 3. meseca i započnite ranu intervenciju u prvih 6 meseci. Uvođenjem UNHS/EHDI programa značajno je pomerena granica otkrivanja oštećenja sluha kod dece čime se omogućava intervencija u najranijem uzrastu. Razvijanjem efikasnih UNHS/EDHI programa fokus nije samo na sprovođenju skrininga kod novorođenčadi, već i u ulaganju u ustanove za dijagnostičku evaluaciju, kvalitetnu ranu rehabilitaciju/habilitaciju slušanja i govora, kao i druge terapeutske usluge. Ukoliko ne postoje audiološke dijagnostičke ustanove sa osobljem obučenim za dijagnostiku i praćenje, rano otkrivanje dece sa oštećenjem sluha neće biti moguće. Takođe, bez habilitacije/rehabilitacije i drugih potpora u učenju za decu i njihove porodice, optimalni razvoj deteta nikada neće biti postignut. U audiološkim centrima se sprovodi audiološka evaluacija, postavlja definitivna dijagnoza i sprovodi rana intervencija za otklanjanje negativnih posledica oštećenja sluha. Posledice nedijagnostikovanog i neprepoznatog oštećenja sluha u ranom detinjstvu mogu biti veoma značajne, sa dokazanim negativnim uticajem na razvoj jezika, kognitivni i emocionalni razvoj, kao i na dečju pismenost i stručni potencijal. Tretman se sastoji u bilateralnoj slušnoj amplifikaciji i habilitaciji/rehabilitaciji slušanja i govora. Ukoliko, tokom prvih meseci rehabilitacije, nema adekvatnog napretka uz slušne aparate pristupa se kohlearnoj implantaciji, koja je najsavremeniji vid tretmana. Ovakvim pristupom deca oštećenog sluha razvijaju slušanje i govor do optimalnog nivoa, lakše se uključuju u sredinu čujućih, postaju samostalna i pohađaju redovnu školu.

Ključne reči: skrining sluha, oštećenje sluha, tretman

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KOHLEARNA IMPLANTACIJA I VESTIBULARNA FUNKCIJA

Bojana Bukurov, Ljiljana Čvorović, Zoran Dudvarski, Nenad Arsović

Najčešći urođeni senzorni deficit je senzorineuralno oštećenje sluha, međutim, prevalencija udruženih vestibularnih oštećenja kod ovih pacijenata je takođe visoka, između 20-70%. Većina ovih pacijenata nikada ne razvije vrtoglavicu. Dodano je utvrđeno na temporalnim kostima primaoca kohlearnih implanata da se nakon određenog broja godina od implantacije u unutrašnjem uvu razvijaju fibroza i endolimfatični hidrops koji dodatno kompromituju vestibularnu funkciju. Iz tog razloga, preporuka je da svako dete sa urođenim oštećenjem sluha mora biti detaljno ispitano u cilju otkrivanja udružene vestibularne patologije i njenog praćenja. Cilj ovog prikaza je osvrtanje na učestalost perifernih vestibularnih oštećenja kod dece i odraslih u programu kohlearne implantacije i skretanje pažnje na značaj procene vestibularne funkcije.

Biće prikazana iskustva Klinike za otorinolaringologiju i maksilofacijalnu hirurgije gde se od 2019. godine sprovodi kompletno vestibulološko testiranje kandidata za kohlearni implant. Pored učestalosti udruženih oštećenja, biće prikazni i nalazi na uzorku dece koja su implantirana pre više od 5 godina.

Ukoliko se radi o udruženoj patologiji, vestibularno oštećenje nastaje najčešće zbog same etiologije, ali može biti i neželjeno, zbog implantacije. Kod dece koja su implantirana pre više od 5 godina, nijedno dete nije imalo odgovor sakulusa (tj. cervikalni vestibularni miogeni potencijali se nisu mogli izazvati). Kod 25% je viđen i nešto niži *gain* polukružnih kanala, dok je 60% pacijenta imalo i hiporefleksiju na kaloričkom testu sa implantirane strane.

Prepoznavanje vestibularnih oštećenja kod dece koja su kandidati za kohlearnu implantatciju i praćenje vestibularne funkcije tokom godina je veoma važno, jer ima direktne efekte na njihov motorni razvoj, održavanje ravnoteže i bezbednost. Ne treba zaboraviti ni uticaj vestibularnog oštećenja na kognitivni razvoj dece i mentalni napor pri svakodnevnim aktivnostima.

Ključne reči: gluvoća, kohlearna imolantacija, vestibularna funkcija

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KOMUNIKACIJSKE VEŠTINE KOD KOHLEARNO IMPLANTIRANE DECE

Mina Nikolić

Kohlearna implantacija predstavlja zlatni standard u tretmanu najtežih senzornih oštećenja sluha, gde se i pomoću savremenih digitalnih slušnih aparata maksimalne izlazne snage ne postiže dobar amplifikovani prag sluha, naročito

u visokofrekventnom registru. Dobar auditivni feedback omogućava maksimalni razvoj auditivne percepcije i sazrevanje slušne funkcije kod dece sa urođenom dubokom nagluvošću. To je osnovni uslov uspešne rehabilitacije i razvoja govorno-jezičkih i kognitivnih sposobnosti kod deteta. Pre primene kohlearnog implanta, komunikacijske veštine veoma teško nagluve i praktično gluve dece bile su značajno lošije od njihovih čujućih vršnjaka i oslanjale su se dominantno na neverbalne oblike komunikacije. Jezičko znanje većine ove dece bilo je oskudno, a govor distorzovan i nerazumljiv za čujuću okolinu. Tokom pune tri decenije pedijatrijske kohlearne implantacije u svetu (od 1990. godine) i dve decenije kod nas (od 2002. godine), veliki broj istraživanja potvrdio je značajne rezultate u vidu bolje percepcije zvuka i govora (bez vizuelne podrške), bolje govorne produkcije, jezičke maturacije i veština čitanja u populaciji kohlearno implantirane dece. Kada su u pitanju urođena i rano stečena oštećenja sluha najbolji rezultati postižu se implantacijom u prve četiri godine života, kada se uz adekvatnu postoperativnu rehabilitaciju sluha i govora, postiže puna zrelost kortikalnih auditivnih zona dok se kasnijom implantacijom do 7. godine, usled postepenog gubitka plastičnosti centralnog nervnog sistema, taj efekat postiže kod manje od polovine dece. Osim preporučenog vremena implantacije, istraživanja pokazuju da slušni uzrast deteta, trajanje i intenzitet postoperativne rehabilitacije slušanja i govora u značajnoj meri utiču na dostignuti nivo komunikacijskih veština i govorno-jezičkog razvoja kohlearno implantirane dece.

Ključne reči: oštećenje sluha, kohlearna implantacija, komunikacija, deca

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