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ORIGINALNI NAUČNI RADOVI

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STATUS OF TRYPTOPHAN METABOLITES IN DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE OF NON-DIABETC ETIOLOGY

STATUS METABOLITA TRIPTOFANA U RAZLIČITIM STADIJUMIMA HRONIČNE BUBREŽNE BOLESTI NEDIJABETESNE ETIOLOGIJE

Ivana ISAKOV[1], Velibor ČABARKAPA[1, 2], Branislava SRĐENOVIĆ ČONIĆ[3, 4], Nebojša KLADAR[3, 4], Branislava ILINČIĆ[1, 2] and Dragan BURIĆ[1, 2]

Summary

Introduction. Modification of tryptophan metabolism during the progression of chronic kidney disease may have significant pathophysiological consequences. The aim of this study was to investigate the status of metabolic products of tryptophan, indoxyl sulfate and kynurenine in different stages of chronic kidney disease. **Material and Methods.** In all participants included in the cross-sectional study (n = 66) with previously diagnosed chronic kidney disease, the parameters of renal function were measured: glomerular filtration rate using radionuclide plasma clearance with 99mTc-labelled diethylene triamine penta-acetate and effective renal plasma flow using 131I-labeled orthoiodohippuric acid. Plasma concentrations of indoxyl sulfate and kynurenine were measured by high-performance liquid chromatography. **Results.** A significant difference was observed in the concentrations of both metabolites between the observed groups (Group II – measured glomerular filtration rate - 15 - 60 ml/min/1.73 m²; n = 36 vs. Group I measured glomerular filtration rate > 60 l/min/1.73 m²; n = 26): indoxyl sulfate 1.07 ± 0.89 vs. 2.44 ± 4.05 μg/ml, p < 0.001; kynurenine 3.15 ± 0.22 vs. 3.21 ± 0.17 μg/ml, p < 0.05. The correlation was statistically significant between glomerular filtration rate and kynurenine – r = -0.38, p = 0.001 and indoxyl sulfate – r = 0.56, p ≤ 0.001; effective plasma renal flow and kynurenine – r = -0.33, p < 0.05 and indoxyl sulfate – r = 0.46, p ≤ 0.001. **Conclusion.** There is a significant difference in the plasma concentrations of indoxyl sulfate and kynurenine in the group of patients with glomerular filtration rate of 15 - 60 ml/min/1.73 m² compared to patients with glomerular filtration rate > 60 ml/min/1.73 m². In patients with chronic kidney disease, plasma concentrations of both metabolites of tryptophan are inversely correlated with the glomerular filtration rate and effective plasma renal flow.

Key words: Kidney Failure, Chronic; Tryptophan; Glomerular Filtration Rate; Chromatography, High Pressure Liquid; Biomarkers; Kynurenine; Indican

Sažetak

Uvod. Poremećaj metabolizma triptofana koji se javlja tokom progresije hronična bubrežne bolesti može imati značajne patofiziološke posledice. Cilj ove studije bio je ispitivanje statusa metabolite triptofana, indoksilsulfata i kinurenina u različitim stadijumima hronična bolesti bubrega. **Materijal i metode.** Svim pacijentima obuhvaćenim studijom preseka (n = 66) sa prethodno dijagnostikovanom hroničnom bolesti bubrega izmereni su parametri za procenu bubrežne funkcije: jačina glomerulske filtracije pomoću plazma klirensa radionuklida99mTc-dietilen triamin pentaacetata i efektivni bubrežni protok plazme pomoću 131 I – ortojodhipurne kiseline. Plazmatske koncentracije indoksilsulfata i kinurenina određene su metodom tečne hromatografije visokih performansi. **Rezultati.** Uočena je značajna razlika u koncentracijama oba metabolite između posmatranih grupa (Grupa II – mGFR – 15–60 ml/min/1,73 m²; n = 36) vs (Grupa I mGFR > 60 ml/min/1,73 m²; n = 26): indoksil sulfat 1,07 ± 0,89 vs 2,44 ± 4,05 μg/ml, p < 0,001; kinurenin 3,15 ± 0,22 vs 3,21 ± 0,17 μg/ml, p < 0,05. Korelacija je bila statistički značajna između jačine glomerulske filtracije i kinurenina – r = -0,38, p = 0,001 i indoksil sulfata r = 0,56, p = < 0,001; efektivni bubrežni protok plazme i kinurenin r = -0,33, p < 0,05 i indoksil sulfata – r = 0.46, p = < 0,001. **Zaključak.** Postoji značajna razlika u plazmatskim koncentracijama indoksilsulfata i kinurenina u grupi pacijenata sa jačinom glomerulske filtracije 15–60 ml/min/1,73 m² u poređenju sa pacijentima sa jačinom glomerulske filtracije > 60 ml/min/1,73 m². Kod pacijenata sa hroničnom bolesti bubrega plazmatske koncnetracije oba metabolita triptofana su u obrnutoj korelaciji sa jačinom glomerulske filtracije i efektivnim bubrežnim protokom plazme.

Ključne reči: hronična bubrežna bolest; triptofan; jačina glomerularne filtracije; tečna hromatografija visokih performansi; biomarkeri; kinurenin; indoksil

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Abbreviations

CKD	– chronic kidney disease
KYN	– kynurenine
GFR	– glomerular filtration rate
IS	– indoxyl sulfate
OAT	– organic anion transporter
CCV	– Clinical Center of Vojvodina
M	– male
F	– female
BMI	– body mass index
FMU	– first morning urine
DTPA	– diethylene triamine penta-acetate
ERPF	– effective renal plasma flow
CysC	– Cystatin C
HPLC	– high-performance liquid chromatography
WHR	– waist to hip ratio
HDL-c	– high density lipoprotein cholesterol
LDL-c	– low density lipoprotein cholesterol
CVD	– cardiovascular disease

Introduction

Chronic kidney disease (CKD) includes a heterogeneous group of kidney structure and function disorders that affects about 7% of the adult population over 30 years of age. It has been estimated that the occurrence of moderate forms of kidney function disorders in people over the age of 65 is present in as many as 30% of the population. Renal damage leads to a decline in the excretory, endocrine and metabolic functions of the kidneys [1]. Regardless of the underlying etiology, CKD is a slowly progressive disease leading to irreversible nephron loss and terminal renal disease followed by dialysis [2].

Various substances that are normally excreted by the kidneys accumulate in patients with CKD, which negatively affects many biological functions and contributes to the development of uremic syndrome. It is of great importance that the intestinal microflora is significantly altered due to the progression of CKD. Fermentation of proteins and amino acids by certain intestinal bacteria results in the formation of various metabolites that are absorbed into the circulation and retained in the body [3–7]. Namely, with the progression of CKD, especially in uremia, there is a breakdown of the intestinal epithelial 'tight junction' barriers, which affects the composition and metabolic activity of the intestinal microflora, leading to an increase in the production of harmful substances (toxins) that enter the circulation and cause systemic inflammation [8]. In advanced stages of CKD, especially in terminal forms of the disease, the diversity of intestinal flora is reduced and the presence of aerobic bacteria, such as Enterobacter and Escherichia coli, is increased, leading to an imbalance in the intestinal ecosystem and finally, the production of protein-binding uremic toxins through the proteolysis of undigested proteins retained in the intestines [9].

Tryptophan is an essential amino acid that must be provided through the diet for the needs of the body. Due to the complexity of tryptophan metabolic pathways, different properties of tryptophan metabolic products are associated with different pathophysiological conditions [10, 11]. Approximately one third of the total tryptophan in the body comes from food, and the rest from protein degradation. Tryptophan metabolism involves three metabolic pathways in the gut: 1) the kynurenine (KYN) pathway, in epithelial and immune cells, 2) serotonin pathway, in enterochromic cells, and 3) indole pathway, associated with the intestinal microflora [12]. Over 95% of absorbed tryptophan is catabolized via the kynurenine pathway, while only 1 – 2% via the serotonin and 2 – 3% via the indole pathways [13].

The KYN is the first stable product and the key point of the kynurenine pathway. Three major transformation pathways diverge from KYN: a) deamination to kynurenic acid b) degradation to anthranilic acid, and c) conversion to 3-hydroxykynurenine [14]. The KYN and its metabolites are excreted in the urine reaching the urine during the glomerular filtration process [15].

Indoxyl sulfate (IS) is produced when dietary tryptophan is metabolized into indole. Indole is absorbed by the intestines and reaches the liver through circulation. After metabolic reactions in the liver, indole becomes IS and re-enters the blood circulation. In normal physiological conditions, IS enters the cells of the proximal tubules via organic anion transporters (OAT): OAT1 and OAT3 localized in basolateral membrane of proximal tubule cells, and then it is excreted from the cells via OAT4 localized in the apical membrane of renal tubular cells [16].

Modification of tryptophan metabolism and accumulation of certain toxic metabolites in the body is one of the most important factors for the development of CKD symptoms such as neurological disorders, bone metabolism disorders, impaired lipid metabolism, endothelial dysfunction leading to atherosclerosis, hypercoagulability, and blood vessel calcification, which is directly related to a higher prevalence of cardiovascular incidents [17–21].

The aim of this study was to examine the status of tryptophan metabolic products, IS and KYN, in patients with different stages of CKD of non-diabetic etiology.

Material and Methods

This cross-sectional study was conducted at the Clinical Center of Vojvodina (CCV) in Novi Sad in the period March – October, 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the CCV; informed consent for participation in the study was obtained from each participant.

The study included a total of 66 participants with previously diagnosed CKD who were being treated at the CCV. Considering various pathophysiological mechanisms that led to the development of CKD, an important factor in the selection of participants was information about previously diagnosed diabetes mel-

litus. The participants were divided into two groups based on the values of glomerular filtration rate (GFR). The first group included 26 participants: male (M) = 11, female (F) = 15 with GFR > 60 ml/min/1.73 m², and the second group included 40 participants: M = 22, F = 18, with GFR between 15 – 60 ml/min/1.73 m².

The exclusion criteria from the study were: a) patients under 20 years of age, b) patients treated for diabetes, c) patients with a kidney transplant, d) patients with GFR < 15 ml/min/1.73 m², e) patients with cardiovascular or cerebrovascular accidents in the previous 3 months, f) patients with inflammatory bowel disease, g) patients with impaired liver function, h) patients with a malignant disease.

All anthropometric measurements were performed with participants lightly dressed and barefoot. Body height was measured with a Harpenden anthropometer (Holtain Ltd, Croswell, UK) with 0.1 cm accuracy, while body weight was measured using an electronic scale with 0.1 kg accuracy. The body mass index (BMI) was obtained by calculation according to the formula: body weight in kilograms divided by the square of body height expressed in meters. Waist and hip circumferences were measured using a flexible measuring tape with a precision of 0.1 cm in a standing position. Waist circumference was obtained by measuring at the level between the lowest point of the costal arch and the upper border of the iliac crest, while the hip circumference was measured around the widest part of the thigh.

Blood pressure was measured using a mercury manometer by the Riva Rocci auscultatory method. Blood samples for analysis were taken from the cubital vein in the morning after 12-hour overnight fast, and the urine sample for processing was the first morning urine (FMU). The samples were taken for processing immediately after sampling.

In order to assess kidney function as accurately as possible, the following parameters were assessed:

– The GFR was measured using a single injection 99mTc-diethylene triamine penta-acetate (DTPA) clearance with a two point sampling approach at 180 min and 240 min post injection (37 MBq) according to the method described by Russell et al.

– Effective renal plasma flow (ERPF) was determined by the isotopic clearance of ^{131}I labeled orthoiodohippuric acid (hippuran) from two blood samples, collected after 20 and 30 minutes, using the Blaufox method. The ERPF values were expressed in ml/min/1.73 m^2 [22].

For the quality control of the radiochemical purity of isotopes (> 95%), paper chromatography was used. For accurate measurement of the sample radioactivity, gamma counter with NaI (Tl) crystals (Captus 3000 by Capintec, USA) was used.

– Cystatin C (CysC) was measured by immunoturbidimetric method using a biochemical analyzer ADVIA 1800, and GFR was calculated using formulas:

If the CysC ≤ 0.8: eGFR (mL/min/1.73 m^2) = 133 x (CysC/0.8)-0.499 x 0.996 years (x 0.932 if female).

If the CysC > 0.8: eGFR (mL/min/1.73 m^2) = 133 x (CysC/0.8)-1.3289 x 0.996 years (x 0.932 if female) [23].

– Urea, creatinine and uric acid were determined by standard biochemical methods using a biochemical analyzer ADVIA 1800 with Siemens commercial kits (Siemens kits, Erlangen, Germany);

– Albumin and creatinine concentrations in FMU sample were determined by immunoturbidimetry method using an Alinity C biochemical analyzer and commercial Abbott kits (Abbott kits, Wiesbaden, Germany). The albumin-creatinine ratio index was calculated as the quotient of the obtained values.

Plasma and FMU samples, i.e., prepared diluted solutions for the determination of IS and KYN, were stored frozen at -20 °C until the moment of analysis, for a maximum of six weeks. The IS and KYN in human plasma and urine samples were measured by high-performance liquid chromatography (HPLC) using an Agilent HPLC 1100 system equipped with a diode array and fluorescent detectors (Agilent Technologies, Santa Clara, California, USA). Separation of analytes was done using Nucleosil RP C18 column (250 mm × 4.6 mm, 5 μm particle size). The quantification included isocratic elution at 1 mL/min flow of a mobile phase consisting of 95% (v/v) acetonitrile and 5% (v/v) acetate buffer (15 mM, pH = 4.5) at 25 °C and subsequent fluorescent detection for IS and ultraviolet (UV) detection at 225 nm for KYN. Prior to sample analysis, the corresponding calibration curves for IS and KYN were obtained under the previously stated experimental conditions, based on which the analytical method was validated.

The sample (human plasma and FMU) preparation included mixing 300 μL of sample and 900 μL of formic acid in acetonitrile (1%, v/v), centrifugation at 3500 rpm for 5 minutes and filtration of the obtained supernatant in the chromatography tube. The injection volume was 5 μL [24, 25].

Complete blood count was performed using a SYSMEX XN hematology counter with Siemens commercial kits (Siemens Health Care, Erlangen, Germany). Laboratory parameters (glucose, lipid status, apolipoprotein AI, apolipoprotein B, vitamin B12, folic acid) were determined by standard biochemical methods using the biochemical analyzer ADVIA 1800 with Siemens commercial kits (Siemens kits, Erlangen, Germany), while insulin was processed using an Alinity analyzer with Abbott commercial kits (Abbott kits, Wiesbaden, Germany).

Statistical analysis was done using the statistical program Statistica 20 (StatSoftInc, Tulsa, OK, USA). The data are shown by mean values ± standard deviation (SD). The F- and T-tests were used to determine statistically significant differences between groups of participants, while the χ^2 test was used for categorical data. Linear regression analysis was used for determining the correlation between different parameters. Values of $p < 0.05$ were considered statistically significant.

Results

A comparison of general characteristics between the two examined groups is given in **Table 1.** The average age was significantly lower in Group I than

Table 1. Characteristics of examinees with different stages of chronic kidney disease
Tabela 1. *Karakteristike ispitanika u različitim stadijumima hronične bubrežne bolesti*

Parameter *Parametar*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/ *II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p-value *p-vrednost*
Gender (male/female)/*Pol (muški/ženski)*	11/15	22/11	0.31
Age (years)/*Starost (godine)*	45.57 ± 16.36	60.18 ± 11.52	***< 0.001***
BMI (kg/m²)/*Indeks telesne mase (kg/m²)*	26.77 ± 3.69	28.35 ± 4.51	0.19
Waist circumference (cm)/*Obim struka (cm)*	91.81 ± 10.44	99.05 ± 13.72	***< 0.05***
WHR/*Odnos struk–kuk*	0.87 ± 0.08	0.93 ± 0.12	***< 0.05***
Hypertension (yes/no)/*Hipertenzija (da/ne)*	12/14	34/6	***0.05***
Systolic blood pressure (mmHg)/*Sistolni krvni pritisak (mmHg)*	132.61 ± 21.56	133.70 ± 26.89	0.86
Diastolic blood pressure (mmHg)/*Dijastolni krvni pritisak (mmHg)*	80.0 ± 11.7	80.0 ± 10.2	0.93
Glucose (mmol/L)/*Glukoza (mmol/L)*	5.28 ± 0.75	5.92 ± 1.71	0.07
HDL cholesterol (mmol/L)/*HDL holesterol (mmol/L)*	1.42 ± 0.45	1.22 ± 0.30	***< 0.05***
LDL cholesterol (mmol/L)/*LDL holesterol (mmol/L)*	3.35 ± 1.60	2.97 ± 0.82	0.21
Triglycerides (mmol/L)/*Trigliceridi (mmol/L)*	1.44 ± 1.00	1.71 ± 1.00	0.27
Apolipoprotein A1 (g/L)/*Apolipoprotein A1 (g/L)*	1.54 ± 0.35	1.42 ± 0.25	0.11
Apolipoprotein B (g/L)/*Apolipoprotein B (g/L)*	1.05 ± 0.40	1.01 ± 0.21	0.56
Insulin (mIU/L)/*Insulin (mIU/L)*	17.44 ± 13.72	23.74 ± 21.14	0.18
Folic Acid (nmol/L)/*Folna kiselina (nmol/L)*	12.13 ± 6.05	13.46 ± 9.02	0.51
Vitamin B12 (pmol/L)/*Vitamin B12 (pmol/L)*	238.07 ± 107.93	316.55 ± 340.49	0.26

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije;* BMI – body mass index/*indeks telesne mase*; WHR – waist to hip ratio/*Odnos struk/kuk*; HDL – high density lipoprotein/*lipoprotein visoke gustine*; LDL – low density lipoprotein/*lipoprotein niske gustine*

in Group II (45.6 ± 16.4 vs. 60.2 ± 11.5 years, p < 0.001). Among Group I participants, waist size and waist to hip ratio (WHR) were significantly lower compared to Group II participants (91.8 ± 10.4 vs. 99.1 ± 13.7 cm, p = 0.03; 0.9 ± 0.1 vs. 0.9 ± 0.1, p < 0.01). There was no significant difference in gender, BMI or in the measured blood pressure values (p = 0.31, p = 0.19, p = 0.93). As a comorbidity, hypertension was more common in Group II patients (12/14 vs. 34/6, p = 0.049). Statistically significant differences were observed between HDL cholesterol values, which were lower in Group II participants (1.42 ± 0.45 vs. 1.22 ± 0.30 mmol/L, p < 0.05).

The parameters used to assess the state of renal function are showed in **Table 2.** All the observed parameters, as expected, showed a statistically significant difference between the two groups (p < 0.001), except for albumin in FMU (p = 0.39).

Table 2. Parameters for the assessment of kidney function
Tabela 2. *Parametri za procenu bubrežne funkcije*

Parameter *Parametar*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/ *II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p-value *p-vrednost*
Urea (mmol/L)/*Urea (mmol/L)*	5.23 ± 1.28	9.52 ± 3.78	***< 0.001***
Creatinine (mmol/L)/*Kreatinin (mmol/L)*	80.53 ± 18.26	128.00 ± 57.54	***0.001***
mGFR – DTPA (ml/min/1.73 m²)/*JGF – DTPA (ml/min/1,73 m²)*	79.7 ± 15.0	39.7 ± 11.8	***< 0.001***
ERPF (ml/min)/*EBPP (ml/min)*	421.9 ± 90.3	250.4 ± 62.6	***0.001***
Cystatine C (mg/L)/*Cistatin C (mg/L)*	1.02 ± 0.19	1.75 ± 0.65	***< 0.001***
GFR Cystatine C/*JGF Cistatin C*	80.76 ± 21.38	42.40 ± 18.06	***< 0.001***
Creatinine FMU (mmol/L)/*Kreatinin PJU (mmol/L)*	7.88 ± 6.34	5.49 ± 2.86	***0.04***
Albumine FMU (g/L)/*Albumin PJU (g/L)*	105.33 ± 276.61	189.95 ± 452.04	0.39
Albumine/Creatinine Ratio (FMU)/*Albumin/kreatinin odnos (PJU)*	24.73 ± 69.46	49.36 ± 134.79	0.39

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; ERPF – effective renal plasma flow/*EBPP – efektivni bubrežni protok plazme*; FMU – first morning urine/*PJU – prvi jutarnji urin; DTPA – dietilen triamin penta acetat*

Table 3. Concentration of indoxyle sulfate and kynurenine in plasma and first morning urine sample
Tabela 3. *Koncentracije indoksil sulfata i kinurenina u plazmi i prvom jutarnjem urinu*

Variables *Varijable*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/*II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p–value *p–vrednost*
Indoxyl sulfate – p (μg/ml)/*Indoksil sulfat – p (μg/ml)*	1.07 ± 0.89	2.44 ± 4.05	***< 0.001***
Indoxyle sulfate FMU (μg/ml)/*Indoksil sulfat PJU (μg/ml)*	16.93 ± 34.80	22.98 ± 50.76	0.655
Kynirenine – p (μg/ml)/*Kinurenin – p (μg/ml)*	3.15 ± 0.22	3.21 ± 0.17	***< 0.05***
Kynurenine FMU (μg/ml)/*Kinurenin PJU (μg/ml)*	2.91 ± 0.49	2.85 ± 0.27	0.906

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; p – plasma/*plazma*; FMU – first morning urine/*PJU – prvi jutranji urin*

Table 4. Correlation analysis of indoxyle sulfate and kynurenine concentrations with the selected parameters
Tabela 4. *Korelaciona analiza koncentracije indoksil sulfata i kinurenina sa selektovanim parametrima*

	Kynurenine *Kinurenin*		Indoxyl sulfate *Indoksil sulfat*	
Parameter *Parametar*	r	p–value *p–vrednost*	r	p–value *p–vrednost*
Age/*Starost*	0.02	0.81	0.1	0.41
Waist circumference/*Obim struka*	0.17	0.90	0.29	***0.01***
WHR/*Odnos struk/kuk*	0.12	0.33	0.27	***0.02***
BMI (kg/m²)/*Indeks telesne mase (kg/m²)*	0.18	0.08	0.39	***< 0.001***
Total cholesterol (mmol/L)/*Ukupni holesterol (mmol/L)*	-0.17	0.16	0.17	0.16
HDL cholesterol (mmol/L)/*HDL holesterol (mmol/L)*	-0.19	0.12	0.19	0.12
LDL cholesterol (mmol/L)/*LDL holesterol (mmol/L)*	-0.20	0.09	0.12	0.35
Triglycerides (mmol/L)/*Trigliceridi (mmol/L)*	0.16	0.21	0.05	0.66
Apolipoprotein A I (g/L)/*Apolipoprotein A I (g/L)*	-0.07	0.55	0.21	0.08
Apolipoprotein B (g/L)/*Apolipoprotein B (g/L)*	-0.12	0.30	0.12	0.34
Urea (mmol/L)/*Urea (mmol/L)*	0.39	***0.001***	0.70	***< 0.001***
Creatinine (mmol/L)/*Kreatinin (mmol/L)*	0.28	***< 0.05***	0.38	***< 0.001***
mGFR – DTPA (ml/min/1.73 m²)/*JGF – DTPA (ml/min/1,73 m²)*	-0.38	***0.001***	-0.56	***< 0.001***
ERBF (ml/min)/*EBPP (ml/min)*	-0.33	***< 0.05***	-0.46	***< 0.001***
Cystatine C (mg/L)/*Cistatin C (mg/L)*	0.30	***< 0.05***	0.69	***< 0.001***
GFR Cystatine C/*JGF Cistatin C*	-0.27	***< 0.05***	-0.54	***< 0.001***
Albumine/Creatinine Ratio (FMU)/*Albumin/Kreatinin odnos (PJU)*	0.15	***0.24***	0.25	***< 0.05***

Legend/*Legenda:* WHR – waist to hip ratio/*odnos struk/kuk*; HDL – high density lipoprotein/*lipoprotein velike gustine*; LDL – low density lipoprotein/*lipoprotein niske gustine*; GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; ERBF – effective renal blood flow/*EBPP – efektivni bubrežni protok plazme*; FMU – first morning urine/*PJU – prvi jutarnji urin*

The comparison of the measured concentrations of IS and KYN in plasma, showed higher plasma concentrations for both metabolites in Group II (IS 1.07 ± 0.89 vs. 2.44 ± 4.05 μg/ml, $p < 0.001$; KYN 3.15 ± 0.22 vs. 3.21 ± 0.17 μg/ml, $p < 0.05$), while the differences in FMU concentrations were insignificant (**Table 3**).

The correlation of metabolites with selected traits is shown in **Table 4.** Both metabolites showed a significant correlation with the parameters that are markers of renal function preservation. The KYN had a moderate correlation with urea, creatinine, eGFR - DTPA, ERPF and CysC ($p < 0.05$), while IS showed a high correlation with the same parameters ($p < 0.001$).

Discussion

Examination of the status of tryptophan metabolites, IS and KYN, in patients with different stages of CKD, showed that plasma concentrations of both metabolites were statistically significantly higher in CKD participants with GFR between 15 – 60 ml/min/1.73 m², compared to participants with GFR higher than 60 ml/min/1.73 m² (IS $p < 0.001$; KYN $p < 0.05$).

Two main causes are generally mentioned as the reasons for the increase in the level of the mentioned metabolites. Firstly, the increased immune activity leads to increased levels of proinflammatory factors, resulting in an increased activity of KYN pathway enzymes, and secondly, a decrease in the renal excretion

of these metabolites due to CKD related impaired renal function, leads to their accumulation in circulation and tissues [1, 26]. Another contributing factor to the altered tryptophan metabolism in CKD is the breakdown of intestinal 'tight junction' barriers, which affects the metabolic activity of the intestinal microflora [8].

The impaired tryptophan metabolism and the consequent accumulation of these metabolites are associated with a number of pathophysiological mechanisms in patients with CKD.

One of the mechanisms refers to the association of toxins produced by tryptophan fermentation and the occurrence of cardiovascular diseases (CVDs) in patients with CKD [19, 27]. Namely, tryptophan metabolites, formed mainly within the indole and KYN pathways, have pro-oxidative, pro-inflammatory, pro-coagulant and pro-apoptotic effects [27].

As a comorbidity, hypertension was, as expected, more common in patients with greater renal impairment. The effect of KYN and its metabolites on blood pressure has not been fully elucidated, because many studies suggest that certain metabolites play contradictory roles in vascular pressure [28, 29]. There are few studies on this topic, and some of them prove that certain metabolites of the KYN pathway may also be part of antihypertensive compensatory mechanisms. Hence, this issue requires further research so that the impact of changes in KYN pathway activity on the development of hypertension can be better understood.

Based on the available data related to our study groups, which refer to the health condition of participants, we noticed some differences in the number of antihypertensive drugs used by the examinees. In Group I (26 participants), 11 patients were without any antihypertnesive therapy, while the rest (n = 15) used 2 drugs on average, while in Group II (40 participants), only 2 patients were without any antihpertensive therapy, and more than half (n = 21) used 3 or more drugs as part of therapeutic protocols.

A correlation analysis showed that both metabolites of tryptophan significantly correlated with the renal function assessment parameters. The IS showed a high correlation with urea, creatinine, DTPA, ERBF, CysC ($r = 0.7$, $r = 0.38$, $r = -0.56$, $r = -0.46$, $r = 0.69$; $p < 0.001$), while KYN had a moderate correlation with the same parameters ($r = 0.39$, $r = 0.28$, $r = -0.38$, $r = -0.33$, $r = 0.30$; $p < 0.05$).

Harmful effects of tryptophan metabolites have been described in some earlier studies. The KYN metabolites can affect the proliferation rate of mesangial cells as well as the level of gene expression in these cells. In addition, elevated levels of KYN and its metabolites are associated with the progression of renal failure and glomerular fibrosis [16]. Also, IS significantly accumulates in the kidneys, inducing inflammatory reactions and enhancing oxidative stress, accelerating glomerular sclerosis and interstitial fibrosis, thus aggravating the decline of renal function [30]. It has been shown that IS specifically has a direct cytotoxic effect on renal tubular epithelial cells; after its cellular uptake by organic anion transporter, IS induces tubular cell necrosis [15]. Together, these mechanisms may contribute to the progression of CVDs and CKD [31].

Our study revealed that waist circumference (91.81 ± 10.44 vs. 99.05 ± 13.72 cm, $p = 0.03$) and WHR (0.87 ± 0.078 vs. 0.93 ± 0.12, $p = 0.01$) were significantly higher in the group with GFR 15 – 60 ml/min/1.73m^2, while the BMI value did not differ significantly between the examined groups. Our data are in line with the results of other authors [32] who emphasize WHR as one of the most important predictive factors for the development of CKD compared to other indices, pointing out that fat redistribution is important for the development of CKD, and not obesity per se.

A correlation analysis of IS and KYN showed a moderate correlation of IS with waist circumference ($r = 0.3$, $p = 0.01$), WHR ($r = 0.27$, $p = 0.02$) and BMI ($r = 0.39$, $p < 0.001$).

Of the metabolic parameters included in the study, only the level of HDL-c showed a significant difference between subjects of Group I and II, whereas lower values were observed in Group II participants (1.42 ± 0.45 vs. 1.22 ± 0.30 mmol/L, $p = 0.03$).

Reduced renal function is known to be associated with modification of lipoprotein structure and lipoprotein metabolism disorders [33–35]. According to some studies, the progressive loss of renal function is associated with increased concentration of IS and a decreased level of HDL-c [36] and a study by Li Wang et al. showed that there is an association between IS and HDL-c levels regardless of renal impairment, i.e., that IS is an independent risk factor for low HDL-c concentrations [37]. A correlating analysis of our results revealed that IS and KYN did not show a significant association with HDL-c values in the participants included in the study.

Conclusion

Based on the results of this study, it can be concluded that there is a significant difference in the concentrations of indoxyl sulfate and kynurenine in relation to different stages of chronic kidney disease, and that it is inversely correlated with measured glomerular filtration rate and measured effective renal plasma flow. Considering the numerous harmful effects of elevated plasma concentrations of indoxyl sulfate and kynurenine, further research is needed for a more comprehensive consideration of all pathophysiological mechanisms of chronic kidney disease involved in the dysregulation of tryptophan metabolism, as well as for finding an appropriate therapeutic model whose timely application would potentially reduce the aforementioned adverse effects, that is, slow down their clinical manifestation.

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PERIOPERATIVE BEVACIZUMAB IN THE TREATMENT OF COLORECTAL CANCER IN PATIENTS WITH LIVER METASTASES

PERIOPERATIVNO LEČENJE BEVACIZUMABOM PACIJENATA SA KOLOREKTALNIM KARCINOMOM I METASTAZAMA U PARENHIMU JETRE

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Summary

Introduction. Patients with colorectal cancer with metastases in the liver parenchyma may benefit from perioperative chemotherapy with biological agents and operative liver resection. **Material and Methods**. This prospective, multicenter, non-interventional study included 191 previously untreated patients with metastatic colorectal cancer and potentially resectable or initially unresectable liver metastases who received bevacizumab plus chemotherapy. The safety profile, as well as progression-free-survival, response rate and conversion rate of initially unresectable metastases to resectable were assessed. **Results.** A total of 40 adverse events were reported in 29/191 patients (15.2%), of which 31 were serious adverse events. Among the serious adverse events, 14 were related to the use of bevacizumab therapy, of which 4 were fatal due to serious adverse events, but only one could be related to bevacizumab therapy. The median progression-free period was 9 months (1 - 28). A high rate of response to the applied therapy, 34.5% and 49%, was recorded in both groups of patients: with initially unresectable and potentially resectable metastases in the liver parenchyma. A significant part of patients with metastatic colorectal cancer and metastases only in the liver parenchyma had a clinical benefit from intensive chemotherapy with bevacizumab (disease control rate of 70%). **Conclusion.** This study confirmed a favourable safety profile and tolerability in terms of the incidence and severity of adverse and serious adverse events. High rates of resectability in both groups of patients, initially unresectable and potentially resectable, reflect the heterogeneity of criteria in decision making about liver resection and emphasize the need for establishing multisciplinary oncology teams and following the generally accepted criteria.

Key words: Bevacizumab; Perioperative Care; Colorectal Neoplasms; Neoplasm Metastasis; Liver Neoplasms; Drug Therapy; Risk Assessment; Treatment Outcome; Antineoplastic Combined Chemotherapy Protocols; Drug-Related Side Effects and Adverse Reactions

Sažetak

Uvod. Pacijenti oboleli od kolorektalnog karcinoma sa metastazama u parenhimu jetre imaju korist od primene perioperativnih hemioterapija uz biološku terapiju i operativne resekcije metastaza u parenhimu jetre. **Materijal i metode.** U ovu prospektivnu, multicentričnu, neintervencijsku studiju uključen je 191 prethodno nelečeni pacijent sa metastatskim karcinomom kolorektuma i potencijalno resektabilnim ili inicijalno neresektabilnim metastazama u jetri koji su primili perioperativno bevacizumab uz hemioterapiju. Bezbednosni profil kao i period bez progresije bolesti, odgovor na primenjenu terapiju kao i konverzija incijalno neresekabilnih metastatskih promena u jetri u resektabilne su takođe procenjivani. **Rezultati.** Zabeleženo je ukupno 40 neželjenih događaja 29/191 (15,2%), od toga 31 ozbiljan neželjeni događaj. Među ozbiljnim neželjenim događajima 14 je bilo u vezi sa primenom bevacizumab terapije od kojih su četiri bila smrtna ishoda, od toga samo se jedan mogao povezati sa terapijom bevacizumabom. Srednji period bez progresije bolesti bio je devet meseci (1–28). Visoka stopa odgovora na primenjenu terapiju, 34,5% i 49% zabeležena je u obe grupe pacijenata sa inicijalno neresektabilnim i potencijalno resektabilnim metastazama u parenhimu jetre. Značajan deo pacijenata sa kolorektalnim karcinomom sa metastazama i metastazama samo u parenhimu jetre, imali su kliničku korist od sprovedene intenzivne hemioterapije bevacizumabom (stopa kontrole bolesti od 70%). **Zaključak.** Ova studija je potvrdila povoljan sigurnosni profil i podnošljivost u pogledu učestalosti neželjenih i ozbiljnih neželjenih događaja. Visoke stope resektibilnosti u obe grupe pacijenata, inicijalno neresektabilnih i potencijalno resektabilnih, odražavaju heterogenost kriterijuma u donošenju odluka o resekciji jetre i naglašavaju potrebu za uspostavljanjem multidisciplinarnih timova i praćenjem opšteprihvaćenih kriterijuma.

Ključne reči: bevacizumab; perioperativna nega; kolorektalne neoplazme; metastaze; neoplazme jetre; farmakoterapija; procena rizika; ishod lečenja; kombinovani antineoplastični hemoterapijski protokoli; neželjeni efekti i neželjene reakcije

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Abbreviations

CRC – colorectal cancer
RR – response rate
PFS – progression free survival
mCRC – metastatic colorectal cancer
CRF – case report form
ITT – intention-to-treat
DFS – disease free survival

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide. Approximately 50% of patients suffering from colorectal cancer will develop metastases, usually in the liver, while 25% have metastases at diagnosis [1]. Survival strongly correlates with the extent of the disease. In recent years, perioperative chemotherapy for patients with liver only metastatic colorectal cancer (mCRC) is commonly used in routine clinical practice. The advantages of perioperative therapy include effects on micrometastases, tumor chemo-sensitivity testing (by radiological and pathological methods), which is important in the choice of adjuvant chemotherapy. Furthermore, by assessing the aggressiveness of the disease, perioperative therapy may indicate patients who will not benefit from the resection [2]. It is known that resection of liver metastases is the best chance for cure of these patients [3] with a five-year survival rate of 25 – 40% [4], which may go to 55% or even higher [5, 6]. Reflecting this, conversion to resectability of liver metastases is a very important step in the treatment of mCRC patients.

Bevacizumab (Avastin, Roche) is a biologic therapy that provides statistically significant increase in response rate (RR), overall survival (OS) and progression free survival (PFS), while, in combination with fluoropyrimidine chemotherapy, provides quality survival of mCRC patients [7]. Bevacizumab inhibits vascular endothelial growth factor, leading to normalization of tumor blood vessels and reduction of intratumoral pressure, which improves the delivery of chemotherapeutic agents to tumor cells [8–11]. Based on numerous clinical evidence and worldwide routine clinical practice experience, bevacizumab, in combination with chemotherapy, has become a standard therapy for mCRC.

Data showed that colorectal cancer patients with mCRC may benefit from perioperative chemotherapy with biological agents and liver resection [12, 13]. Resectability rate may be increased by aggressive approach with triplet chemotherapy consisting of doublet chemotherapy and monoclonal antibody.

It is important to select patients with resectable metastases and those with initially unresectable disease in whom the metastases may become resectable after a major response has been achieved with combined chemotherapy. The role of perioperative treatment may therefore be to convert initially unresectable liver metastases of mCRC to resectable and to increase the resectability rate.

Monitoring of bevacizumab efficacy in routine practice with safety monitoring should enable proper dosing and treatment duration. Desired outcomes of perioperative usage of combination of chemotherapy with bevacizumab may lead to higher resectability rate, as well as RR and PFS.

The aim of this study was to demonstrate that in treatment of mCRC, according to local label and local standard of care, bevacizumab represents a valuable perioperative therapy option.

The primary objective was to assess the safety profile of bevacizumab combined with chemotherapy regimens. The secondary objective was to evaluate RR (including rate of conversion from primary unresectable to resectable) and PFS.

Material and Methods

This open-label, multicenter non-interventional phase IV study evaluated the safety and efficacy of bevacizumab in the perioperative treatment of mCRC patients.

Study was conducted at 6 clinical sites in Serbia. The patients were recruited from March 2009 to October 2012. The last subject's last visit was in August 2014.

The target population included previously untreated mCRC patients, aged > 18, eligible for bevacizumab treatment per local label and clinical practice. A total of 191 patients signed informed consent forms for participation in the study. The main exclusion criterion was contraindication to bevacizumab therapy as per locally approved prescribing information.

All study procedures were conducted in accordance with routine clinical practice. Before the initiation of therapy, patients were evaluated in terms of resectability of liver metastases. Assessment of resectability was performed using the following criteria: remnant liver volume ≥ 30%, lesions near and/or invading vascular and/or biliary vessels, and feasibility for removal of all liver lesions. Additionally, the Fong clinical risk score was used to assess the risk of recurrence using the following parameters: involvement of lymph nodes, appearance of metastases within 12 months from primary tumor surgery, carcinoembryonic antigen levels, number of liver metastases, and sites of metastases. Based on the final score, patients were classified as low, medium and high risk patients.

Patients enrolled in the study received preoperative chemotherapy with bevacizumab, every two or three weeks.

Bevacizumab was administered as an intravenous infusion at a dose of 5 mg/kg once every 2 weeks, up to maximum 10 cycles. Standard chemotherapy regimens were used: FOLFOX4, (oxaliplatin 85 mg/m^2 on day 1, leucovorin 200 mg/m^2, fluorouracil IV bolus 400 mg/m^2, then fluorouracil continued infusion for 22 hours 600 mg/m^2), FOLFIRI (irinotecan 180 mg/m^2 on day 1, leucovorin 200 mg/m^2, fluorouracil bolus 400 mg/m^2, then fluorouracil

continued infusion for 22 hours 600 mg/m^2), XELOX (oxaliplatin 130 mg/m^2 on day 1, capecitabine 1000 mg/m^2, 1 – 14 days), XELIRI (irinotecan at 175 mg/m^2 on day 1, capecitabine 1000 mg/m^2, 1 – 14 days). After four cycles of bevacizumab-containing regimens, tumor assessments were performed. In the majority of cases, radiology evaluation was done using computed tomography, while magnetic resonance imaging and positron emission tomography and computed tomography were used on demand. Radiology assessment was performed as per local standard of care, using Response Evaluation Criteria in Solid Tumors 1.1.

Clinical assessments included RR evaluation, evaluation of resectability, PFS and safety monitoring according to routine practice. Patients whose status did not convert to resectable could continue with therapy for up to 10 cycles. Patients who were evaluated as resectable underwent surgery after mminimum 4 weeks following the last dose of bevacizumab. After surgery, patients could have received chemotherapy, but without bevacizumab, as per local regulations.

All patients were followed until progression of the disease, unacceptable toxicity, lost to follow up, death of any cause, or withdrawal of informed consent. The following data were analyzed: baseline patient characteristics, previous treatment, and current treatment, duration of treatment, conversion to resectability, RR and therapy outcome, PFS, and frequency and grade of adverse events/severe adverse events.

All patient data were recorded in paper case report forms (CRF) and in a timely manner validated by the responsible specialist. Data entered in the CRF were matched with data entered in the medical history of each patient. Statistical analysis was performed based on clinical database for this study. All analyses were performed in intention-to-treat (ITT) population.

Descriptive statistical analysis was performed including all baseline patient characteristics. All subjects enrolled in the study who received at least one dose of study medication were included in analysis. Categorical data were analyzed using a chi-square test and Fisher's exact test, as appropriate.

The incidence and severity of adverse events were assessed in order to determine the safety and tolerability of bevacizumab. Sample size was calculated according to gathered data about the safety and tolerability of bevacizumab.

The analysis of ITT population included all patients who received at least one dose of the study drug and had subsequent post baseline assessment. All adverse events reported during the observational period were included in the analysis of the safety data.

Results

Baseline characteristics are presented in **Tables 1** and **2**. At initial presentation, metastatic disease was found in 41.9% of patients. Previous adjuvant treatment was received by 63 patients (33%). Disease free survival (DFS) in patients who were initially diagnosed as Dukes B or C was 12 months **(Table 3)**. Mean sum of the longest diameters of liver metastases was 7.7 cm. In the study, 95% of

Table 1. Demographic characteristics of patients
Tabela 1*. Demografske karakteristike pacijenata*

Baseline characteristics/*Osnovne karakteristike*	No./Br. = 191 (%)
Gender/*Pol*	
Male/*Muški*	120 (62.8)
Female/*Ženski*	71 (37.2)
Age/*Uzrast*	
Mean/*Srednja vrednost*	59.31 ± 10.23
Median/*Medijana*	61
Minimum/*Minimum*	27
Maximum/*Maksimum*	78

Table 2. Performance status and initial stage of patients at diagnosis
Tabela 2*. Performans status i inicijalni stadijum pacijenata u momentu dijagnoze*

ECOG Performance status/*Opšte stanje pacijenta*	
0	149 (78%)
1	40 (21%)
Unknown/*Nepoznato*	2 (1%)
Stage at initial diagnosis/*Stadijum kod inicijalne dijagnoze*	
Dukes B	43 (22.5)
Dukes C	67 (35.1)
Dukes D	80 (41.9)
Carcinoma in situ	1 (0.5)

Legend: ECOG – Eastern Oncology Cooperative Group/*Legenda: ECOG – Istočna kooperativna onkološka grupa*

Table 3. Disease free survial
Tabela 3. *Prikaz vremena bez bolesti kod pacijenata*

DFS (months)/*Vreme bez bolesti (meseci)*	
Mean/*Srednja vrednost*	15.84 ± 14.7
Median/*Medijana*	12
Minimum/*Minimum*	1
Maximum/*Maks*imum	96
Age distribution (years)/*Starosna distribucija (godine)*	N = 191 (%)
Age (years)/*Starost*	
18 - 64	126 (66%)
65 - 84	65 (34%)
85 years and over/*85 godina i stariji*	0 (0)

patients received oxaliplatin-based regimen, mostly FOLFOX4 or XELOX, only 5% received irinotecan-based chemotherapy.

In regard to resectability evaluation, 89% of patients were evaluated as potentially resectable with remnant liver volume of ≥ 30%. In 79% of patients, potential resection could be done with microscopically negative margins (R0) and 5.8% of patients had metastases surrounding important vascular and biliary vessels. In terms of prognostic parameters, assessment was done in 98% of patients: 45% were classified as low risk, 52% as medium, and 1% of patients were classified as high risk.

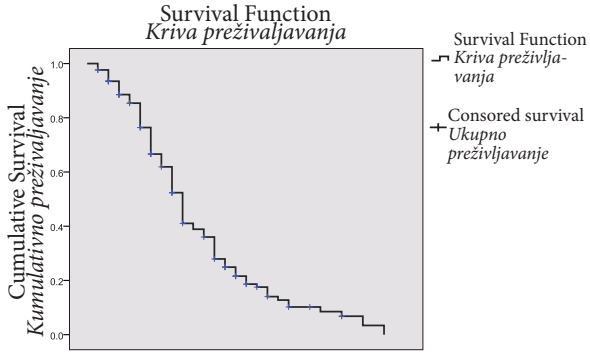
Based on these parameters, all included patients were classified as potentially resectable (162 patients) and initially unresectable (29 patients), that could be converted to resectable.

Patients with initially unresectable liver metastases of mCRC

The mean number of initial bevacizumab cycles was 4 (1 – 8). Complete remission was achieved in 7%, partial response in 31%, stable disease (SD) in 31%, and progressive disease was recorded in 13.8% of patients, while the response was not assessed or data were missing in 17.2% of patients. Four patients (13.8%) continued bevacizumab therapy after the first evaluation, but on second evaluation they were confirmed as unresectable. Ten patients (34.5%) had undergone liver resection. A microscopically negative margins resection was performed in 4 patients (40%, or 13.8% of all unresectable patients), without recorded perioperative complications, and 2 patients continued therapy after resection.

Patients with potentially resectable liver metastases of mCRC

The mean number of initial bevacizumab cycles was 4.5 (1 – 12). A complete remission was achieved in 5.6%, PR in 25.3%, SD in 42%, and disease progression was recorded in 17.9 of patients, while the response was not assessed or data were missing in 9.2% of patients. Thirty-four patients (21%) continued bevacizumab therapy after the first evaluation, but on second evaluation only 1 patient was considered for liver resection, after the 7th cycle. Eighty patients (49.4%) had undergone liver resection. A microscopisally negative margins resection was performed in 61 patients (76%), transitional perioperative complications were recorded in 4 patients, and 21 patients continued therapy after resection. In terms of PFS, median PFS in ITT population was 9 months (1 – 28) **(Graph 1)**.



Graph 1. Median disease free survival in intention-to-treat population
Grafikon 1. *Medijana vremena bez progresije bolesti kod populacije sa namerom lečenja*

Adverse events

In this study, adverse events were reported in 29/191 patients (15.2%). Of 40 reported adverse events, 31 were serious **(Table 4)** and 9 non-serious **(Table 5)**. A detailed description of all adverse events is listed below. Among serious adverse events, 14 were designated as related to bevacizumab therapy, of which the majority (7/14) were vascular disorders, which all resolved with recovery. There were four fatal outcomes due to serious adverse events. One fatal outcome occurred in a patient with febrile neutropenia that was not related to bevacizumab treatment. The second fatal outcome occurred in a patient with multiple serious adverse events (diarrhea, fatigue, fever, abdominal pain, acute renal failure), and this outcome was reported as related to bevacizumab treatment. The third fatal event was sudden death, not related to bevacizumab treatment. The fourth fatal outcome

Table 4. Summary of serious adverse events
Tabela 4. *Pregled ozbiljnih neželjenih efekata*

System Organ Class/*Klasa sistema organa*	Treatment Group/*Grupa na terapiji* No./*Br.* = 191				
Preferred Term/*Odabrani termin*	n (%)	Number of Events/*Broj neželjenih dejstava*			
		Total	TR	Fatal	TR Fatal
Number of subjects with at least one SAE *Broj pacijenata sa bar jednim ozbiljnim neželjenim dejstvom*	23 (12)	31	14	4*	1
Infections and infestations/*Infekcije i infestacije*					
Tuberculosis/*Tuberkuloza*	1 (0.5)	1	0	0	0
System Organ Class *Klasa sistema organa*	Treatment Group N = 191/*Grupa na terapiji* No./*Br. = 191*				
Preferred Term/*Odabrani termin*	n (%)	Number of Events/*Broj neželjenih dejstava*			
		Total	TR	Fatal	TR Fatal
Blood and lymphatic system disorders/*Poremećaji krvi i limnog sistema*					
Febrile neutropenia/*Febrilna neutropenija*	1 (0.5)	1	0	1	0
Neutropenia/*Niski leukociti*	5 (2.6)	5	1	0	0
Thrombocytopenia/*Niski trombociti*	1 (0.5)	1	0	0	0
Vascular disorders/*Vaskularni poremećaji*					
Deep vein thrombosis/*Duboka venska tromboza*	1 (0.5)	1	0	0	0
Embolism/*Embolija*	1 (0.5)	1	1	0	0
Hypertension/*Povišen krvni pritisak*	3 (1.5)	3	3	0	0
Peripheral artery thrombosis/*Periferna arterijska tromboza*	1 (0.5)	1	1	0	0
Thrombophlebitis/*Upala vena*	1 (0.5)	1	1	0	0
Respiratory, thoracic and mediastinal disorders/*Respiratorni poremećaji*					
Pleural effusion/*Pleuralni izliv*	1 (0.5)	1	0	0	0
Pulmonary embolism/*Plućna embolija*	1 (0.5)	1	1	0	0
Respiratory failure/*Respiratorna insuficijencija*	1 (0.5)	1	0	0	0
Gastrointestinal disorders/*Gastrointestinalni poremećaji*					
Abdominal pain/*Bol u trbuhu*	1 (0.5)	1	1	1	1
Diarrhea/*Proliv*	2 (1)	3	1	1	1
System Organ Class/*Klasa sistema organa*	Treatment Group N = 191/*Grupa na terapiji No./Br. = 191*				
Preferred Term/*Odabrani termin*	n (%)	Number of Events/*Broj neželjenih dejstava*			
		Total	TR	Fatal	TR Fatal
Gastrointestinal necrosis/*Gastrointestinalna nekroza*	1 (0.5)	1	1	0	0
Ileus/*Ileus*	1 (0.5)	1	0	0	0
Renal and urinary disorders/*Poremećaji urogenitalnog trakta*					
Acute kidney injury/*Akutna bubrežna insuficijencija*	1 (0.5)	1	1	1	1
General disorders and administration site conditions/*Opšti poremećaji i stanja na mestu primene*					
Fatigue/*Umor*	2 (1)	2	1	1	1
Impaired healing/*Ne zarastanje rana*	1 (0.5)	1	0	0	0
Sudden death/*Iznenadna smrt*	1 (0.5)	1	NR**	1	NR**
Investigations/*Ispitivanja*					
High body temperature/*Povišena telesna temperatura*	1 (0.5)	1	1	1	1
Injury, poisoning and procedural complications *Povrede, trovanja i proceduralne komplikacije*					
Post procedural complication *Komplikacije nakon procedure*	1 (0.5)	1	0	1	0

Legend: *1 fatal is without reported relationship with bevacizumab; **NR – Not reported; N = number of subjects at risk; n = number of subjects with an event; TR – treatment related; Fatal – Number of events with fatal outcome; TR Fatal – Number of treatment-related events with fatal outcome

*Legenda: *1 smrtni ishod bez prijavljene veze sa bevacizumabom; **NR – Nije prijavljeno; N = broj rizičnih subjekata; n = broj subjekata sa neželjenom reakcijom; TR – povezano sa lečenjem; Fatal – Broj reakcija sa smrtnim ishodom; TR Fatal – Broj reakcija u vezi sa lečenjem sa smrtnim ishodom*

Table 5. Non-serious adverse events
Tabela 5. *Ostali neželjeni efekti*

System organ classification *Klasa sistema organa*	Non serious adverse events *Ostali neželjeni efekti*	n (%) *n (%)*	No. of Events *Br. neželjenih efekata*	Grade (N) NA *Stepen (N)*		
				1	2	3
Blood and lymphatic system disorders *Poremećaji krvi i limnog sistema*	Neutropenia *Neutropenija*	1 (0.5)	1		1	
	Thrombocytopenia *Trombocitopenija*	1 (0.5)	1		1	
Vascular disorders *Vaskularni poremećaji*	Hypertension *Hipertenzija*	1 (0.5)	1			1
General disorders and administration site conditions *Opšti poremećaji i stanja na mestu primene*	Disease progression *Progresija bolesti*	1 (0.5)	1			1
Gastrointestinal disorders *Gastrointestinalni poremećaji*	Constipation *Konstipacija*	1 (0.5)	1		1	
	Diarrhea *Dijareja*	2 (1)	2	1	1	
Renal and urinary disorders *Poremećaji urogenitalnog trakta*	Proteinuria *Proteinurija*	2 (1)	2	1	1	

occurred as a consequence of post procedural complication and was not reported as related to bevacizumab therapy.

Discussion

Preoperative chemotherapy is increasingly used in real-life clinical practice prior to surgical resection of liver mCRC [12, 13]. A large number of published papers pointed out the benefits of neoadjuvant chemotherapy in these patients in terms of potential downstaging and conversion to resectable disease, leading to metastases resection and potential cure. On the other hand, chemotherapy can cause damage to the liver: blue liver (oxaliplatin) and steatohepatitis or yellow liver (irinotecan), which is associated with perioperative morbidity and even mortality in patients undergoing surgery [14]. The addition of bevacizumab to preoperative chemotherapy regimens does not increase the morbidity or mortality related to liver resection and significantly prolongs survival in patients with mCRC [15]. Therefore, to a large extent, bevacizumab is used in combination with chemotherapy regimens in patients with potentially resectable liver metastases [16].

On the other hand, as an angiogenesis inhibitor, bevacizumab has its own side effects - hemorrhage, hypertension, wound healing complications, bowel perforation, arterial thromboembolism, which in turn can delay surgery or increase postoperative morbidity and mortality.

The primary and secondary objectives of this study were achieved. Bevacizumab demonstrated a favourable safety and tolerability in regard to the incidence and severity of adverse and serious adverse events reported during the course of the study. There were no new safety signals recorded in this study. This observational study confirmed a high percentage of patients who achieved a clinical benefit (70% of disease control rate) after beginning of aggressive triplet therapy, including bevacizumab, for patients with liver only mCRC (both initially unresectable and resectable/potentially resectable). High resectability rate, 34.5% and 49%, both in initially unresectable and potentially resectable patients, shows heterogeneous criteria in decision making about liver resection, and highlights the need for establishing and following generally accepted criteria. The role of specially trained and educated surgeons remains very important. Overall, bevacizumab confirmed that it is a valuable perioperative therapy option for mCRC patients.

In addition, bevacizumab demonstrated PFS and RR which are in line with doses recorded in other studies. Our results are in agreement with the results of already published studies on the treatment of mCRC. In the study by T. Gruenberger and associates [17] the overall RR was obtained in 59% of cases, a stable disease was achieved in 38%, while 3% of patients had a disease progression. On the other hand, Wong and colleagues found an objective RR in 78% of cases, stable disease was achieved in 16%, while disease progression occurred only in 7% of cases [18]. Encouraging are also the results of a large observational cohort study (BRITE), which included 46 centres and a total of 1953 patients, who received bevacizumab in the first line therapy for mCRC. In this study, complete remission was achieved in 12.3% of patients, partial response in 35.8%, stable disease was found in 30.5% of patients, while disease progression occurred in 21.3% of patients [19]. Comparing our results with the results of the BRITE study, we noticed similarity in terms of stable disease and disease progression, while in our country slightly higher rate of partial response and lower rate of complete remission were reported.

Limitations

Heterogeneous resectability criteria between centres may lead to diverse classification of patients between potentially resectable and unresectable.

Conclusion

Overall, bevacizumab confirmed that it is a valuable perioperative therapy option for metastatic colorectal cancer patients. Based on the results of studies conducted so far, and based on the results of our trial, we can conclude that the combination of bevacizumab with standard chemotherapy regimens increases the resectability rate of metastatic colorectal cancer with high probability. Given that we are in the era of biological target therapy, its use offers new possibilities and modalities of cancer treatment.

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SPEECH CAPACITY IN PATIENTS WITH VOICE DISORDERS BEFORE AND AFTER SURGICAL TREATMENT OF VOCAL FOLD TUMORS

KAPACITET GOVORA KOD PACIJENATA SA POREMEĆAJEM GLASA PRE I POSLE HIRURŠKOG TRETMANA IZRAŠTAJA NA GLASNICAMA

Mila VESELINOVIĆ[1, 2], Slobodan MITROVIĆ[1, 2], Renata ŠKRBIĆ[1], Vesela MILANKOV[1] and Daniela TAMAŠ[1]

Summary

Inroduction. Pathological characteristics of voice and speech in persons with benign and malignant vocal fold tumors present as different variations in the voice pitch, intensity and quality. The aim of the study was to determine the speech capacity of persons with malignant and benign vocal fold tumors before and after surgical treatment and to establish if there are differences in the speech capacity in relation to the type of tumor. **Material and Methods**. The sample included 67 subjects who were divided into two groups: group I – subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema and group II - subjects with malignant tumors, aged 23 to 74 years (mean age 55.43; standard deviation 11.95). Acoustic voice analysis, maximum phonation time of the vowel /a/, analysis of temporal speech organization, and sentence melody analysis were used for measuring the speech capacity. **Results**. Before surgery, the speech capacity in both groups of patients was almost equal, without statistical significance between the compared groups. After surgery, there was a statistically significant difference between the speech capacity in the examined groups, with better speech capacity observed in group I ($t = -3.807$, $p < 0.001$). The study did not show an isolated effect of time or tumor type on the speech capacity, but showed a combined effect ($F = 10.079$, $p = 0.002$). **Conclusion.** The proposed method for the assessment of the speech capacity before and after surgical treatment of vocal fold tumors is a useful tool for the prediction of the voice outcome and in planning rehabilitation procedures.

Key words: Larynx; Neoplasms; Vocal Cords; Vocal Cord Dysfunction; Voice; Speech-Language Pathology; Speech Acoustics; Speech Production Measurement

Sažetak

Uvod. Patološke karakteristike glasa i govora kod osoba sa benignim i malignim tumorima na glasnicama sagledavaju se kao različite varijacije visine, intenziteta i kvaliteta govornog glasa. Cilj rada bio je utvrđivanje govornog kapaciteta osoba sa malignim i benignim tumorima na glasnicama, pre i posle hirurškog lečenja i konstatovanje da li postoje razlike u govornom kapacitetu u odnosu na vrstu tumora. **Materijal i metode.** Uzorak je činilo 67 ispitanika podeljenih u dve grupe: I grupa − benigni tumori (polipi, ciste) i Rajnkeovi edemi i II grupa − ispitanici sa malignim tumorima, starosti od 23 do 74 godine (prosečna starost 55,43 godine; standardna devijacija 11,95 godina). Kao mera kapaciteta govora u istraživanju je korišćena akustička analiza glasa, maksimalno vreme fonacije samoglasnika /a/, analiza vremenske organizacije govora i analiza melodije rečenice. **Rezultati.** Pre operacije govorni kapacitet u obe grupe ispitanika bio je skoro jednak, bez statističke značajnosti između upoređenih grupa. Nakon operacije uočena je statistički značajna razlika između govornog kapaciteta ispitivanih grupa, pri čemu je bolji govorni kapacitet uočen u I grupi ($t = -3,807$, $p < 0,001$). Studija nije pokazala izolovani efekat vremena ili vrste tumora na kapacitet govora, ali je pokazala kombinovani efekat ($F = 10,079$, $p = 0,002$). **Zaključak.** Predloženi način procene govornog kapaciteta pre i posle operativnog lečenja tumora glasnica predstavlja koristan alat kojim bi se moglo predvideti očekivano stanje glasa i planirati rehabilitacioni postupak.

Ključne reči: larinks; neoplazme; glasne žice; disfunkcija glasnih žica; glas; govorno jezička patologija; govorna akustika; merenje govornog kapaciteta

Introduction

Voice is a result of laryngeal sound production which is primarily an auditory component of speech that allows the expression of emotions and feelings in personal, social and professional relationships [1, 2]. As part of the speech capacity, voice can be quantified by using acoustic voice parameters, including fundamental frequency, intensity, quality, melody [3] and so on. Thus, voice is associated with prosody [4], emotions [3, 5, 6], temporal organization of speech [7–9], articulation of sounds, phonemes, and melody of speech [10].

Voice quality can be impaired due to various pathological conditions that occur primarily at the level of the glottis [11, 12]. Tumors on the vocal folds lead to voice quality disorders, dysphonia due to glottis occlusion disorders; disorders of vocal stiffness (mobility); discrepancies in the mechanical characteristics of the two vocal folds. Acoustic phe-

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Abbreviations
MPT a – maximum phonation time of the vowel /a/

nomena of the voice are caused by: hoarseness - asymmetry of vocal fold vibrations; roughness - sound quality that has been related to the amplitude modulation, sometimes caused by more than two sound sources; pneumophonic coordination (dominance of respiratory noise in the voice) due to greater insufficient occlusion of the glottis. Inflammatory processes, tumors, scarring and other changes may lead to the surface layer rigidity, which has very unfavorable effects on the voice [13–18].

All changes in voice quality are perceived as different variations in pitch, intensity, and spoken voice quality [19]. The development of technologies that deal with the synthesis [20] and conversion of speech today is associated with greater opportunities for quantitative speech analysis [21].

In everyday clinical practice, a simple way to measure the impact of voice and its impairment on speech production is to calculate the speech capacity, which can be expressed as a standardized summary score of variables which are the measure of speech production capacity.

The aim of the study was to determine the speech capacity of persons with malignant and benign vocal fold tumors before and after surgical treatment and to establish if there are differences in the speech capacity in relation to the type of tumor.

Material and Methods

A total of 70 subjects participated in the study, but the sample included 67 subjects who met the inclusion criteria of the study. Subjects with benign tumors, pseudo-tumors or malignant tumors of the vocal folds were divided into two groups. Group I included 36 subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema of the vocal folds, aged from 23 to 74 years (M = 52.94; SD = 13.02). Group II included 31 subjects with vocal fold malignant tumors, aged from 38 to 73 years (M = 58.32; SD = 10.03). The study was conducted from June 2016 to May 2017 at the Clinic of Ear, Nose and Throat Diseases of the Clinical Center of Vojvodina, and it was part of a PhD thesis*.

Prior to conducting the research, consents of the Ethics Committee of the Clinical Center of Vojvodina and the Ethics Committee of the Faculty of Medicine of the University of Novi Sad were obtained. Subjects were offered to participate in the study after surgical treatment of vocal fold tumors. They were given information about the course of the study in writing and a written consent to be signed by those who wanted to participate in the research. Information about the participants were taken according to the research protocol. Subjects were divided into two groups based on the nature of the vocal fold tumors. Postoperative analysis of voice and speech was performed seven days following the surgical treatment. The research was conducted as a prospective study.

To check whether they met the inclusion criteria, each subject first underwent a screening assessment of cognitive abilities using the Mini Mental State Examination by Folstein et al. 1975 [22], and only subjects with orderly cognitive functions were included. Subjects also completed an anxiety questionnaire in order to exclude subjects with severe anxiety (General anxiety disorder-7, Spitzer et al.) [23]. The quality of articulation was checked using the Triage Articulation Test, by Kostić and Vladisavljević, 1981 [24]. These tests were not performed postoperatively and they were not considered when calculating the speech capacity.

In the next phase, subjects who met the inclusion criteria were examined using the following instruments:

– Acoustic analysis of voice and speech (isolated vowel /a/), and analysis of a selected short statement - a sentence that has all the vowels of the Serbian language, "I'll be back soon" by Kašić [25]. The recording was performed using a Pioneer DM-DV 15 microphone, and the subjects were instructed to pronounce the intended vowel and the selected sentence at a comfortable intensity and pitch at a distance of 5 cm from the mouth. The analysis was performed using the Praat program for acoustic speech analysis (Praat: www.praat.org), and the following data were used: pitch, intensity, micro perturbation of pitch, micro perturbation of intensity, and the presence of noise in the voice during isolated vowel production, pitch and intensity of short statement.

– Maximum phonation time of the vowel /a/ (MPT a) measured using a stopwatch showing the longest period during which a patient can sustain phonation of a vowel sound /a/ after maximum inhale.

– The temporal speech organization was analyzed during the reading of the phonetically balanced text. First, the subjects read the whole text, and the reading time was measured with a stopwatch. The text was then read again for one minute. The number of syllables and the number of inhalations while reading the text in one minute were counted (subjects were first instructed to read the text not to express their reading ability, but speech);

– The sentence melody analysis was done using a subscale of the Test for the Assessment of Sentence Accent and Melody (quantitative speech assessment) by Kostić and Sovilj [26]. The instruments used before were also used after the vocal fold surgery. On average, the test lasted between 30 and 40 minutes.

Speech capacity is a standardized summary score of variables selected in this study as a measure of capacity. According to the theoretical framework of this research, the following variables were included in the summary speech capacity: The basic voice pitch observed during the pronunciation of the vowel /a/, isolated or during continuous speech; Rapid pitch perturbations of the isolated vowel - jitter; Voice intensity during the pronunciation of the vowel /a/, isolated or during continuous speech; Rapid perturbations of isolated voice intensity - shimmer; Harmonic to noise ratio (HNR) for iso-

lated vowel /a/ pronunciation; The MPT of the vowel /a/; The MPT ratio for sounds /s/ and /z/ - (S/Z ratio); Melody of speech.

Each of these variables is assessed from 1 – 3, where 1 means a good result and 3 - a bad (pathological) result. The values of the total capacity range from 10 to 30.

In the data processing, basic descriptive statistical parameters for qualitative and quantitative assessment of the obtained results were used. We also assessed the statistical significance of the obtained results, as well as the levels of statistical significance. Statistical data analysis was done using the SPSS version 20 for Windows, and the obtained results are presented in tables and graphs with necessary comments and analysis of the obtained results.

After checking the normality of the distribution of data obtained by measuring the variables of the speech capacity, we found that the distribution of data does not correspond to normal, so these scores were standardized before further analysis.

Results

Table 1 shows descriptive data of mean speech capacity in subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema (Group I) ranging from 19.528 to 20.528 and in subjects with vocal fold malignant tumors (Group II) with mean speech capacity ranging from 20.032 to 22.258. These data show that the speech capacity had improved in subjects with benign tumors, while in subjects with malignant vocal fold tumors (Group II), the speech capacity increased after surgical treatment, which indicates reduced speech capacity.

The Student's t-test was used to analyze the difference between the speech capacity before and after surgery in both groups of subjects. Before the surgery, subjects with malignant tumors had a slightly better speech capacity, but without a statistically significant difference. After surgery, there was a statistically significant difference between the speech capacity in the examined groups; the subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema had a better speech capacity ($t = -3.807$; $p < 0.001$).

Table 2 shows the results of the t-test for dependent samples, that is the difference between the average values of Z scores before and after surgery.

In both examined groups, there was a statistically significant difference between the speech capacity before and after surgery (**Table 3**).

In the Group I, there was a statistically significant improvement in the speech capacity after surgery ($p = 0.005$). However, in Group II, the speech capacity after surgery was statistically significantly worse compared to the measured capacity before surgery ($p = 0.007$). In the Group I, a moderate correlation was obtained between the first and second measurement ($r = 0.558$, $p < 0.001$). In the Group II, no correlation was found between the measured speech capacity before and after surgery.

A two-way analysis of variance was used to analyze the effects of interaction. **Table 4** shows the effects of interaction of dependent variables on the speech capacity.

The obtained results show that there is no isolated effect of time or type of tumor on the speech capacity, but there is a combined effect ($F = 10.079$, $p = 0.002$) **(Graph 1)** showing the difference in the obtained scores between the groups and the interac-

Table 1. Speech capacity of subjects before and after surgery in relation to the type of tumor
Tabela 1. *Izmerene vrednosti kapaciteta govora ispitanika pre i nakon operacije u odnosu na izraštaje*

Group/*Grupa*	Time/*Vreme*	N/*Br.*	Min/*Min*	Max/*Maks*	Mean/*Srednja vrednost*	Standard deviation *Standardna devijacija*
Group I/*Grupa I*	Before/*Pre*	36	15	25	20.528	2.602
	After/*Posle*	36	14	25	19.528	3.184
Group II/*Grupa II*	Before/*Pre*	31	17	25	20.032	2.198
	After/*Posle*	31	16	28	22.258	2.594

Legend: Group I – benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema; Group II – malignant tumors
Legenda: Grupa I – benigni tumori, lezije glasnica (polipi, ciste) i Rajnkeovi edemi; Grupa II – maligni tumori

Table 2. Relationship between the tumor type and measurement time
Tabela 2. *Interakcija između vrste izraštaja i vremena merenja*

Time/*Vreme*	Tumor Type *Vrsta izraštaja*	N/*Br.*	Z Mean *Z Srednja vrednost*	Standard deviation *Standardna devijacija*	t/*t*	p/*p*
Before/*Pre*	Group I/*Grupa I*	36	0.095	1.076	0.834	0.407
	Group II/*Grupa II*	31	-0.110	0.909		
After/*Posle*	Group I/*Grupa I*	36	-0.393	0.991	-3.807	0.000
	Group II/*Grupa II*	31	0.457	0.808		

Legend: Group I – benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema; Group II – malignant tumors
Legenda: Grupa I – benigni tumori, lezije glasnica (polipi, ciste) i Rajnkeovi edemi; Grupa II – maligni tumori

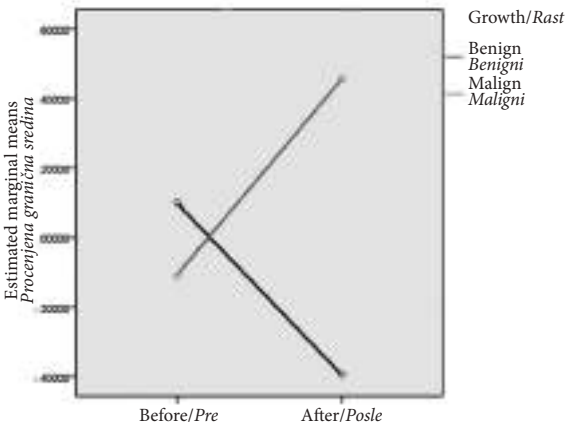
Table 3. Differences between the speech capacity of subjects before and after surgery
Tabela 3. *Razlike između kapaciteta govora ispitanika pre i posle operacije*

Group/*Grupa*	Time/*Vreme*	N/*Br.*	Z Mean *Z Srednja vrednost*	Standard deviation *Standardna devijacija*	r	p (r)	t/*t*	p/*p*
Group I/*Grupa I*	Before/*Pre*	36	0.095	1.076	0.558	0.000	3.006	0.005
	After/*Posle*	36	-0.393	0.991				
Group II/*Grupa II*	Before/*Pre*	31	-0.110	0.909	0.197	0.288	-2.894	0.007
	After/*Posle*	31	0.457	0.808				

Legend: Group I – benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema; Group II – malignant tumors
Legenda: Grupa I – benigni tumori, lezije glasnica (polipi, ciste) i Rajnkeovi edemi; Grupa II – maligni tumori

Table 4. Interaction of variables of the speech capacity
Tabela 4. *Interakcija varijabli u kapacitetu govora*

	Mean Square/*Prosečan kvadrat*	F	p	Eta
Before – After/*Pre – posle*	0.043	0.047	0.829	0.000
Tumors/*Izraštaji*	3.376	3.653	0.058	0.028
Before – After *tumors/*Pre – posle *izraštaji*	9.315	10.079	0.002	0.072



Graph 1. Total speech capacity before and after surgery of vocal fold malignant tumors and vocal fold benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema
Grafikon 1. *Ukupni kapacitet govora pre i posle operacije kod malignih izraštaja na glasnicama i benignih izraštaja na glasnicama, lezija glasnica (polipa, cisti) i Rajnkeovih edema*

tions of the variables. Since the lines in the graph intersect, it can be concluded that there is a combined effect of the type of tumor and time of measurement on the speech capacity.

Although initially subjects in Group I had an equal speech capacity as subjects in Group II, after surgery the speech capacity was statistically significantly better in the Group I, while in Group II the speech capacity was statistically significantly worse.

Discussion

Before surgery, the speech capacity in both groups of subjects was almost equal; the subjects in the Group I had slightly better speech capacity, but without statistical significance in the compared groups. Pathological values of voice pitch in subjects in the Group I and uniform values of other variables that were taken into account when calculating the speech capacity gave a slightly better overall result in the Group II. Similar results in terms of pitch were reported by other authors. Martins et al. [27] reported that subjects with Reinke's edema of the vocal folds before surgery (M = 149.8 Hz) had lower values of pitch. Results of voice pitch in subjects with benign and pseudo-tumors of the vocal folds are shown by Coyle et al. [28] who found that fundamental frequency is within the reference values, with an average of 124.32 Hz. A study by Chotigavanich et al. [29] examined the acoustic characteristics of the voice in subjects with vocal fold malignant tumors before and after endoscopic surgery of T1 glottis carcinomas and found an average voice value of 147.32 Hz before surgery. Klauznicer [30] found an increase to 235.8 Hz in subjects with malignant tumors of the vocal folds. Increased rigidity of the fixed vocal folds affects the increase in pitch. Therefore, the voice pitch in subjects with vocal fold benign tumors is reduced and the voice pitch in subjects with vocal fold malignant tumors is increased; there is an approximation in voice pitch in men and women and between the examined groups.

In terms of pitch and intensity micro-perturbations of voice before surgery, the groups are uniform and similar results are reported by other authors. Martins et al. [27] found that before surgery, voice pitch micro-perturbations were 2.9% on average. The jitter parameter values before surgery of vocal fold polyps were also examined by Petrović-Lazić et al. [31] who found that before surgery in patients with vocal fold polyps the mean value of this parameter was 1.873% and that there was a statistically significant deviation of this parameter compared to the control group of healthy subjects. Pathological values of jitter were found by Verma et al. [17] in a study conducted pre-operatively in 100 subjects with dysphonia caused by benign and pseudo-tumors of the vocal folds. The reason for this finding was explained by the existence of inadequate periodicity

in the vibration of the vocal folds due to the existence of tumors. The jitter parameter values were also pathological in patients with vocal fold malignant tumors, as confirmed by Staníkova et al. [32]. The melody of speech is closely related to the pitch, that is, the fundamental frequency of the voice. It depends on the speed of vibration of the vocal folds, their tension and the change in tension [33].

Due to all the above, the speech capacity in the examined groups is uniform before surgery. In patients with polyps, Sahin et al. [12] found that voice therapy before surgery can increase the results of surgical outcome and lead to the improvement of the speech capacity.

After surgical removal of vocal fold tumors in the Group I, there was a statistically significant improvement in the speech capacity. However, in the Group II, after unilateral cordectomy, the speech capacity was statistically significantly worse compared to the measured capacity before surgery. The reason for this is greater loss of vocal fold tissue than in subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema in line with the principle of surgical radicalism that puts the phonation function off, through the possibility of reconstruction of the remaining laryngeal tissue, postoperative edema of reconstructed tissue, length of general anesthesia, postoperative pain, sparing voice and speech in order not to burden the structures of the reconstructed larynx. Similar postoperative results, in terms of changes in voice pitch, in both benign and malignant tumors are reported by other authors [29, 31, 34].

In our research, voice in the Group I was still partially disturbed after surgery, while the speech capacity values in the Group II changed from borderline values to pathological values after surgery, so the speech capacity deteriorated.

The reason why there is no significant improvement in the speech capacity in the Group I can certainly be found in the fact that these are changes in the vocal folds that disrupt phonation automatism over time. Spontaneously, in the short postoperative time in which the measurements were performed as part of this research, phonation automatism cannot be changed, regardless of the fact that the anatomical preconditions for better occlusion and vibration are significantly better than after removal of one vocal fold due to a malignant tumor.

Removal of vocal folds in patients with malignant tumors disrupts the "phonation valve", which turns partially impaired speech capacity into pathological.

Postoperatively, there was a statistically significant difference between the speech capacity in the examined groups, with better speech capacity in the Group I. Tissue loss after surgery of malignant tumors is greater because it aims to remove the malignant process that has affected the vocal fold. Unilateral cordectomy is the removal of one vocal fold, which prevents early postoperative occlusion of the remains of the glottis and the reconstructed part of the larynx, which results in shorter tone phonation, a greater number of pauses during reading and speech production, poorer melody, soundless segments of speech after surgery, as well as incomprehensible speech production. Some subjects from this group used whispering speech. On the other hand, the advancement of surgical treatment techniques in the Group I, aims to preserve the existing and improve the phonation function of the vocal folds. Less invasive techniques provide better overall voice quality. Also, reconstruction techniques of tissue excision sites, such as the "flap technique" after removal of Reinke's edema, significantly improve the postoperative voice parameters [35].

When observing the overall speech capacity in all 67 subjects included in the study, regardless of the type of tumor and comparison of the results before and after surgery, there is no statistically significant correlation in terms of the speech capacity. Also, the comparison of overall results of the speech capacity in 67 subjects with benign and malignant vocal fold tumors, without analyzing the variables before and after surgery, showed no statistically significant correlation between the speech capacities in all the examined subjects. This is due to the fact that the speech capacity before surgery was equal in both groups of subjects, and after surgery, the speech capacity in the subjects with malignant tumors worsened, and the speech capacity in subjects with benign tumors has improved. So, the obtained results suggest that there is no isolated effect of time or type of tumor on the speech capacity, but that there is a combined effect.

These results were expected, because vocal fold tumors of any origin cause loss of glottis occlusion to a greater or lesser extent, increasing the mass of the vocal folds and affecting the vocal fold vibratory cycle. The research shows that voice quality is affected by the extent and localization of glottis surgery [32]. Pseudo-tumors, such as Reinke's edema, increase the vocal fold mass and affect the vibration process [27]. On the other hand, surgical treatment of benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema is less invasive than surgical treatment of malignant lesions, the structure of the vocal folds remains preserved, and surgical techniques used to treat these changes on the vocal folds aim to preserve the phonation function. Mechanical injuries that occur during microsurgery may lead to structural changes in the layers of the vocal cords [36]. In cordectomy, as a treatment option for early glottic carcinoma, and due to vocal fold scarring, the parameters that we examined as components of the speech capacity were consequently deteriorating [37].

Malignant tumors have a greater impact on pathological perturbations of vocal fold vibration (jitter) in the early stage, and invasion of tumors into deeper layers causes vocal fold stiffness with the existence of a-vibration segment of the vocal fold [18]. After surgical treatment of vocal fold malignant tumors, when they are removed together with the pathological tissues the normal architecture of the glottis is disturbed and there is an increased incompatibility between the two hemi-larynxes. After surgery of malignant tumors, changes in phonation are inevitable, regardless of whether the vo-

cal folds are reconstructed or neo-folds are formed during voice therapy. In order to provide fast and complete vocal fold recovery after surgery, early inclusion of vocal therapy with mechanical stimulation is suggested [37].

Conclusion

Preoperatively, the speech capacity in subjects with benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema as well as in subjects with malignant tumors is partially impaired or pathological. After surgical removal of benign tumors, vocal cord lesions (polyps, cysts) and Reinke's edema, there is a statistically significant improvement in the speech capacity. There is no isolated effect of time or type of tumor on the speech capacity, but there is a combined effect. The proposed method for the assessment of the speech capacity before and after surgical treatment of vocal cord tumors is a useful tool for the prediction of the voice outcome and in planning rehabilitation procedures.

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THE AUDIO RECORDED COGNITIVE SCREEN FOR BRIEF SCREENING OF COGNITIVE IMPAIRMENT IN PATIENTS WITH PSYCHOSIS – A PILOT STUDY

KOGNITIVINI SKRINING AUDIO-ZAPISOM KOD OBOLELIH OD PSIHOZE − PILOT STUDIJA

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Summary

Introduction. Disorders of cognitive functioning in patients with psychosis can manifest in different domains. The disorders vary depending on the severity, from mild to severe, and on the severity of symptoms within the underlying disease. The aim of this study was to examine the possibility of using the Audio Recorded Cognitive Screen in patients with schizophrenia. **Material and Methods.** The study included a total of 61 subjects divided into two groups: 31 subjects with schizophrenia and 30 healthy controls. All subjects completed the Audio Recorded Cognitive Screen to assess the cognitive status in five domains of cognitive functioning. **Results.** The Audio Recorded Cognitive Screen showed a good reliability index (α = 0.85). Multivariate analysis of variance confirmed the differences between the two groups in all examined cognitive domains (F (6.53) = 26.719, p < .001). The partial eta squared results were as follows: object naming 0.159; immediate recall 0.531; delayed recall 0.585; visuospatial functions 0.334; attention 0.644; and verbal fluency 0.590. These results indicated significant differences between the two groups. **Conclusion.** The Audio Recorded Cognitive Screen is a feasible tool for the detection of neurocognitive impairment in individuals with schizophrenia. However, it is necessary to conduct further research in larger samples and use additional assessment instruments in this population.

Key words: Cognition Disorders; Cognition; Auditory Perception; Psychotic Disorders; Schizophrenia; Neuropsychological Tests; Psychometrics

Sažetak

Uvod. Poremećaji u oblasti kognitivnog funkcionisanja kod obolelih od psihoze se mogu manifestovati u različitim domenima. Poremećaji mogu varirati u zavisnosti od težine, od blagih do izraženih, i od izraženosti simptoma u sklopu osnovne bolesti. Cilj istraživanja je da ispita mogućnost upotrebe Kognitivnog skrininga audio-zapisom (*Audio Recorded Cognitive Screen*) kod pacijenata sa šizofrenijom. **Materijal i metode.** Istraživanjem je obuhvaćen 61 ispitanik. Podeljeni su u dve grupe: 31 ispitanik sa šizofrenijom i 30 ispitanika zdrave populacije. Za procenu kognitivnog statusa je korišćen Kognitivni skrining audio zapisom koji procenjuje pet domena kognitivnog funkcionisanja. **Rezultati.** Korišćen instrument je pokazao dobru pouzdanost (α = 0,85). Multivarijantan analiza varijanse je potvrdila postajanje razlika između dve grupe u svim ispitivanim domenima (F (6,53) = 26,719, p < ,001). Rezultati parcijalnog eta kvadrata su sledeći: Imenovanje 0,159; Neposredno pamćenje 0,531; Odloženo pamćenje 0,585; Vizuoprostorne funkcije 0,334, Pažnja 0,644 i Verbalna fluentnost 0,590. Navedeno potvrđuje velike razlike između dve ispitivane grupe. **Zaključak.** Kognitvini skrining audio zapisom je primenjiv na popualciju osoba sa šizofrenijom za utvrđivanje kognitivnog statusa. Međutim, neophodno je sprovesti dalju analizu (istraživanje) na većem uzorku i uz korišćenje dodatnih instrumenata procene na ovoj populaciji.

Ključne reči: kognitivni poremećaji; kognicija; auditivna percepcija; psihotički poremećaji; šizofrenija; neuropsihološki testovi; psihometrija

Introduction

Schizophrenia is a complex mental disorder often associated with cognitive deficit that is an integral part of the clinical picture and represents a direct manifestation of schizophrenia neuropathology [1]. Cognitive impairment is a core feature of schizophrenia that usually predates the onset of clinical manifestations [2]. It has been reported that the incidence and intensity of these deficits vary and that about 75% of patients with schizophrenia have deficits in two domains, but about 25% have no neurocognitive deficits [3]. Although it could be expected that the manifestation of neurocognitive deficits correlates with the clinical symptoms of the disease, previous studies have shown that the correlation was low [4, 5] and that the deficits were more related to disorganized and negative symptoms, such as alogia or apathy [6]. Neurocognitive deficits tend to be persistent and are relatively independent during changes in the clinical condition of the patient. Deficits vary moderately with the severity and acuteness of psychotic symptoms, but do not disappear even in a good remission. It is considered that the new-generation antipsychotics, although indirectly beneficial, do not affect cognitive impairment significantly. Cognitive improvement after treatment with antipsychotics, and con-

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Abbreviations

ARCS – Audio Recorded Cognitive Screen
HAT – Hunter attentional task

sequently improved functional outcome, is regarded primarily an effect of withdrawal of psychotic symptoms and consequently more efficient use of cognitive resources [7, 8].

Neurocognitive deficits in schizophrenia may occur even several years before the onset of psychotic symptoms [8]. Social withdrawal and neurocognitive deficits are the earliest signs of schizophrenia, or have the highest correlation with the subsequent development of schizophrenia [9]. The results of neuropsychological studies of schizophrenia show generalized dysfunction. However, certain cognitive domains are more severely impaired, including: memory [10], especially deficits in working memory and verbal memory; attention [11], in particular selective attention; visuospatial ability [10, 12], executive function, manifesting problems characteristic for frontal lobe defects [13]. Verbal abilities are relatively preserved, but also impaired in comparison with healthy population [14]. Since neurocognitive functions include complex behavior, planning and communication, impairment in these areas may affect the quality of life and social functioning of people with schizophrenia [15, 16].

Numerous instruments with different levels of reliability, sensitivity and specificity have been proposed for the purpose of neurocognitive functioning assessment in patients with schizophrenia [17–19].

The aim of this pilot study was to estimate the validity of a brief screening of neurocognitive deficits in patients with schizophrenia using the Audio Recorded Cognitive Screen (ARCS). This was expected to validate the use of this instrument in the clinical practice to better assess neurocognitive deficits in patients with schizophrenia and thereby contribute to the development of effective rehabilitation and create more precise support programs, in order to improve the patients' overall psychosocial functioning.

Material and Methods

We conducted a comparative study between patients with schizophrenia and persons without a mental disorder. The sample included 31 subjects with schizophrenia (study group: 20 men and 11 women) and 30 subjects without a mental disorder (control group: 12 men and 19 women). Patients with schizophrenia fulfilled all the criteria of the International Classification of Diseases (ICD)-10 [20] and the diagnosis was made by a psychiatrist at the clinic/hospital. The average age of study subjects was 41.47 years (SD = 11.38), ranging from 20 to 60 years. Evaluation was conducted at follow-up visits. Selection criteria included a six-month stable treatment. Among controls, the average age was 36.70 years (SD = 13.55), ranging from 18 to 60 years. The controls did not have any psychological or physical diseases, or any previous mental disease. In both groups the subjects met the inclusion criteria of being literate and having normal hearing and sight, while the exclusion criterion was the presence of any head trauma or neurological disease. Using the t-test for independent samples, the age difference between the study and control groups was tested and there was no statistically significant difference ($t = 1.42$, $p = 0.161$).

As regards the level of education, about 20% of the study group completed only eight years of formal education and 16% completed 11 years of formal education. As opposed to controls, who had a college (23.3%) or university (30%) education in over 50%, the percentage of subjects with higher education in the study group was only 15%. Pearson's χ^2 test showed that the groups statistically significantly differed in regard to education; overall, controls had a higher level of education ($\chi^2 = 12.67$, $p < ,05$).

The instrument used in this research was the ARCS [21] for the detection of cognitive impairment or dementia in diseases such as Alzheimer's disease, multiple sclerosis, schizophrenia, or traumatic brain injury. Earlier studies showed the ARCS to be a useful brief assessment tool for examining the cognitive deficits associated with psychosis [22]. The ARCS has eight subtests that measure five cognitive domains: memory (short-term and long-term), verbal fluency, language (object naming), visuospatial functions (clock drawing) and attention - Hunter attentional task (HAT), HAT-A and HAT-B. The ARCS test/instrument is unsupervised, because it employs an audio device (computer, MP3 player, CD player). Before testing, the subjects receive clear instructions on how the testing is performed and what it requires. The key advantage of the ARCS is ease of use. It can be applied by professionals of all profiles. An additional advantage of the ARCS is that it takes about 3 minutes for a clinician to perform scoring and generate a cognitive profile of a respondent. The ARCS has good validity and reliability; in previous studies the reliability ranged from 0.70 HAT-B to 0.88 for total ARCS. It also has excellent sensitivity (92%) and specificity (90%) [22]. In our study, Cronbach's Alpha was 0.85, indicating a good reliability of the instrument that provides further interpretation of the results obtained.

The study was approved by the Ethics Committee of the Clinical Center of Vojvodina and it was conducted in accordance with the Helsinki Declaration.

Results

Descriptive statistics for the ARCS individual subtests and domain scores are presented in **Table 1**. In the study group, the distribution of responses on delayed recognition memory test exceeded the value of 2 (kurtosis 2.45), which deviates from normal distribution according to the criteria proposed by George and Mallery [23]. In the control group, the distribution of scores on the writing speed test (kurtosis = 10.44 and skewness = 2.89) exceeded the borderline values. Having a negative value, the distribution of scores was skewed to the left, which indicated that the test was very easy for the control group

Table 1. ARCS scores
Tabela 1. *Skorovi kognitivnog skrining audio-zapisa*

	Study group/*Eksperimentalna grupa*						Control group/*Kontrolna grupa*					
	Mean *Srednja vrednost*	SD *Standardna devijacija*	Min *Min*	Max *Maks*	Skew *Indikator zakrivljenosti*	Kurt *Indikator zaravnjenosti*	Mean *Srednja vrednost*	SD *Standardna devijacija*	Min *Min*	Max *Maks*	Skew *Indikator zakrivljenosti*	Kurt *Indikator zaravnjenosti*
Immediate recall *Neposredno pamćenje*	15,52	6,2	1	26	-0,47	-0,23	26,6	3,9	18	34	-0,08	-0,30
Visuospatial ability *Vizuoprostorne funkcije*	2,42	2,9	0	10	1,38	1.00	7,03	3,6	0	10	-0,78	-1,23
Writing speed *Brzina pisanja*	10,90	7,6	0	24	-0,25	-1,08	21,2	5,0	0	24	-2,89	10,44
Verbal fluency *Verbalna fluentnost*	18,55	7,5	5	35	0,28	-0,38	37,3	8,0	22	55	0,03	-0,39
Language (object naming) *Jezik (imenovanje predmeta)*	6,97	2,1	2	10	-0,90	0,15	8,43	1.0	7	10	0.30	-0.92
Delayed recall *Odloženo pamćenje*	8.19	3.9	0	12	-.093	-0.13	11.5	0.7	10	12	-1.18	-0.20
Attention/*Pažnja*	12.81	7.2	0	27	-0.39	-0.63	30.0	7.6	11	40	-0.37	-0.32

Legend: Skew - skewness, Kurt - kurtosis, SD – standard deviation
Legenda: Skew – Indikator zakrivljenosti, Kurt – indikator zaravnjenosti, SD – standardna devijacija

and that values (kurtosis) were grouped mainly around the arithmetic mean, which was quite high (M = 21.27). Significant deviation from the normal distribution was recorded also on the test of delayed recognition (false positive). Subjects in this group reported a very small number of false positives and they were grouped around the arithmetic mean, which means that the test was generally easy for them (i.e., they had a small number of false positives).

Multivariate variance analysis (MANOVA) was used to analyze differences between the groups on a series of cognitive functions. Prior to the analysis, separate scores were created for cognitive functions by adding the scores of the ARCS subtests, and then calculating the average value. Six cognitive domains were distinguished: immediate recall, delayed recall, visuospatial ability, language, attention, and verbal fluency. The model was significant ($F(6.53) = 26.719$, $p < .001$), indicating that there were significant differences between the two groups. Further analysis of the results revealed differences between the two groups in all cognitive domains **(Table 2)**.

The arithmetic means presented in **Table 2** show that controls had higher scores in all cognitive functions. In addition, it shows the effect size (partial eta squared) which indicates that in object naming test the effect size was moderate, while in immediate

Table 2. Comparison between the groups regarding the cognitive domains
Tabela 2. *Poređenje između grupa u odnosu na kognitivne domene*

		Mean *Srednja vrednost*	Standard deviation *Standardna devijacija*	F test *F test*	p/*p*	Effect size *Veličina efekta*
Immediate recall *Neposredno pamćenje*	SG	5.17	2.08	65.54	0.00	0.53
	CG	8.86	1.34			
Delayed recall *Odloženo pamćenje*	SG	3.97	3.09	81.73	0.00	0.58
	CG	9.83	1.67			
Visuospatial ability *Vizuoprostorne funkcije*	SG	2.42	2.91	29.08	0.00	0.33
	CG	7.03	3.69			
Language *Jezik*	SG	6.97	2.19	10.93	0.00	0.15
	CG	8.45	1.02			
Attention *Pažnja*	SG	9.96	5.81	104.73	0.00	0.64
	CG	26.87	6.96			
Verbal fluency *Verbalna fluentnost*	SG	6.18	2.52	83.63	0.00	0.59
	CG	12.32	2.67			

Legend/*Legenda*: SG - study group/*eksperimentalna grupa*; CG - control group/*kontrolna grupa*

recall, delayed recall, visuospatial ability, attention and verbal fluency, the effect was large, suggesting great differences between the two groups.

Discussion

Similar to the results of previous studies, our study shows that patients with schizophrenia exhibit neurocognitive deficits in different domains [14]. The application of the ARCS enabled us to analyze in which domains the deficits were most pronounced. Also, it took less than 1 h to assess impairments in five domains, which is attractive for clinical application [24]. Determination of the specificity of the neurocognitive profile in schizophrenia is made by comparison with controls, whether they are healthy subjects or patients with some other mental disorder (unipolar and bipolar affective disorders). All studies found significant differences in all domains, with the effect size varying from 0.75 to 1.5 [25], which was also confirmed in our study where all cognitive domains were affected by schizophrenia and the effect size ranged from moderate to severe. In patients with schizophrenia, we found prominent deviations in all domains, whereas differences greater than standard deviations were found in the domains of memory, attention and language. These data are in line with previous studies in which these domains were identified as the most affected areas in these patients [14]. Memory is the most frequently analyzed domain, being one of the most problematic ones, because it is manifested by rapid deterioration. Observing memory as a complex function, previous research has shown that schizophrenia usually seriously affects functional memory, and working memory deficits can be several standard deviations below normal [26]. Furthermore, it has shown that working memory has the strongest relationship with functional disability [27]. The overall cognitive functioning and deficits in memory, attention and executive functions in particular, along with the present symptomatology, are in a direct correlation with the functional outcome of treatment, i.e., social functioning of people suffering from schizophrenia [28]. In regard to visuospatial ability, unlike other studies which reported a small difference in the performance among subjects [28], our data indicate significantly lower achievement in subjects with schizophrenia compared with controls. Although there is evidence in the literature that this domain is the least impaired in schizophrenia, our study showed lower achievement also in the domain of attention, which could in part justify poorer functioning in tasks requiring visuospatial analysis and organization and constructive abilities. Sanz and associates [28] found a moderate correlation between years of education and visuospatial/constructive abilities and attention. In addition, cognitive abilities, functions such as language, are relatively preserved in patients with schizophrenia; however, in our study they were deficient compared with the control group.

Taking into account the limited size of our sample, as well as the low educational level among the subjects, this aspect should certainly be further examined and the results should be interpreted with caution. It should be kept in mind that in the group of patients with schizophrenia, the patterns of deficit depend on both the premorbid functioning and positive or negative symptomatology.

Conclusion

Cognitive function has gained a paramount importance in studying the etiology, course and treatment outcome of schizophrenia. Recognition of functional effects of cognitive deficits is important for planning therapeutic guidelines so that, in addition to pharmacotherapy, attention should be paid to cognitive rehabilitation in order to reduce the degree of functional disability of the patients. In relation to the application of the Audio Recorded Cognitive Screen for the detection of cognitive impairment in schizophrenia, our results show that this instrument has good and reliable characteristics and is useful in the clinical practice.

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ASSESSMENT OF THE LEVEL OF PHYSICAL ACTIVITY IN THE FINAL YEAR STUDENTS OF THE FACULTY OF MEDICINE IN RELATION TO THE STUDENTS OF THE FACULTY OF AGRICULTURE DURING THE COVID-19 EPIDEMIC

PROCENA STEPENA FIZIČKE AKTIVNOSTI STUDENATA ZAVRŠNE GODINE MEDICINSKOG FAKULTETA U ODNOSU NA STUDENTE POLJOPRIVREDNOG FAKULTETA TOKOM COVID-19 EPIDEMIJE

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Summary

Introduction. The coronavirus pandemic has affected various aspects of human life around the world and, among other things, the quality and level of physical activity, which is one of the main preventive mechanisms for many chronic diseases, for both young and adults. It has been shown that physical activity improves mental health and quality of life. Due to the strict measures during the pandemic, people spent more time at home, inactive, sitting or lying for long periods of time. The aim of this study is to assess the level of physical activity of students during the coronavirus disease 2019 epidemic and to determine the impact of these measures on its level. **Material and Methods.** The study was conducted as a retrospective study including a total of 60 students, aged 22 to 30 years, of which 30 students were final year students of the Faculty of Medicine in Novi Sad and 30 students of the Faculty of Agriculture in Novi Sad. The International Physical Activity Questionnaire was used to assess the level of physical activity. **Results.** The strictest epidemiological measures affected the level of physical activity of students, mostly reducing the level of light-intensity physical activity. Students of the Faculty of Agriculture were more physically active during the quarantine, especially regarding moderate physical activities. There is no statistically significant association between gender, body mass index, and length of study with the level of physical activity during the epidemic. **Conclusion.** Students of the Faculty of Medicine showed a lower level of physical activity during the strict epidemiological measures compared to the following period. Students of the Faculty of Agriculture were more physically active during the quarantine compared to the following period and compared to the students of the Faculty of Medicine; they were more active both during and after the strict measures during the coronavirus disease 2019 epidemic.

Key words: COVID-19; Pandemics; Quarantine; Students; Exercise; Leisure Activities; Sedentary Behavior; Body Mass Index; Surveys and Questionnaires

Sažetak. Pandemija koronavirusa uticala je na različite sfere ljudskih života širom sveta, između ostalog i na kvalitet i nivo fizičke aktivnosti koja predstavlja jedan od glavnih preventivnih mehanizama za brojne hronične bolesti, kako mladih, tako i odraslih. Dokazano je da fizička aktivnost utiče na poboljšanje mentalnog zdravlja i kvaliteta života. Najstrože mere tokom pandemije dovele su do češćeg boravka u kući, pasiviziranja, dužeg sedenja i boravka u ležećem položaju. Cilj ove studije je da proceni stepen fizičke aktivnosti studenata tokom COVID-19 epidemije i utvrdi uticaj najstrožih epidemioloških mera na njen nivo. **Materijal i metode.** Studija je sprovedena u vidu retrospektivne studije na uzorku od 60 studenata, starosti od 22 do 30 godina, od kojih su 30 ispitanika studenti završne godine studija Medicinskog fakulteta Novi Sad, a 30 studenti Poljoprivrednog fakulteta Univerziteta u Novom Sadu. Za procenu nivoa fizičke aktivnosti, korišćen je Međunarodni upitnik za fizičke aktivnosti. **Rezultati.** Najstrože epidemiološke mere uticale su na stepen fizičke aktivnosti studenata, najviše se odrazivši na pad nivoa fizičke aktivnosti lakog intenziteta. Studenti Poljoprivrednog fakulteta su bili u većem stepenu fizički aktivni za vreme karantina, posebno pri obavljanju umerenih fizičkih aktivnosti. Ne postoji statistički značajna povezanost pola, indeksa telesne mase i dužine studiranja sa nivoom fizičke aktivnosti za vreme epidemije. **Zaključak.** Studenti Medicinskog fakulteta pokazali su niži stepen fizičke aktivnosti tokom najstrožih epidemioloških mera u odnosu na kasniji period. Studenti Poljoprivrednog fakulteta su bili fizički aktivniji tokom karantina u odnosu na kasniji period i u poređenju sa studentima Medicinskog fakulteta bili su aktivniji i tokom i nakon najstrožeg perioda COVID-19 epidemije.

Ključne reči: COVID-19; pandemija; karantin; studenti; fizička aktivnost; slobodne aktivnosti; sedeće aktivnosti; indeks telesne mase; ankete i upitnici

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Abbreviations

IPAQ – International Physical Activity Questionnaire
CVD – cardiovascular disease
COVID-19 – coronavirus disease 2019
BMI – body mass index
MET – metabolic equivalent of task

Introduction

Physical activity is defined as any movement of the body produced by skeletal muscles that results in energy expenditure. Exercise is considered a subset of physical activity that is planned, structured and repetitive and its ultimate goal is to improve or maintain physical fitness [1].

Physical activity has an essential role in the prevention and treatment of many chronic diseases, both in young people and adults. Increasing physical activity is a key component to reducing morbidity and mortality [2].

There is ample evidence from clinical studies that moderate exercise has a protective effect on the development of cardiovascular disease, atherosclerosis and many other chronic diseases [3–4]. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide [5] and risk factors for CVD include obesity, smoking, low levels of high-density lipoprotein, high total cholesterol, triglycerides, and high blood pressure [6].

It was found that the symptoms of depression in adolescents are inversely proportional to the level of physical activity.

Numerous studies have shown that people who maintain good physical fitness, among other benefits, have a longer life expectancy, their mortality rate is three times lower, especially between the ages of 50 and 70 [7]. A major obstacle for the development of effective methods for promotion of physical activity among all sections of the population is the lack of knowledge about the benefits of regular physical activity [8].

The World Health Organization declared a pandemic of the coronavirus disease 2019 (COVID-19) on March 11, 2020 [9]. The Director-General of the World Health Organization, Tedros Adhanom Ghebreyesus, called on all countries to activate and improve emergency response systems. Due to the crisis in countries around the world, measures were introduced to combat the pandemic of this contagious disease.

With enforcement of the anti-epidemic measures (temporary suspension of educational institutions, limited work hours, and recommendations for working from home, temporary measure of staying at home for people aged 65 and over, as well as closing sports facilities and open spaces for training) routine outdoor activities became limited, including regular physical activity.

Combating the spread of the virus was an urgent priority for public health, but there were not enough public health guidelines on what people could or should do to maintain their daily exercise or physical activity habits. It was necessary to prevent increase in sedentary behavior due to extended stay at home, i.e. spending a lot of time sitting or lying for long periods of time (watching TV, using mobile devices, playing games) and reducing regular physical activity. Such behavior over a long period of time may lead to muscle relaxation, loss of fitness, decreased circulation, anxiety, sleep disorders, depression, weight gain, increase in blood pressure and blood sugar, and potential worsening of chronic diseases.

There is a strong health dimension for physical activity at home. Carrying out regular physical activities and routine exercise in a safe home environment is an important strategy for maintaining healthy life during a coronavirus crisis.

Material and Methods

The retrospective study included a total of 60 students, male and female, aged 22 to 30 years. The examinees were divided into two groups. The first group included final year students of the Faculty of Medicine, while the second included final year students of the Faculty of Agriculture in Novi Sad. In both groups, male and female students were equally distributed, i.e. included 15 students of both sexes. Participation in the study was voluntary.

The study used a standardized questionnaire: the International Physical Activity Questionnaire (IPAQ) as well as a modified physical activity questionnaire in which the questions referred to a seven-day period at a time when the most stringent anti-epidemic measures were in force. All examinees, along with completed surveys, provided data on their height, body weight, age, gender, length of studying, and based on the first two parameters, the body mass index (BMI) was calculated for each subject.

Within the study, examinees independently completed the IPAQ.

International Physical Activity Questionnaire

The IPAQ assesses the level and types of physical activity carried out during everyday life. Within this study, examinees were surveyed through IPAQ 1 and IPAQ 2 questionnaires about the time they spent performing certain physical activities in the previous 7 days (IPAQ 1), and during 7 days at the time of the most severe epidemiological measures (IPAQ 2). The IPAQ has been widely used to assess the degree of physical activity in adult patients aged 15 to 69 years. The questionnaire can be used within clinical trials as well as in population surveys that compare levels of physical activity among populations internationally [11].

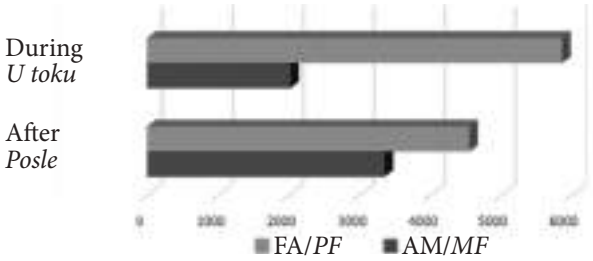
There are two versions of this questionnaire. The first is a short version, consisting of 7 questions, while the second is a longer version with 27 questions on physical activity. The short version collects data on all three levels of physical activity - vigorous (e.g. lifting loads, fast cycling ...), moderate (e.g. carrying less loads ...), light (light walking), but also about the time spent in a sitting position.

Table 1. Average values and basic characteristics of distribution
Tabela 1. *Prosečne vrednosti i osnovne karakteristike distribucije*

Questions/*Pitanja*	Descriptive characteristics/*Deskriptivne karakteristike*							
	Min *Min*	Max *Maks*	Mean/*Srednja vrednost*	Standar deviation *Standarna devijacija*	Sk	Ku	T-test/*T-test*	p/*p*
Question No 1 After *Pitanje broj 1 Posle*	0	7	2.25	1.86	0.33	-0.91	0.42	0.67
Question No 2 After *Pitanje broj 2 Posle*	0	480	71.28	86.26	2.71	9.45	1.02	0.30
Question No 3 After *Pitanje broj 3 Posle*	0	7	2.63	1.75	0.41	-0.12	1.05	0.29
Question No 4 After *Pitanje broj 4 Posle*	0	240	68.71	58.70	0.85	0.02	0.88	0.38
Question No 5 After *Pitanje broj 5 Posle*	1	7	5.90	1.62	-1.48	1.22	6.81	0.00
Question No 6 After *Pitanje broj 6 Posle*	15	350	81.750	64.64	1.97	1.53	2.00	0.05
Question No 7 After *Pitanje broj 7 Posle*	1	13	5.51	2.66	0.80	0.50	1.56	0.12
Question No 1 During *Pitanje broj 1 Posle*	0	7	2.41	2.51	0.68	-0.90	0.42	0.67
Question No 2 During *Pitanje broj 2 Posle*	0	720	93.70	169.9	2.40	4.94	1.02	0.30
Question No 3 During *Pitanje broj 3 Posle*	0	7	3.01	2.11	0.54	-0.71	1.05	0.29
Question No 4 During *Pitanje broj 4 Posle*	0	600	83.33	117.4	2.87	8.81	0.88	0.38
Question No 5 During *Pitanje broj 5 Posle*	0	7	4.01	2.50	-0.32	-1.18	6.81	0.00
Question No 6 During *Pitanje broj 6 Posle*	0	500	62.500	90.81	3.07	1.108	2.00	0.05
Question No 7 During *Pitanje broj 7 Posle*	0	15	6.20	3.69	0.53	-0.27	1.56	0.12

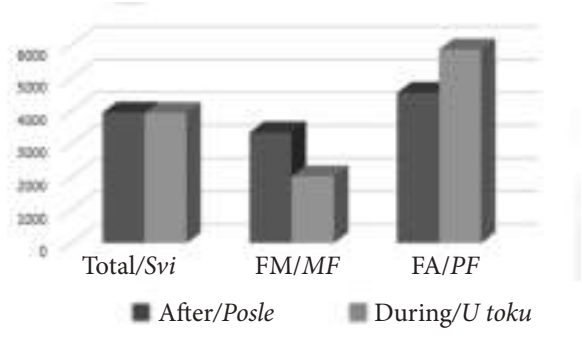
Legend: *Sk (Skjunis) curvature indicator; Ku (Kurtosis) indicator of flatness
Legenda: *Sk *(Skjunis) indikator zakrivljenosti; Ku (Kurtoza) Indikator zaravnjenosti*

The so-called metabolic equivalent of task (MET) is used to measure each of these levels of physical activity. One MET represents the basal rate of oxygen consumption and the associated caloric expenditure. Each physical activity can be represented as a product of one MET.



Graph 1. Total score on the scale - during and after the anti-epidemic measures
Grafikon 1. *Ukupan skor na skali - tokom i nakon antiepidemijskih mera*
FA – Faculty of Agriculture, FM – Faculty of Medicine
PF – Poljoprivredni fakultet; MF – Medicinski fakultet

The determined average value of MET in healthy adults is:
– 3.3 - for light physical activity/walking



Graph 2. Total score on the scale - during and after the anti-epidemic measures, differences between faculties
Grafikon 2. *Ukupan skor na skali - tokom i nakon antiepidemijskih mera, razlike između fakulteta*
FA – Faculty of Agriculture, FM – Faculty of Medicine
PF – Poljoprivredni fakultet; MF – Medicinski fakultet

Table 2. Correlation coefficients and significance level
Tabela 2. *Koeficijenti korelacije i nivo značajnosti*

Physical activity/*Fizička aktivnost*		BMI/*Indeks telesne mase*
Total *Svi*	After/*Posle* During/*Tokom*	0.13 -0.06
Faculty of Medicine *Medicinski fakultet*	After/*Posle* During/*Tokom*	0.35 0.07
Faculty of Agriculture *Poljoprivredni fakultet*	After/*Posle* During/*Tokom*	-0.06 -0.24

****p* < .001, ***p* < .01, **p* < .05*

– 4 - for moderate physical activity
– 8 - for intense/vigorous physical activity.

Results in MET-minutes for a certain level of physical activity are obtained by multiplying the average MET value by the duration of physical activity expressed in minutes.

The total MET score, which is the sum of MET-minute scores for the previous 7 days, is used to classify examinees into 3 categories according to the total level of physical activity:

Category 1: Low level of physical activity
The people in this category are considered to be physically inactive or have a low level of physical activity. This category implies the lowest level of physical activity, i.e. persons who do not meet the criteria for the second and third category.

Category 2: Moderate level of physical activity
This category includes examinees who meet one of the following 3 criteria:
– 3 or more days of high-intensity activity, minimum 20 minutes per day, or
– 5 or more days of moderate-intensity physical activity or 30 minutes of walking per day, or
– 5 or more days of any combination of walking, moderate or high-intensity activities accumulating at least 600 MET-minutes per week.

Category 3: High level of physical activity
It is necessary to meet any of the following 2 criteria:
– High-intensity activities at least 3 days a week accumulating at least 1500 MET-minutes a week, or
–7 or more days of any combination of walking, moderate or high-intensity activities accumulating at least 3000 MET-minutes per week.

Results

There were 60 examinees, of whom 50% were male. The gender distribution was identical in the groups attending the two faculties. The age range was 22 - 30 years, while the median age in the groups was 24.22 years, standard deviation = 1.96.

In regard to body height of the examinees, it ranged from 155 - 200 cm; the median body height was 176.40 cm; the median body height of the students of the Faculty of Medicine was 177.14 cm, while in the students of the Faculty of Agriculture it was 175.66 cm. The average body weight of the examinees was predominantly in the range of 74 - 75 kg, and the BMI was in the range 23 - 24. It can be concluded that the two groups of students were aligned regarding the parameters taken into account.

The internal consistency of the Physical Activity Scale, at the end and during the anti-epidemic measures, was evaluated by calculating the Cronbach's alpha coefficient.

Based on the gathered data, it was determined that the reliability of the Physical Activity Scale was $\alpha = 0.839$ after the anti-epidemic measures were lifted, and during the anti-epidemic measures it was 0.822, which points to good reliability. Results indicate that physical activity was approximately uniform during anti-epidemic measures, as well as after they were lifted **(Table 1)**.

The results of the T-test for dependent samples showed that there were no significant differences between answers regarding the time during and after anti-epidemic measures, except for the question number 5: How many days have you walked for at least 10 minutes? The results show that after the anti-epidemic measures were lifted, there were significantly more days when the students walked for at least 10 minutes.

The differences between answers regarding the period during and after the anti-epidemic measures were calculated using a series of T-tests for dependent samples in students of the Faculty of Medicine and students of the Faculty of Agriculture.

When it comes to the first four questions, the results of the T-test showed that the differences in the average values regarding the same question concerning the period during and after the anti-epidemic measures did not differ significantly among students of the Faculty of Medicine. However, statistically significant differences were noticed in answers to the last three questions, indicating that the students of the Faculty of Medicine walked significantly less during the anti-epidemic measures and were sitting significantly more.

The results of the T-test **(Graph 1)** showed that the differences in the average total scores on the scale of physical activity did not differ significantly when all the students were observed. Analyzing only the students of the Faculty of Medicine, we found that the total score of physical activity was

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
MEĐUNARODNI UPITNIK ZA FIZIČKE AKTIVNOSTI

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. **During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?**
_ days per week
☐ No vigorous physical activities **Skip to question 3**

2. **How much time did you usually spend doing vigorous physical activities on one of those days?**
_ hours per day
_ minutes per day
☐ Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. **During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.**
_ days per week
☐ No moderate physical activities **Skip to question 5**

4. **How much time did you usually spend doing moderate physical activities on one of those days?**
- hours per day
_ minutes per day
☐ Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. **During the last 7 days, on how many days did you walk for at least 10 minutes at a time?**
days per week
☐ No walking **Skip to question 7**

6. **How much time did you usually spend walking on one of those days?**
_ hours per day
_ minutes per day
☐ Don't know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. **During the last 7 days, how much time did you spend sitting on a week day?**
_ hours per day
_ minutes per day

☐ Don't know/Not sure

significantly higher after the anti-epidemic measures were lifted, compared to the period during the anti-epidemic measures. The total score of physical activity among students of the Faculty of Agriculture was higher during the anti-epidemic measures, but the difference between the two scores was not statistically significant.

Differences in the in the overall score on the Scale of Physical Activities during and after the anti-epidemic measures in the students of the Faculty of Medicine and the Faculty of Agriculture, were examined with the help of a series of T-tests for independent samples. The results of the T-test **(Graph 2)** showed that the students of the Faculty of Agriculture had a higher level of physical activity during and after the anti-epidemic measures than students of the Faculty of Medicine. After the measures were lifted, this difference was small and not statistically sig-

nificant, while it was evident that students of the Faculty of Agriculture had much higher physical activity during anti-pandemic measures.

No gender differences were found in students' physical activity before and during the anti-epidemic measures. When the students of the Faculty of Medicine and the students of the Faculty of Agriculture were studied separately, it was also found that there were no statistically significant gender differences in physical activity during and after the anti-epidemic measures.

In order to determine whether there was a correlation between BMI and the overall score of the Physical Activity Scale during and after anti-epidemic measures, Pearson's correlation coefficient was applied. When it comes to the correlation between BMI of examinees and the Physical Activity Scale score **(Table 2)**, the results showed that there was no significant correlation during and after the anti-epidemic measures. The analysis was conducted on individual samples in relation to the faculty and the same findings were obtained in both groups.

Discussion

From the very beginning, the new coronavirus pandemic has posed an important health challenge for populations around the world, taking into account specific anti-epidemic measures. In addition to morbidity and mortality directly related to COVID-19 disease, many states have adopted measures that have also had important physical and psychological effects on individuals [12, 13]. In order to control the spread of the disease, social interactions were restricted and stay at home was recommended.

A large part of the population was forced to stay indoors for weeks, which was accompanied by a decrease in physical activity and adoption of sedentary behavior, staying longer in a sitting or lying position that requires energy expenditure $\leq$ 1.5 MET [14].

With the shutdown of sports facilities, gyms and parks, the recommendations of the World Health Organization regarding the level of physical activity (at least 150 minutes of moderate to strong or 75 minutes of intense physical activity per week, or a combination of them in adults), [15] became difficult to achieve. In this situation, the use of exercise programs at home has proved useful as a mean of combating the harmful effects of increased physical inactivity. A large number of international and national institutions have published a series of recommendations emphasizing the importance of remaining physically active during the quarantine period, including numerous recommendations for exercise at home [16].

This research included 60 examinees, of which 30 were students of the Faculty of Medicine and the other 30 were students of the Faculty of Agriculture. Out of the total number, male and female students were evenly distributed - 30 examinees of each.

A study including 214 male and 295 female biomedical students of the University of Queensland, assessed the impact of quarantine measures on diet and physical activity, yielded the following results: 30% fewer examinees of both sexes achieved a "sufficient" level of physical activity, defined by at least 150 minutes during at least five sessions, compared to the previous two years. Over the next six to eight weeks compared to the first study, despite mitigating measures, there was a higher food intake in females and reduced levels of physical activity in both sexes [17]. Our research also proves that there is no gender difference in the level of physical activity during and after anti-epidemic measures.

A study conducted at three Italian universities showed that, among other parameters, female gender is associated with achieving recommended levels of physical activity, even during the anti-epidemic measures [18]. A study of 3.800 healthy adults in Spain found that during the anti-epidemic measures, men were more likely to reduce physical activity than women (21% vs. 9%) [19]. The average BMI in the total sample was 23.50, and individually - 23.04 in medical students and 23.95 in agricultural students.

The research we conducted showed that there were no statistically significant differences in the levels of physical activity between students with different values of BMI, neither during nor after the anti-epidemic measures were lifted.

When analyzing the answers of the examinees related to 7 questions about the physical activity during the most severe anti-epidemic measures, as well as 7 questions related to the previous 7 days, in the total sample of 60 students, no significant differences were found in the level of physical activity during and after anti-epidemic measures, except for the question related to light physical activity (walking longer than 10 minutes). Students from both faculties claimed that they spent significantly fewer days walking during anti-epidemic measures.

When comparing the level of physical activity among medical students, during and after the COVID-19 anti-epidemic measures, especially in light of all three levels of activity, a significantly lower level of physical activity of lighter intensity was registered (walking longer than 10 minutes), as well as much longer time spent sitting during anti-epidemic measures. Similar results were obtained in a survey of students in Italy, where it was observed that all sedentary behaviors were significantly increased, and that all physical activities were significantly reduced during the "lockdown" [18]. The same results were recorded among students of the Faculty of Agriculture in the segment of issues related to light physical activity, with the difference that during anti-epidemic measures this group of examinees spent significantly longer time performing physical activities of moderate intensity.

The results of this study showed that the total score of students of the Faculty of Medicine indicates that their level of physical activity was significantly lower during the anti-epidemic measures than after they were suspended. Among the students of the Faculty of Agriculture, we noticed that the total score of physical activity during the anti-epidemic measures was

higher, but with a statistically negligible difference between the two periods. The results indicate that students of the Faculty of Agriculture both during and after the anti-epidemic measures had a higher level of physical activity than students of the Faculty of Medicine, which is especially pronounced in the period of the most severe anti-epidemic measures.

The obtained results are supported by the answers to the last question in the survey, in which students of both faculties were given the freedom to state what activities they practiced and how they spent their free time during the curfew and quarantine. The largest number of medical students answered that they spent that period studying (50%), while watching movies/series/playing games was present in the same percentage as performing any form of physical activity on a daily basis (40%), among which physical activities outside the home, such as cycling and walking and exercising at home, were evenly represented.

Among the students of the Faculty of Agriculture, statistical data indicate that 13.3% spent their time studying, 46.6% of them spent part of their time in front of the TV, and as many as 70% of examinees spent the quarantine period doing some physical activities, most often mentioning various household chores, field work, growing fruits and vegetables.

Conclusion

Students of both the Faculty of Medicine and the Faculty of Agriculture had a lower level of physical activity during the most severe coronavirus disease 2019 anti-epidemic measures, especially when performing activities of light intensity.

There is a difference in the level of physical activity of medical and agricultural students during the strict coronavirus disease 2019 anti-epidemic measures, because it was proven that agricultural students were significantly more physically active during the anti-epidemic measures, especially when performing moderate-intensity physical activities.

There are no significant gender differences in the results related to the level of physical activity during the coronavirus disease 2019 epidemic.

There are no statistically significant differences in the level of physical activity of students during the coronavirus disease 2019 epidemic in relation to the length of study.

There are no statistically significant differences in the body mass index and the results related to the level of physical activity during the coronavirus disease 2019 epidemic.

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REVIEW ARTICLES
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THE ROLE OF METABOLIC SYNDROME IN THE DEVELOPMENT OF OSTEOARTHRITIS

ULOGA METABOLIČKOG SINDROMA U NASTANKU OSTEOARTROZE

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Summary

Introduction. Knee osteoarthritis is a progressive degenerative disease of the entire joint that leads to functional limitations and reduced quality of life. The end-stage of the disease is associated with disability and a significant burden both for the patient and the society. **Osteoarthritis and metabolic syndrome.** Metabolic syndrome is a group of cardiovascular risk factors including diabetes and hyperglycemia, abdominal obesity, hypercholesterolemia, and hypertension. The adverse effects of the metabolic syndrome are associated with worsening of the clinical manifestations and disease prognosis through the combined effects of metabolic disorders. It has also been suggested that individual components of the metabolic syndrome may be an independent risk factor for knee osteoarthritis. **Osteoarthritis and diabetes mellitus.** Experimental and epidemiological evidence supports the role of type II diabetes mellitus in the pathogenesis of osteoarthritis. Chronic hyperglycemia leads to oxidative stress and excessive production of proinflammatory cytokines, while insulin resistance can act locally and systemically through chronic low-grade inflammation. **Osteoarthritis and hypertension.** The mechanism that explains the relationship between osteoarthritis and hypertension is unclear. Several potential pathways for subchondral bone damage due to hypertension have been described. **Osteoarthritis and dyslipidemia.** Experimental studies suggest that dyslipidemia may be involved in the pathophysiological process of osteoarthritis, while epidemiological studies show heterogeneous results. **Conclusion**. Patients with knee osteoarthritis require a holistic approach in which the emphasis is not only on symptomatic pain relief, but also on the treatment of metabolic disorders.

Keywords: Metabolic Syndrome; Osteoarthritis; Risk Factors; Diabetes Mellitus, Type 2; Dyslipidemias; Hyperglycemia; Hypertension; Inflammation; Obesity;

Sažetak

Uvod. Osteoartroza kolena predstavlja degenerativno oboljenje kompletnog zgloba koje dovodi do funkcionalnih ograničenja i smanjenog kvaliteta života. Krajnji stadijum bolesti je povezan sa invaliditetom i značajnim opterećenjem kako za samog pacijenta, tako i za društvo. **Osteoartroza i metabolički sindrom.** Metabolički sindrom predstavlja skup nekoliko kardiovaskularnih faktora rizika– dijabetes melitus i hiperglikemija našte, abdominalna gojaznost, hiperholesterolemija i hipertenzija. Glavni efekat metaboličkog sindroma može biti pogoršanje kliničkih manifestacija i prognoze bolesti putem udruženog uticaja metaboličkih poremećaja. Sugerisano je i da bi pojedinačne komponente metaboličkog sindroma mogle biti nezavisan faktor rizika za osteoartrozu kolena. **Osteoartroza i dijabetes melitus.** Eksperimentalni i epidemiološki dokazi podržavaju ulogu dijabetesa melitus tip II u patogenezi osteoartroze. Hronična hiperglikemija dovodi do oksidativnog stresa, te prekomerne proizvodnje proinflamatornih citokina, dok insulinska rezistencija može delovati lokalno i sistemski putem hronične inflamacije niskog stepena. **Osteoartroza i hipertenzija.** Mehanizam koji bi objasnio vezu između osteoartroze i hipertenzije je nejasan. Opisano je nekoliko potencijalnih načina za oštećenje subhondralne kosti usled povišenog krvnog pritiska. **Osteoartroza i dislipidemija.** Eksperimentalne studije sugerišu da bi dislipidemija mogla biti uključena u patofiziološki proces nastanka osteoartroze, dok epidemiološke studije pokazuju heterogene rezultate. **Zaključak.** Pacijenti sa osteoartrozom kolena zahtevaju holistički pristup u kom akcenat neće biti samo na simptomatskom ublažavanju bolova, već i na lečenju metaboličkih poremećaja.

Ključne reči: metabolički sindrom; osteoartroza; faktori rizika; dijabetes melitus, tip 2; dislipidemije; hiperglicemija; hipertenzija; inflamacija; gojaznost

Introduction

Osteoarthritis (OA) is a progressive degenerative disease of the entire joint, since it affects the articular cartilage, meniscus, ligaments, bone, and synovium [1–4]. This rheumatic disease most often has a multifactorial etiology and affects peripheral joints (knee, hip, hand) and/or spine, but it can affect any other joint [5, 6]. The OA involves molecular, anatomic, and/or physiologic disorders such as abnormal joint metabolism, cartilage degradation, bone remodelling, osteophyte formation, and inflammation [2,

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Abbreviations

OA – osteoarthritis
MetS – metabolic syndrome
HDL – high-density lipoprotein
LDL – low-density lipoprotein
DM II – diabetes mellitus type II
BP – blood pressure
BMI – body mass index

4]. Pain, stiffness, and limited mobility are common functional limitations in patients with OA, which affect the ability to perform daily activities [7, 8] and reduce the quality of life [9]. Knee OA affects about 250 million people worldwide, and this number is expected to increase due to the obesity epidemic and population aging [1, 9]. Mechanical pressure is considered to be the primary cause of knee OA. It mostly affects the elderly population, obese people, and women are at higher risk of developing knee OA than men [9]. Also, it has been reported that women with knee OA have a poorer functional status and a more pronounced functional decline compared to men [10].

The pathophysiological mechanisms leading to OA are still unclear, although there is general agreement on the impact of biomechanical and excessive mechanical loading to the joints [11]. Early changes that affect the osteochondral junction play an important role considering the fact that they represent possible mediators of pain and structural progression of OA. The loss of osteochondral integrity removes the barrier between intra-articular and subchondral compartments, exposing subchondral bone and its innervation to unbalanced biochemical and biomechanical influences [12]. Other risk factors that may contribute to an increase in the incidence of OA include genetic, metabolic and neuroendocrine factors, [11] and it is currently considered that there are several disease phenotypes: post-traumatic, age-related OA, genetic, and metabolic syndrome (MetS)-related OA [13].

The incidence and prevalence of OA increase with age, while prolonged life expectancy also increases the need for knee arthroplasty, so the rate of these interventions in the United States is expected to increase by 143% by 2050 [14]. Data on the incidence and prevalence of OA in Serbia are limited. In the period from 2013 to 2014, the waiting list for total hip and knee arthroplasty increased by 20%, and the demand for such procedures is expected to rise [15]. Total knee arthroplasty is an effective treatment for OA, but in conditions of limited access to health care, less expensive therapy options are needed. Treatment of metabolic disorders could be one of the potential ways to slow down the progression of knee OA [16].

Osteoarthritis and metabolic syndrome

Most patients with knee OA are overweight or obese. The risk of developing OA is twice as high in obese individuals compared with individuals with a normal body mass index (BMI) (< 25) odds ratio 1.98, [17] and obesity is the main risk factor for OA [18–20]. Its strongest association is with knee OA, followed by hip OA [18] while the link between high BMI and hand OA remains unclear [19]. A combination of disorders that constitute the MetS has also been reported to be associated with an increased risk for knee, hand, and lumbar spine OA [18]. Hypertension, dyslipidemia, and hyperglycemia are more common in obese patients, indicating that these metabolic disorders are inseparably linked [16]. According to the International Diabetes Federation (IDF) consensus, MetS implies central obesity and any of the following two criteria: elevated triglycerides ≥ 150 mg/dL (1.7 mmol/L) or prior specific treatment, reduced high-density lipoprotein (HDL) cholesterol < 50 mg/dL (1.29 mmol/L) in women/< 40 mg/dL (1.03 mmol/L) in men or previous specific treatment, high blood pressure (BP) - systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension, raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type II diabetes mellitus (DM II) [21]. The MetS is a significant public health problem that continues to grow rapidly due to urbanization and sedentary lifestyle, as well as increased caloric intake and consequent increased incidence of obesity [12]. It has been estimated that MetS affects 10 - 40% of the population, depending on diagnostic criteria, sex, age, lifestyle, diet and physical activity [2]. The main effect of MetS may be the worsening of the disease, especially in terms of clinical manifestations. Also, promoting the progression of structural damage through the cumulative impact of metabolic disorders may worsen the prognosis [18].

Numerous studies have recently been conducted to investigate the association between MetS and OA [9, 11, 22–24] but the association between MetS and OA remains controversial. While some studies suggest an association rather than a causal link, others point out that there is no association between MetS and OA [18, 22, 23]. There is still a consideration that OA is exclusively a consequence of the increased load on the joints due to excess weight [25]. It is undisputed that the mechanical effect caused by increased body weight can affect the development of knee OA in patients with MetS, but in recent years the emphasis has been on the metabolic effects of MetS [9]. The strongest epidemiological association between OA and MetS is found in knee OA, since higher body weight leads to a greater load on the supporting joints. However, obesity increases the risk of developing OA on joints that do not carry weight, like the hand [8, 9].

Systemic adipose tissue includes subcutaneous adipose tissue, visceral adipose tissue, intramuscular and intermuscular adipose tissue. An excess in visceral adipose tissue which is known as central obesity [26] plays an important role in the pathogenesis of OA, since it can promote systemic and local inflammation [24, 27]. Increased amount of adipose tissue in the body leads to metainflammation through the production of adipokines and cytokines which can modulate the immune system and

induce the synthesis of proinflammatory and catabolic mediators. This process may lead to chondrocyte dysfunction and accelerated progression of OA [27]. "Metabolic inflammation" was described by Hotamisligil et al. [28] in the late 1990s. They found that adipose tissue in obese mice and humans released higher amounts of tumor necrosis factor-α than in non-obese mice and humans. It remains unclear whether metainflammation is the cause or consequence of metabolic diseases or both considerations are true [25]. In addition to chronic inflammation, it is important to emphasize the influence of insulin resistance and the production of abnormal adipokines (tumor necrosis factor-α, interleukin-1, interleukin-6, leptin, and adiponectin) [22]. Adipokines, which are mainly secreted by white adipose tissue, have shown a systemic pro-inflammatory effect in many diseases [29]. Several studies compared adipokine levels in synovial fluid and blood in patients with and without OA. Good correlations were found between adipokines levels in synovial fluid and BMI. Higher levels of adipokines in synovial fluid and serum in OA patients were associated with disease severity and progression [25, 30].

The heterogeneity of results in current literature may be due to methodological differences, such as differences in the definition of MetS, different criteria for the diagnosis of OA, different age, gender and ethnicity of respondents [11]. Some studies did not consider the influence of factors that may contribute to the outcome such as smoking, physical activity level, or use of medications like antihypertensives and statins [13, 29]. Two reasons related to the design of the study are cited as a common lack of studies that have reported the association between MetS and OA. The first is the non-adjustment of results for body weight or BMI, while the second is the fact that most published studies were designed as cross-sectional studies [22].

Osteoarthritis and dysglycemia

Metabolic processes play an important role in the physiological turnover and remodelling of synovial joint tissue in both healthy and affected joints. Persistent hyperglycemia promotes oxidant production, which results in increased catabolism of the osteoarthritic cartilage matrix [17]. It is considered that the link between the two diseases is related to the adverse role of glucose excess due to accumulation of advanced glycation end products, oxidative stress and promotion of systemic inflammation [31]. Due to the accumulation of advanced glycation end products, the brittleness and stiffness of collagen increase, and the joint becomes additionally sensitive to mechanical stress [32]. It has been reported that cartilage is softer in diabetics and consequently submissive to damage [17]. Increased insulin resistance may contribute to the development of osteophytes and sclerosis of the subchondral bone [31].

Type II diabetes mellitus is a highly prevalent metabolic disorder with multifactorial etiology caused by an inadequate β-cell response to progressive insulin resistance [27, 33]. The incidence and prevalence of DM II have almost doubled within the last two decades, and a high incidence of DM II has been reported among patients with knee OA [27, 34]. It was reported that 47.3% of patients with DM II have some form of arthritis. Although an association between DM and OA has been shown, a causal link has not been well established [33]. Increased incidence of musculoskeletal diseases in patients with DM may be explained by common risk factors [27].

The association between DM II and OA has been investigated in several meta-analyses [31, 32]. Epidemiological and experimental evidence suggests that DM II may be an independent risk factor for the development and progression of the DM-induced OA phenotype [35]. The DM has also been reported to be a strong predictor of severe OA, regardless of age and body mass index [34]. Veronese et al. [33] found that after adjustment for age, BMI, and other potential risk factors, the DM II doubles the risk of severe OA which requires arthroplasty (hazard ratio = 2.1; 95% confidence index 1.1,3.8; $p = 0.023$). Additionally, DM II may harm the outcome of arthroplasty as it leads to an increased risk of postoperative mortality, reduces functionality, and increases the risk of infection and the need for revision arthroplasty [33]. To the contrary, the results of other studies did not support the link between DM and OA [22, 23].

Osteoarthritis and hypertension

Knee OA and cardiovascular disease are tightly associated with sedentary lifestyle and obesity, [17] but less attention has been paid to the association between hypertension and OA [25]. The mechanism that would explain the association between OA and hypertension is unclear. Both diseases have a high incidence and one of the possible explanations is common risk factors such as aging, obesity and chronic inflammation [29, 36]. It has been shown that multiple genes are involved in both OA and hypertension. It has also been reported that polymorphism in vitamin D receptors may be linked with decreased bone mineral density, OA, and hypertension [29]. Additionally, the proinflammatory cytokine interleukin-6 plays a significant role in the pathogenesis of both diseases [29, 37, 38]. The prevalence of hypertension is higher in patients with OA compared to the population without OA, and poorly regulated hypertension is related to the severity of OA. It has been suggested that hypertension may favour OA through its proatherogenic influence on the subchondral bone [25]. In their meta-analysis, Zhang and al. [29] found a significant relationship between hypertension and both radiographic and symptomatic knee OA. Also, a recent longitudinal study has shown that after adjustment for several confounders, patients with knee OA had a 13% higher chance of developing hypertension (hazard ratio 1.13; 95% confidence index: 1.01–1.26; $p = 0.03$) [39, 40].

Bone tissue is well supplied with blood vessels, and vascularization is closely related to growth, re-

newal and metabolic processes. There are several ways in which vascular pathology leads to damage to the integrity of the subchondral bone resulting in the development of OA [12]. A few mechanisms have been proposed to explain the association between OA and hypertension, [39] including the promotion of microvasculature remodelling [36]. It is considered that blood vessels are narrowing over time, which results in restriction of blood flow to the subchondral bone. Due to impaired blood flow, nutrient delivery is reduced and cartilage begins to deteriorate [39]. In addition to subchondral bone ischemia and osteocyte apoptosis, a decreased blood flow may induce osteoclastic bone resorption [41]. It should be emphasized that hypertension confers a prothrombotic state and microvascular thrombosis further exacerbates the already damaged subchondral bone perfusion [36].

Osteoarthritis and hyperlipidemia

While experimental studies suggest that lipid disorders may be related to the pathophysiology of OA, epidemiological studies show heterogeneous results [13]. Low-density lipoprotein (LDL) cholesterol may be involved in the pathophysiological processes associated with OA through the induction of synovial inflammation, cartilage destruction and ectopic bone formation [42]. The accumulation of cholesterol in cartilage can disrupt the efflux function of cartilage, leading to OA [25, 29]. It has been shown that dietary cholesterol intake leads more frequently to spontaneous cartilage damage in mice [42]. Study in ApoE knockout mice has shown that higher levels of LDL cholesterol in serum correspond to higher levels of pro-inflammatory cytokines [43]. On the other hand, in vivo studies have shown that reduced HDL cholesterol levels play a significant role in the pathogenesis of OA [42]. Also, it seems that higher levels of HDL cholesterol appear to protect against early knee bone-marrow lesions [25].

In their meta-analysis, Baudart et al. [13] showed that the prevalence of dyslipidemia was statistically significantly higher among patients with OA (30%) compared to patients without OA (8%) and that people with OA had 1.98 times higher risk of developing dyslipidemia compared to people without OA. The heterogeneity of epidemiological studies may be due to the use of different definitions of OA and different definitions of dyslipidemia [13].

Conclusion

Holistic treatment of patients with osteoarthritis should be based on an individualized approach including identification and management of risk factors. In addition to symptomatic pain relief and support for weight reduction, it is necessary to treat metabolic disorders, which will eventually lead to an improved biomechanical and metabolic condition of the joint. It is necessary to raise the level of awareness about the importance of primary and secondary prevention of metabolic syndrome to slow down the epidemic of knee osteoarthritis. In the era of diseases related to age and obesity, combined preventive measures are necessary to prevent complications and reduce treatment costs.

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MALIGNANT PLEURAL EFFUSION IN PATIENTS WITH OVARIAN CANCER

MALIGNA PLEURALNA EFUZIJA KOD PACIJENTKINJA SA OVARIJALNIM KARCINOMOM

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Summary

Introduction. Ovarian cancer is the most lethal gynecological cancer. The most common manifestation of thoracic metastasis is pleural effusion. Pleural effusion with positive cytology is regarded as stage IVa of the International Federation of Gynecology and Obstetrics classification, and the overall five-year survival in these patients is less than 20%. We analyzed the data of patients with ovarian cancer who were treated at the Oncology Institue of Vojvodina, in order to establish the incidence of malignant pleural effusions, laterality of pleural effusions, and clinical manifestations. **Material and Methods.** The study included 731 patients with ovarian cancer who were treated at the Oncology Institue of Vojvodina from January 2012 to May 2020. The obtained data were compared with data found in the literature in the same period. **Results.** The incidence of malignant pleural effusion in our study was 5.75%; right-sided pleural effusion was found in 57.15% of patients, 33.33% of patients had effusion on the left side, and 9.52% had bilateral effusions. Thus, unilateral effusion was found in 90.48% of cases, and bilateral in only 9.52%. The most common symptom was dyspnea, reported in 33 patients (78.6%). **Conclusion.** The incidence of malignant pleural effusion in our study was most similar to data found by Zamboni et al. published in 2015; the right side was the dominant side of pleural effusions. The most common symptoms were dyspnea, shortnes of breath and chest pain.

Key words: Pleural Effusion, Malignant; Ovarian Neoplasms; Neoplasm Metastasis; Thoracic Neoplasms; Signs and Symptoms; Neoplasm Staging; Incidence

Sažetak

Uvod. Karcinom jajnika je najletalniji ginekološki karcinom. Najčešća prezentacija torakalnih metastaza je pleuralni izliv. Pleuralni izliv sa pozitivnom citologijom se označava kao stadijum IVa klasifikacije Internacionalne federacije za ginekologiju i opstetriciju i ukupno petogodišnje preživljavanje za ove pacijentkinje je manje od 20%. Analizirali smo podatke pacijentkinja sa ovarijalnim karcinomom, koje su lečene u Institutu za onkologiju Vojvodine, da bismo utvrdili incidenciju malignog pleuralnog izliva, stranu lokalizacije izliva i kliničke manifestacije. **Materijal i metode.** Analizirali smo podatke 731 pacijentkinje sa dijagnostikovanim ovarijalnim karcinomom, lečene u Institutu za onkologiju Vojvodine od januara 2012. do maja 2020. godine. Dobijeni podaci su upoređeni sa podacima koje smo pronašli u literaturi iz istog perioda. **Rezultati.** Incidencija malignog pleuralnog izliva u našoj studiji iznosila je 5,75%, dominantna strana izliva je bila desna, kod 57,15% pacijentkinja, dok je 33,33% imalo izliv sa leve strane, a 9,52% na obe strane. Pleuralni izliv je bio unilateralan u 90,48% slučajeva i bilateralan u samo 9,52%. Najčešći simptom bio je dispnea, prijavljen kod 33 pacijentkinje (78,6%). **Zaključak.** Incidencija u našem istraživanju je najsličnija podacima koje smo našli u studiji Zamboni i saradnika iz 2015. godine, dominantna strana izliva je bila desna. Najčešći simptomi su dispnea, kratak dah i bol u grudima.

Ključne reči: maligni pleuralni izliv; tumori jajnika; metastaze; tumori grudnog koša; znaci i simptomi; stadijumi tumora; incidenca

Introduction

Ovarian cancer is the most lethal gynecological cancer. After endometrial cancer, it is the most common, and it has the same distribution worldwide. In Serbia, 820 cases of ovarian cancer are newly diagnosed every year, and it is the leading cause of death among all malignant gynecological tumors [1]. The most common histological type is epithelial (95%) (high-grade serous carcinoma in 70 – 80% of cases) adenocarcinoma. In 80% of cases it is discovered in advanced stages, International Federation of Gynecology and Obstetrics (FIGO) stage III or IV, due to the lack of symptoms in earlier stages [2].

Omentum and peritoneum are the most common metastatic sites of ovarian cancer, while lymphatic and hematogenous metastases occur in only 2 – 3% of patients. The most common extra-abdominal metastases are malignant pleural effusion and pleural nodules [3]; metastatic ovarian carcinoma is the fourth

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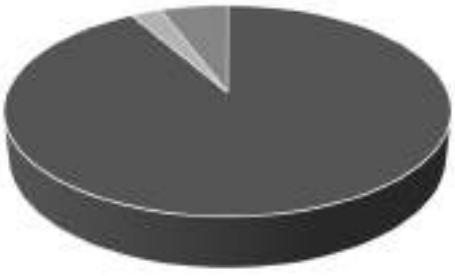
Abbreviations

MPE – malignat pleural effusion
FIGO – International Federation of Gynecology and Obstetrics
VEGF – vascular endothelial growth factor
CT – computed tomography
MRI – magnetic resonance imaging
VATS – video-assisted thoracoscopic surgery

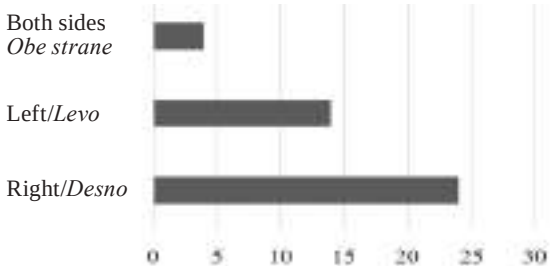
leading cause of malignat plerual effusions (MPE) [4]. Pleural effusion with positive cytology is regarded as FIGO IVa, which means that the overall five-year survival in these patients is less than 20% [2].

The most common presentation of thoracic metastases in these patients is pleural effusion, while pulmonary parenchymal metastases, lymphangitis, and nodal involvement are less common [5]. The MPE is defined as the accumulation of a significant amount of exudate in the pleural space, accompanied by the presence of malignant cells or tumor tissue [6]. There are several theories about the pathophysiology of MPE; currently it is believed that a combination of increased fluid production due to fluid extravasation from hyper-permeable parietal or visceral pleural and/or tumor vessels and impaired lymphatic outflow underlie the development of MPE [7]. Direct cause of pleural effusion is the impaired lymphatic drainage of the pleural space due to obstruction of the lymphatic system at any point from the stroma of the parietal pleura to the mediastinal and internal mammary lymph nodes, or by direct tumor involvement of the pleura. Indirect cause of pleural effusion includes inflammatory response inducing increased microvascular permeability as a result of pleural tumor invasion into the structures of the lymphatic system, resulting in increased entry rate of liquid into the pleural space [4]. The cytokine vascular endothelial growth factor (VEGF) plays a vital role in the induction of further vascular leakage, which has been shown not only in pleural effusions but also in ascites [8]. Also, important mechanism is transdiaphragmatic lymphatic drainage of peritoneal fluid (ascites) into pleura. Positive pleural cytology is an indicator in the diagnosis of MPE; unfortunately, about 30% of cytological pleural fluid results are false-negative [8].



91.65% (patients without pleural effusion)
91,65% (pacijentkinje bez pleuralne efuzije)
2.6% (patients with pleural effusion, less than 2 cm, seen on chest x-ray or thorax CT/MRI)/*2,6% (pacijentkinje sa pleuralnom efuzijom, manjom od 2 cm, viđenom na rendgenskom snimku grudnog koša ili kompjuterizovane tomografije/magnetne rezonantne tomografije)*
5.75% (patients with cytology confirmed MPE)
5,75% (pacijentkinje sa citološki potvrđenom malignom pleuralnom efuzijom)

Graph 1. Ovarian cancer and prevalence of pleural effusion
Grafikon 1. *Ovarijalni karcinom i prevalencija pleuralne efuzije*



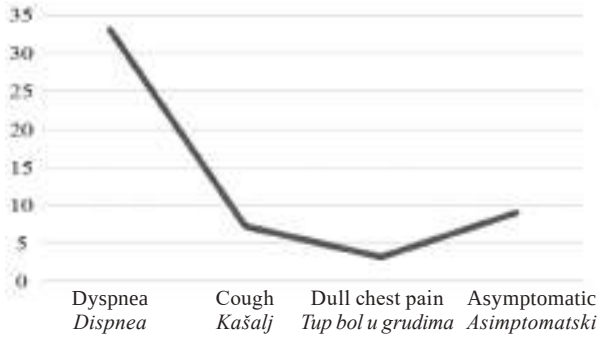
Graph 2. Distribution of MPE and laterality of pleural effusion
Grafikon 2. *Distribucija maligne pleuralne efuzije po stranama pleurane šupljine*

Material and Methods

We analyzed the data of 731 patients with ovarian cancer who were treated at the Oncology Institue of Vojvodina from January 2012 to May 2020, in order to establish the incidence of MPE, laterality of pleural effusion, and clinical manifestations. These data were compared with the data found in the literature in the same period.

Results

The study included 731 patients. A total of 61 patients with stage IVa were diagnosed with pleural effusion, seen on chest x-ray or computed tomography (CT)/magnetic resonance imaging (MRI). Pleural effusion with positive cytology was found in 42 patients (5.75%) confirming MPE. Among them, 29 patients were treated with thoracentesis and 13 needed thoracic drainage due to reaccumulation of effusion. Diagnostic thoracentesis was not performed in 19 (2.6%) patients with CT/MRI pleural effusion diameter less than 2 cm **(Graph 1)**.



Graph 3. Symptoms in patients with MPE
Grafikon 3. *Simptomi kod pacijenata sa malignom pleuralnom efuzijom*

Right-sided pleural effusions were more common; 24 patients (57.15%) had right-sided, 14 patients had left-sided (33.33%), and 4 (9.52%) had bilateral effusions. Thus, unilateral effusion was found in 90.48% of MPE cases, and bilateral in only 9.52% **(Graph 2)**.

The symptoms that were associated with MPE were dyspnea, cough and pain, while 9 patients were asymptomatic. The most common symptom was dyspnea, reported in 33 patients, out of whom 7 also reported cough, and 3 a dull chest pain **(Graph 3)**.

The incidence of MPE in our study was 5.75%; in most cases effusions were unilateral (90.48%) and right-sided (57.15%). The leading symptom of MPE was dyspnea, reported in 33 (78.6%) patients.

Discussion

In 2012, Porcel et al. published a paper on clinical implications of pleural effusions in ovarian cancer. They showed that in a sample of 364 patients, in 14% of cases ovarian cancer was the cause of pleural effusion. According to their study, ovarian cancer was the third most common cause of MPE, after breast (34%) and lung cancer (14.5%). Pleural effusion was unilateral in 77%, mostly right-sided (60%), bilateral in 23%, and two thirds of effusions occupied half or more of the hemithorax. Shortnes of breath was the leading symptom. After chest x-ray, all of the patients underwent a diagnostic thoracocentesis [9].

A research of Zamboni et al., on prognostic factors for the survival in patients with MPE, included 165 patients with MPE, and showed that ovarian cancer was the cause of effusion only in 3.6% of cases. The patients presented with the following symptoms: dyspnea, dull chest pain and nonproductive cough, while 15% of patients were asymptomatic. In 52% of cases, the effusions were large, affecting two tirds or more of the hemithorax, and in 33% they were massive. The diagnosis was confirmed by thoracocentesis as a standard method, and in a small percentage of cases by pleural biopsy, and if needed by video-assisted thoracoscopic surgery (VATS) or thoracotomy. Patients with ovarian cancer that caused MPE had the best median survival (21 months) compared to those with other primary tumors [10].

In the research of Perez Warnisher et al., who analyzed the characteristics of patients with MPE as debut of gynecologic malignancy, 17% of all MPE were associated with gynecological cancer, mostly with ovarian cancer. The most common symptoms in these patients with MPE were dyspnea (82%), cough (32%) and chest pain (25%). In 89% of patients thoracocentesis was performed for diagnosis, while in a small percentage they underwent blind pleural biopsy and thoracoscopy. All patients had a positive citology. In 64% of cases, MPE with ovarian cancer was located in the right hemithorax and ovarian adenocarcinoma was the most frequent primary tumor [11].

In 2018, Shitai et al. studied thoracic manifestations of gynecological tumors, and in a sample of 100 patients with ovarian cancer with thoracic manifestations, 19% of patients had pleural effusion, 38% had a lung mass, and lymphangitic carcinomatosis was found in 11% of cases. Respiratory symptoms included shortness of breath in most cases, cough, chest pain, and sometimes wheezing and hemoptysis. The diagnosis of pleural effusion was made using ultrasound of the pleural space, chest radiography and pleural fluid aspiration. This study showed that pleural effusion is the second most common thoracic manifestation of ovarian malignanices, while the most common was lung mass [12].

A research of Khotimah et al. from 2018, investigated pleural and lung metastases in patients with ovarian cancer who were treated in "Dr. Soetomo Hospital" in Indonesia from 2014 – 2015. It showed that there were only 1.7% of patients with stage IVa ovarian cancer. Only 5 of 292 patients had pleural effusion, of whom 4 patients were diagnosed initially, and only 1 was diagnosed in a 6-mounth period from the initial diagnosis of ovarian cancer. All patients underwent thoracocentesis, and they had positive cytology of the pleural fluid [13].

According to Skok et al., who investigated MPE and its managment, in a literature review from 2019, MPE was found in 18 – 20% of all ovarian cancer patients, that is much higer than in our study. In 33 – 35% of cases, it was also the most common peritoneal manifestation of epithelial ovarian cancer.

In 15% of newly diagnosed patients, MPE is the first clinical sign of the disease, and it presents in 77% of cases ipsilaterally and in 23% bilaterally. Patients who are suffering from ovarian cancer and have MPE have almost twice longer median survival in comparison to other cancer patients with MPE, with 21 months on average **(Table 1)** [2].

Table 1. Distribution of MPE in patients with ovarian cancer (OC)
Tabela 1. *Distribucija maligne pleuralne efuzije (MPE) kod pacijentkinja sa ovarijalnim karcinomom (OK)*

Incidence of MPE in patients with OC *Incidencija MPE kod pacijentkinja sa OK*		OC as the cause of MPE *OK kao uzročnik MPE*	
Perez Warnisher et al. 2016	17%	Porcel et al. 2012	14%
Shitai et al. 2018	19%	Zamboni et al. 2015	3.6%
Khotimah et al 2018	1.7%		
Skok et al. 2019	18 - 20%		

Table 2. Symptoms, localization and diagnosis of MPE
Tabela 2. *Simptomi, lokalizacija i dijagnostika maligne pleuralne efuzije*

Research *Istraživanje*	Symptoms *Simptomi*	Localization *Lokalizacija*	Diagnostic procedure *Dijagnostička procedura*
Porcel et al. 2012	Shortnes of breath *Kratak dah*	77% unilateral (right-sided 60%)/*77% unilateralna (desna strana 60%)*	Chest x-ray thoracocentesis *Rendgenografija i torakocenteza*
Zamboni et al. 2015	Dyspnea, dull chest pain and nonproductive cough 15% asymptomatic/*Dispnea, tup bol u grudima i neproduktivan kašalj 15% asimptomatično*	52% two thirds of hemithorax 33% massive *52% dve trećine hemitoraksa 33% masivno*	Thoracocentesis VATS thoracotomy *Torakocenteza VATS torakotomija*
Perez Warnisher et al. 2016	Dyspnea (82%), cough (32%) and chest pain (25%) *Dispnea (82%), kašalj (32%) and bol u grudima (25%)*	64% right hemithorax *64% desni hemitoraks*	89% Thoracocentesis pleural biopsy thoracoscopy *89% torakocenteza pleuralna biopsija torakoskopija*
Shitai et al. 2018	Shortness of breath, cough, chest pain, wheesing and hemoptysis/ *Kratak dah, kašalj, bol u grudima, wheesing i hemoptizije*	No data *Bez podataka*	Ultrasound, chest x-ray and pleural fluid aspiration *Ultrazvuk, rendgenografija i aspiracija pleuralne tečnosti*
Khotimah et al. 2018	No data *Bez podataka*	No data *Bez podataka*	Thoracocentesis *Torakocenteza*
Skok et al. 2019	No data *Bez podataka*	77% ipsilaterally and 23% bilaterally/*77% ipsilateralno i 23% bilateralno*	No data *Bez podataka*

Porcel et al., in their research from 2012, found a much higer rate of ovarian cancer causing MPE than Zamboni et al. in 2015. In regard to the incidence of MPE in patients with ovarian cancer, three papers published in 2016, 2018 and 2019 reported similar incidence, from 17 – 20%, while Khotimah et al. reported a much lower incidence of only 1.7%. Our research is betwen the study of Khotimah et al. and other studies published in 2016, 2018, and 2019, with an incidence of 5.75%. The localization of MPE in most studies was unilateral, and in two researches (Zamboni and Perez Warnisher) the right side was dominant, like in our study. According to Skok et al., about 25% of MPEs were bilateral.

The most common symptoms found in these studies were dyspnea, shortnes of breath and chest pain, that is in line with the results of our research **(Table 2)**.

Conclusion

The most common manifestation of thoracic metastasis in patients with ovarian cancer is pleural effusion, while pulmonary parenchymal metastases, lymphangitis, and nodal involvement are less common. The incidence of malignat pleural effusion in our study was 5.75%, and this finding is the most similar to data reported by Zamboni et al. from 2015.

In most cases pleural effusion was unilateral (90.48%), with right-sided dominance (57.15%) and these data were similar to data that we found in other analyzed studies.

The leading symptom of malignat pleural effusion was dyspnea, reported in 78.6% of patients. The most common symptoms found in the literature were dyspnea, shortness of breath and chest pain, and these data correspond to data in our study.

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ROLE AND SIGNIFICANCE OF TRACHEOTOMY IN INTENSIVE CARE UNITS IN CORONAVIRUS DISEASE 2019-POSITIVE PATIENTS

ULOGA I ZNAČAJ TRAHEOTOMIJE U JEDINICAMA INTENZIVNE NEGE KOD COVID-19 POZITIVNIH PACIJENATA

Ivan ĐORĐEVIĆ[1] and Dejan STOJAKOV[2]

Summary

Introduction. The role of tracheotomy in the treatment of patients with prolonged intubation in intensive care units is known and confirmed. In light of the global pandemic of severe acute respiratory syndrome coronavirus-2 infection and consequent coronavirus disease 2019, we present our experiences with tracheotomy in infected patients. **Material and Methods**. A retrospective observational study of patients treated in intensive care units at the Clinical Hospital Center “Dr. Dragiša Mišović Dedinje” was carried out in the period from March 21, 2020 to May 14, 2020. **Results**. A total of 970 coronavirus disease 2019-positive patients were treated and out of that number, 116 patients were treated in intensive care units (12%), of which 49 patients (42%) were on non-invasive mechanical ventilation and 67 patients (58%) on intensive mechanical ventilation. The average age of the patients was 59.3 years; the youngest patient was 46, and the oldest 73 years old. Tracheotomy was performed in 24 patients (21%), in 13 males (54.1%) and 11 females (45.9%). The mean time from intubation to tracheotomy was 11.6 days. Of the 24 tracheotomized patients, 12 had a successful decannulation (50%) and were discharged from intensive care units, 6 had a lethal outcome, and 6 patients were in treatment. **Discussion**. All the patients underwent tracheotomy in the hospital room, because we considered that any transfer and manipulation of these severe patients may lead to worsening of the generally serious condition. Although some guidelines recommend that it would be ideal to know the coronavirus disease 2019 status before any invasive procedure, we believe that this is not necessary, especially considering the clinical picture of patients during the pandemic, as well as computed tomography findings in the lungs. **Conclusion**. Tracheotomy has an important place in the treatment of patients with severe coronavirus disease 2019 infection since it provides easier maintenance of the airway, and in the recovery phase leads to easier transition of patients from mechanical ventilation to spontaneous breathing. The decision on the day when the tracheotomy will be performed is strictly individual and depends on the general condition of the patient, and the use of thermocautery does not affect the course of treatment and the final outcome.

Key words: Tracheotomy; Intensive Care Units; COVID-19; SARS-CoV-2; Risk Factors; Aerosolized Particles and Droplets; Personal Protective Equipment; Clinical Protocols; Prognosis

Sažetak

Uvod. Uloga traheotomije u lečenju dugotrajno intubiranih pacijenata u jedinicama intenzivnog lečenja je poznata i potvrđena. U svetlu svetske pandemije SARS-CoV-2 infekcije i posledične COVID-19 bolesti želimo da prikažemo naša iskustva sa traheotomijama kod zaraženih pacijenata. **Materijal i metode.** Urađena je retrospektivna opservaciona studija pacijenata lečenih u jedinicama intenzivnog lečenja u Kliničko-bolnički centar „Dr Dragiša Mišović-Dedinje“ u periodu od 21. 3. 2020−14. 5. 2020. godine. **Rezultati.** Lečeno je ukupno 970 pacijenata pozitivnih na COVID-19, a od ovog broja u jedinicama intenzivnog lečenja lečeno je 116 pacijenata (12%), od toga je 49 bolesnika (42%) bilo na neinvanzivnoj mehaničkoj ventilaciji a 67 bolesnika (58%) na intenzivnoj mehaničkoj ventilaciji. Prosečna starost pacijenata bila je 59,3 godine, najmlađi pacijent imao je 46 godina, a najstariji 73 godine. Traheotomija je urađena kod 24 pacijenta (21%), i to kod 13 osoba muškog pola (54,1%) i 11 kod osoba ženskog pola (45,9%). Prosečno vreme od intubacije do traheotomije bilo je 11,6 dana. Od 24 pacijenta, kod 12 je uspešno urađen dekanilman (50%) i otpušteni su iz jedinica intenzivne nege; kod šest je došlo do letalnog ishoda, a kod šest pacijenata lečenje je bilo u toku. **Diskusija.** Kod svih pacijenata je traheotomija urađena u bolesničkom krevetu jer smo smatrali da svaki transfer, prebacivanje i manipulacija oko ovih teških bolesnika može dovesti do pogoršavanja opšteg teškog stanja. Iako neki vodiči preporučuju da bi bilo idealno znati COVID status pre bilo kakve invazivne procedure, smatramo da to nije neophodno, posebno ako se ima u vidu klinička slika pacijenta u vreme pandemije, kao i nalaz kompjuterizovane tomografije pluća. **Zaključak**. Traheotomija ima značajno mesto u lečenju pacijenata sa teškim oblikom COVID-19 infekcije i ogleda se u lakšem održavanju disajnog puta, a u fazi oporavka dovodi do lakšeg prevođenja pacijenata sa mehaničke ventilacije na spontano disanje. Odluka o danu kada će se izvršiti traheotomija je strogo individualna i zavisi od opšteg stanja pacijenta, a primena termokautera ne utiče na tok i krajnji ishod lečenja.

Klučne reči: traheotomija; jedinice intenzivne nege; COVID-19; SARS-CoV-2; faktori rizika; kapljice i čestice aerosola; lična zaštitna oprema; klinički protocol; prognoza

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Abbreviations

COVID-19	– coronavirus disease 2019
ICU	– intensive care unit
SARS-CoV-2	– severe acute respiratory syndrome coronavirus-2
CT	– computed tomography
PCR	– polymerase chain reaction
NIV	– non-invasive ventilation
IMV	– invasive mechanical ventilation

Introduction

Tracheotomy is an operative procedure known since ancient times. It is assumed that Asclepiades was the first to perform it, which was later confirmed by Galen and Aretaeus.

Tracheotomy is an integral and often necessary part of treatment of patients with malignant tumors of the larynx and pharynx, and its role in the long-term treatment of intubated patients in intensive care units (ICUs) is known and confirmed. In light of the global pandemic of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, and consequent coronavirus disease 2019 (COVID-19), we presented our experiences with tracheotomy in infected patients.

Tracheotomy is defined as an aerosol-generating procedure and healthcare workers are at high risk of infection despite adequate Personal Protection Equipment 1. As a result, numerous guidelines for safe tracheotomy in COVID-19 patients emerged very quickly after the outbreak of the pandemic. All these guidelines are based on knowledge and experience with previous epidemics of viruses H5N1-bird flu, H1N1- swine flu, and MERS-CoV - Middle East respiratory syndrome-related corona virus.

Most of the world's tracheotomy guidelines agree that it would be ideal to know the COVID-19 status before surgery. In situations where this is impossible or limited, and the clinical picture, laboratory tests and computed tomography (CT) findings speak in favor of COVID-19, one should act as if COVID-19 was confirmed [1]. Then, it is necessary to take all personal protective measures of the team involved in the tracheotomy, as well as the staff at the ICU.

All tracheotomies were performed in hospital rooms, in order to avoid transportation to the operating room and manipulation of these severe patients, as well as to avoid worsening of the already serious general condition of patients.

The decision on the need to perform tracheotomy was made jointly by the anesthesiologist, intensivist, and surgeon, and the time of surgery was determined for each patient, depending on the general condition and severity of the clinical picture of the patient with COVID-19.

Guided by all preventive measures recommended for work with COVID-positive patients, as well as recommendations given by several world teams [1–4], all personnel protection measures have been taken, a team of experienced doctors has been appointed, and the number of people participating in the procedure and the tracheotomy itself has been reduced to eliminate the possibility of aerosolization of the virus.

Lung CT has a very important place in the diagnosis and monitoring of COVID-19-positive patients, given the characteristic findings and high sensitivity and specificity. For that purpose, the Total CT Severity score: CO-RADS 6 COVID-19 stage was introduced in the Clinical Hospital Center "Dr. Dragiša Mišović Dedinje", which is an excellent prognostic factor for monitoring and outcome of the disease.

Indications for tracheotomy in COVID-19 patients

The indications for tracheotomy included:
- Separation from mechanical ventilation
- Clearance of secretions
- Acute respiratory distress syndrome
- Pulmonary edema
- Reducing the need for sedation
- Reintubation
- Extracorporeal membrane oxygenation.

Types of tracheotomy in COVID-19 patients

As in non-COVID conditions, there are two options for performing tracheotomy - open, surgical tracheotomy, and percutaneous dilatation tracheotomy. Given the possibilities, equipment, experience and anticipated duration of the procedure, preference was given to open surgical tracheotomy, rather than to percutaneous dilatation tracheotomy.

Material and Methods

The team performing tracheotomy includes two most experienced surgeons, an anesthesiologist, and two nurse practitioners. All the other personnel working in the ICU must be at an adequate distance or absent, all in order to reduce the possibility of infection during the surgery. All team members must have the highest level of personal protection. It is recommended that the operation be performed in rooms with negative pressure, if it is possible.

The biggest problem when performing tracheotomy in intubated patients is that the lung ventilation must be achieved through a respirator, and at the moment of opening the trachea, there is a great possibility of aerosolization. Some authors report the presence of SARS-CoV-2 virus in the air up to 3 hours after exposure [2]. For this purpose, the procedure has been modified and adjusted.

The preparation of patients is the same as in any surgical tracheotomy; maximum extension of the neck, with the head in retroflexion, and a maximum exposure of the neck is of key importance. Preparation of the operation field is standard and involves wiping the field with antiseptic solution (povidone-iodine) and covering it with sterile compresses. In order to reduce the possibility of spreading the virus, a high-power aspirator is necessary.

All patients underwent a tracheotomy using a horizontal incision of the skin and platysma, about

2 - 3 cm in length, then a vertical incision of the deep neck fascia, movement of the pothioid muscles laterally and presentation of thyroid isthmus. In all patients, the isthmus was dissected, ligated, and cut, and then moved to the side for better visualization of the anterior tracheal wall. Before opening the tracheal window, a complete neuromuscular block is necessary, in order to eliminate the cough reflex and swallowing.

When the anterior wall of the trachea is exposed and adequate hemostasis is achieved, the anesthesiologist stops mechanical ventilation, discharges the cuff from the tube and inserts the tube as caudally as possible, inflates the cuff again and continues with mechanical ventilation, which takes place lower than where the trachea will open. Constant patient monitoring is required. The anterior wall is opened with a scalpel and scissors by a horizontal intercartilaginous incision between 2nd and 3rd rings and then two vertical incisions are made, thus creating a flap (Bjork flap) whose base is below and which is then fixed to the skin with three non-absorbable sutures.

The anesthesiologist then stops the mechanical ventilator, dispenses cuff on the tube and gently pulls the tube above the window on the trachea, the surgeon inserts a tracheal cuff cannula, inflates the cuff and connects it to the mechanical lung ventilation system. The cannula is fixed either with skin sutures or neck straps provided for fixation.

We believe that this way of performing tracheotomy reduces the risk of infection in the team performing the operation, the time needed for the procedure is shorter than with other methods of tracheotomy (percutaneous dilatation tracheotomy), and the Bjork flap technique itself allows quick and efficient replacement of tracheal cannula in ICU by nurses or other staff, at times when a surgeon is not available.

We are proud to point out that none of the team members who participated in tracheotomies showed signs of SARS CoV-2 infection.

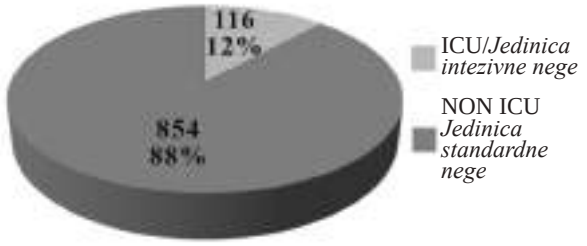
A retrospective observational study of patients treated in ICU at the Clinical Hospital Center "Dr. Dragiša Mišović Dedinje" was performed in the period from March 21, 2020 to May 14, 2020. All tracheotomized patients were COVID-19-positive, as evidenced by nasopharyngeal swabs by polymerase chain reaction (PCR). The average age of patients was 59.3 years; the youngest patient was 46, and the oldest 73 years old.

Results

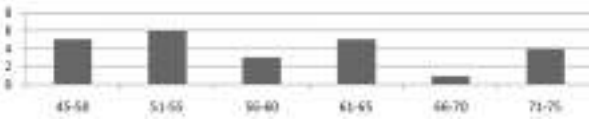
In the aforementioned period, a total of 970 COVID-19-positive patients were treated at the Clinical Hospital Center "Dr. Dragiša Mišović Dedinje" and out of them, 116 patients were treated in the ICUs (12%) **(Graph 1)**.

A total of 24 patients were operated, 13 males (54.1%) and 11 females (45.9%).

The average age of patients was 59.3 years; the youngest patient was 46, and the oldest 73 years old. The age distribution of patients is shown in **Graph 2**.



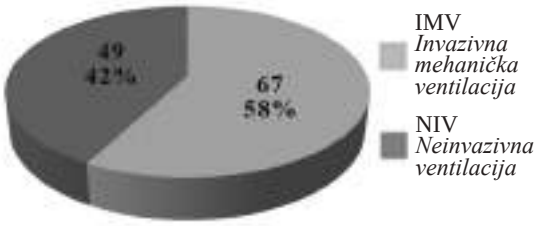
Graph 1. Number of treated patients in standard care and ICUs in the period from March 21, 2020 to May 14, 2020
***Grafikon 1**. Broj lečenih pacijenata u jedinicama standardne nege i intenzivne nege u periodu od 21.03.2020-14.05.2020.*



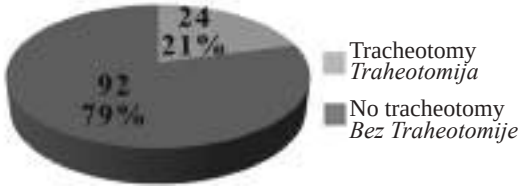
Graph 2. Age distribution of operated patients
***Grafikon 2**. Starostna struktura operisanih pacijenata*

Of the total number of patients in ICUs, 116 patients were on some type of mechanical ventilation; 49 patients (42%) were on non-invasive mechanical ventilation (NIV) and 67 patients (58%) on intensive mechanical ventilation (IMV), that is, they were intubated and on artificial ventilation. The ratio of patients on invasive and non-invasive mechanical ventilation is shown in **Graph 3**.

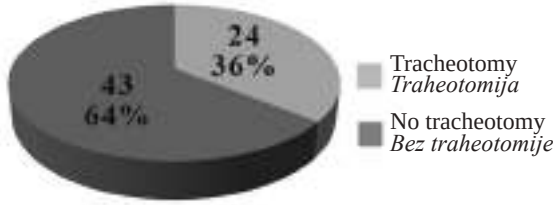
Surgical tracheotomy was performed in 24 patients treated in ICUs (21%).



Graph 3. Number of patients on non-invasive ventilation and on invasive mechanical ventilation
***Grafikon 3**. Broj pacijenata na neinvanzivnoj ventilaciji i na intenzivnoj mehaničkoj ventilaciji*



Graph 4. Total number of patients treated in ICUs (N = 116) and number of tracheotomies performed (N = 24)
***Grafikon 4**. Ukupan broj lečenih pacijenata u jedinicama standardne nege (N=116) i broj urađenih traheotomija (N=24)*



Graph 5. Number of intubated patients on IMV and number of tracheotomies
Grafikon 5. *Broj intubiranih pacijenata na intenzivoj mehaničkoj ventilaciji i broj traheotomija*

However, if the number of intubated patients (N = 67) who underwent tracheotomy is examined, the percentage is higher and amounts to 36% **(Graph 5)**.

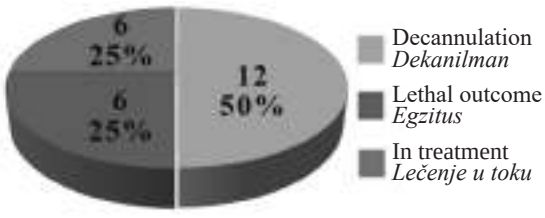
Of the operated patients, 15 had bilateral pneumonia, two (N = 2) patients had unilateral pneumonia, four (N = 4) had acute respiratory distress syndrome, one (N = 1) showed atelectasis, and one (N = 1) only had an enhanced bronchovascular pattern, all confirmed by chest X-ray.

Lung CT was performed in 11 patients and CO-RADS 6 COVID-19 score was determined, but due to technical reasons and severe general condition, lung CT was not performed in 13 patients. Total CT severity score in tracheotomized patients was 6 – 16, and average 11.8.

In regard to comorbidities, most intubated patients in ICUs (65.2%) had numerous comorbidities, (most often arterial hypertension, diabetes mellitus, hyper or hypothyroidism, gastritis, obesity, conditions after mastoidectomy, Hodgkin's lymphoma, gout, renal calculi, etc.) in addition to pneumonia caused by SARS-CoV-2, leading to serious conditions and the need for endotracheal intubation and mechanical ventilation. In our study, presence of associated chronic diseases was not determined in 8 patients (34.7%).

The average time from intubation to tracheotomy was 11.6 days, and 3 tracheotomies were performed within 7 days (early tracheotomy) on the second and fourth day due to pneumomediastinum, and in one patient on the third day due to severe deterioration and complete dependence on mechanical ventilation.

Of the 24 tracheotomized patients, successful decannulation was performed in 12 (50%) and they were discharged from ICUs, 6 had a lethal outcome, and 6 patients were still in treatment **(Graph 6)**.



Graph 6. Total treatment outcome at the end of the study period
Grafikon 6. *Ukupan ishod lečenja pacijenata na kraju posmatranog perioda*

Postoperative bleeding complication occurred in one patient (N = 1) and it was repaired during the same day. Partial pneumothorax occurred as a complication of mechanical ventilation in two patients (N = 2) and pneumomediastinum in two (N = 2), which was also successfully surgically resolved. The average time to replace the tracheal cannula was about the 10th day (+/- 3 days).

Discussion

Since the SARS-CoV-2 pandemic first appeared abroad, there are many unknowns about the treatment of COVID-19-positive patients. Of particular importance is the fact that a large number of patients need to be treated in ICUs, and the rapid tendency of the virus to spread and transmit is of particular concern. Therefore, it is necessary to apply all personal protection measures when treating these patients, and special care is necessary during any intervention or surgery.

All patients underwent tracheotomy in the hospital room, because we thought that any transfer and manipulation in these seriously sick patients could lead to a worsening of the generally severe condition. The authors claim that the incidence of patients in intensive care units is 3 – 15%, so our data of 12% are in line with the expected statistics [1].

Although some guidelines [2–9] recommend that it would be ideal to know COVID status before any invasive procedure, and even recommend postponing it until a positive PCR test is obtained, we believe that this is not necessary, especially considering the clinical picture of patients during the pandemic, and since CT findings in the lungs are highly specific and sensitive to COVID-19.

There are numerous controversies in the treatment of the most severe COVID patients in ICU, especially regarding the use of tracheotomy, given that even in conventional conditions there is no consensus on optimal timing of tracheotomy in intubated patients in ICUs.

Timing of tracheotomy

European guidelines for tracheotomy in COVID-19-positive patients recommend tracheotomy as early as possible, while guidelines from the United Kingdom, United States of America, Singapore and South Africa have a less aggressive approach, i.e. recommend tracheotomy after 14 days of intubation [4–6, 10–14].

Recent studies indicate that the results of early versus late tracheotomy are almost identical, so a decision is necessary for each patient individually [15, 16]. Although we had in mind the existing recommendations, individual approach was necessary for all patients, so the timing of tracheotomy was determined after consultations between the anesthesiologist and surgeon.

McGrath BA, et al. recommend that the "optimal window" to perform a tracheotomy in ICU in these patients is between 16 – 28 days, which corresponds to the 16th to 30th day from the onset of symptoms.

The first antibodies that can be detected in the blood is from 3 – 7 days, and at the same time, the detectability of SARS CoV-2 by PCR is reciprocally declining. Therefore, the authors recommend this time period for tracheotomy, since the virus disappeared from the upper respiratory tract and the body has created enough antibodies against the virus [15].

However, other authors believe that early tracheotomy, by the 10th day of intubation, is not less effective than late tracheotomy. On the contrary, early tracheotomy separates patients from respirators more easily and efficiently, reduces the use of sedatives, and makes the tracheobronchial tree hygiene easier and better [6, 17].

Types of tracheotomy (open or percutaneous)

In the Clinical Hospital Center "Dr. Dragiša Mišović Dedinje", according to the possibilities and experience, the team of doctors gives preference to classical surgical tracheotomy versus percutaneous dilatation tracheotomy, due to safety and simplicity of procedure, avoiding possible complications and shortening the time necessary to perform tracheotomy. Other authors also prefer the open technique [4–6], without diminishing the possibility of applying percutaneous dilatation tracheotomy [14, 15].

Bjork flap tracheotomy

The Bjork flap tracheotomy technique means that the anterior wall of the trachea is sutured to the skin, so it is consider safer for all other manipulations around the tracheal cannula and manipulation with it. Aspiration through the cannula is usually done by nurses in ICU, and in case of necessary faster replacement of the tracheal cannula, an extremely simple and safe way is provided. Bjork flap is found in all guidelines on tracheotomies in COVID-19 patients [1, 3, 10, 12].

Use of electrocautery

There is controversy about the use of electrocautery during tracheotomy in COVID 19-positive patients. Only 6 guidelines are currently discussing this issue. The recommendation of 5 guidelines is the use of cold dissection and the use of sutures for hemostasis, and only 1 recommends the minimum use of electrocautery [3–7]. We used an electrocautery during the surgery and we believe that with the use of an adequate powerful aspirator it is possible to use an electric knife for tracheotomy.

Tracheal cannula replacement time

The average time for tracheal cannula replacement was on the 10th day (+/- 3 days), which again depends on each patient individually, depending on the local hygiene and care of the tracheobronchial tree, as well as the general condition of the patient.

Conclusion

Although the severe acute respiratory syndrome coronavirus-2 pandemic is still ongoing, many researchers are working to find an adequate treatment modality for coronavirus disease 2019-positive patients.

1. Tracheotomy has a significant place in the treatment of patients with severe coronavirus disease 2019;
2. Tracheotomy has an important place in the treatment of patients with severe coronavirus disease 2019 infection since it provides easier maintenance of the airway, and in the recovery phase leads to easier transition of patients from mechanical ventilation to spontaneous breathing;
3. The decision on the day when a tracheotomy will be performed is strictly individual and depends on the general condition of the patient;
4. The use of thermocautery does not affect the course of treatment and the final outcome of the treatment.

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ELECTROCARDIOGRAPHIC FEATURES OF PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

ELEKTROKARDIOGRAFSKE KARAKTERISTIKE PACIJENATA SA HIPERTROFIČNOM KARDIOMIOPATIJOM

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Summary

Introduction. Hypertrophic cardiomyopathy is a disorder of the myocardium characterized by asymmetric or symmetric left ventricular hypertrophy. It is often an inherited disorder with an autosomal dominant pattern. The aim of this study was to evaluate the electrocardiographic characteristics of patients with hypertrophic cardiomyopathy, as well as to assess the accuracy of current electrocardiographic criteria for left ventricular hypertrophy used as indicators of hypertrophic cardiomyopathy. **Material and Methods.** This retrospective study was conducted using hospital medical records of 42 patients with the diagnosis of hypertrophic cardiomyopathy. Detailed electrocardiography analysis, apart from all the usual parameters, included the calculation of indices used to diagnose left ventricular hypertrophy including Sokolow augmented vector left, Cornell voltage, Cornell product, and Sokolow-Lyon index. **Results.** Sinus rhythm was present in 95.2% of patients, while atrial fibrillation was found in 4.8%. The majority of patients presented with left axis deviation. A slight positive correlation was found between the Sokolow augmented vector left index and posterolateral wall thickness ($r = 0.475$; $p < 0.05$), and also between the Cornell voltage index and posterolateral wall thickness ($r = 0.368$; $p < 0.05$). A borderline positive correlation was found between the Cornell product index and posterolateral wall thickness ($r = 0.290$; $p = 0.063$). Interventricular septum thickness showed no significant correlation with any of the electrocardiographic indices of left ventricular hypertrophy. **Conclusion.** In patients with hypertrophic cardiomyopathy, the Sokolow augmented vector left and Cornell voltage indices were the best indicators of posterolateral wall hypertrophy, whereas none of the examined indices correlated well with the interventricular septum thickness.

Key words: Cardiomyopathy, Hypertrophic; Electrocardiography; Hypertrophy, Left Ventricular; Atrial Fibrillation; Echocardiography; Correlation of Data

Sažetak

Uvod. Hipertrofična kardiomiopatija predstavlja poremećaj srčanog mišića, koji karakteriše asimetrična ili simetrična hipertrofija miokarda leve komore. Hipertrofična kardiomiopatija je najčešće nasledni poremećaj koji se prenosi autozomno dominantno. Cilj ovog istraživanja bio je procena elektrokardiografskih karakteristika pacijenata sa hipertrofičnom kardiomiopatijom kao i provera preciznosti postojećih elektrokardiografskih kriterijuma koji se koriste kao pokazatelji hipertrofije leve komore. **Materijal i metode.** Ovu retrospektivnu studiju sproveli smo koristeći medicinsku dokumentaciju grupe od 42 pacijenta sa potvrđenom dijagnozom hipertrofična kardiomiopatija. Detaljna elektrokardiografska analiza, osim svih uobičajenih parametara, obuhvatila je i izračunavanje indeksa koji se koriste za dijagnozu hipertrofije leve komore uključujući *Sokolow augmented vector left, Cornell voltage, Cornell product* i *Sokolow-Lyon* indeks. **Rezultati**. Sinusni ritam je zabeležen kod 95,2% pacijenata, dok je atrijalna fibrilacija bila prisutna kod 4,8%. Srčana osovina je u najvećem broju slučajeva pokazala devijaciju ulevo. Dobijena je blaga pozitivna korelacija između *Sokolow augmented vector left* indeksa i debljine posterolateralnog zida ($r = 0,475$; $p < 0,05$), kao i između *Cornell voltage* indeksa i debljine posterolateralnog zida ($r = 0,368$; $p < 0,05$). Granično značajna pozitivna korelacija je dobijena između *Cornell product* indeksa i debljine posterolateralnog zida ($r = 0,290$; $p = 0,063$). Debljina interventrikularnog septuma nije imala značajnu korelaciju ni sa jednim elektrokardiografskim indeksom hipertrofije leve komore. **Zaključak**. Kod pacijenata sa hipertrofičnom kardiomiopatijom, vrednosti *Sokolow augmented vector left* i *Cornell voltage* indeksa najbolje pokazuju nivo hipertrofije posterolateralnog zida, dok nijedan od ispitivanih indeksa nije povezan sa debljinom interventrikularnog septuma.

Ključne reči: hipertrofična kardiomiopatija; elektrokardiografija; hipertrofija leve komore; atrijalna fibrilacija; ehokardiografija; korelacija

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited disease of the myocardium with a prevalence of approximately 1 in 500 adults [1], which most often presents between the third and fifth decade. It is a disorder of the myocardium that

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Abbreviations

BMI – body mass index
ECG – electrocardiography
HCM – hypertrophic cardiomyopathy
LV – left ventricle
aVL – augmented vector left
NYHA – New York Heart Association

is characterized by asymmetric or symmetric hypertrophy of the left ventricle (LV) with no apparent reason. In order to make the diagnosis, any abnormal loading conditions, such as aortic stenosis and long-lasting arterial hypertension, as well as other systemic and metabolic diseases that may cause myocardial hypertrophy must be excluded [2, 3].

The HCM is caused by a genetic mutation that is transmitted with an autosomal dominant pattern, caused most commonly by a single mutation in one of the sarcomere protein genes, which can be present in either thick or thin filament genes [4, 5]. The two most common are the thick filament mutation myosin-binding protein C and β-myosin heavy chain, which are responsible for approximately three-quarters of the identified mutations in HCM patients [6].

Clinical presentation of HCM is often completely asymptomatic, while symptoms may include fatigue, dyspnea, chest pain, palpitations, syncope, and in the worst case even sudden cardiac death [7–9]. Septal hypertrophy may lead to obstruction of LV outflow tract, while other complications include myocardial fibrosis, microvascular ischemia, and deterioration of cardiac function [10].

The diagnosis of HCM is based mainly on imaging techniques including electrocardiography, computed tomography and cardiac magnetic resonance imaging. Electrocardiography (ECG) is used as a diagnostic algorithm primarily in the detection and follow-up of heart rhythm disorders that may range from atrial tachyarrhythmias, premature supraventricular and ventricular complexes, to malignant arrhythmias like ventricular tachycardia [11]. Nonetheless, there are specific ECG signs that indicate the presence of LV hypertrophy, based on which several criteria have been established. The best known ECG criteria for LV hypertrophy are Sokolow-Lyon, Sokolow augmented vector left (aVL), Cornell voltage, Cornell product, Romhilt and Siegel [12, 13].

The aim of this study was to evaluate the ECG characteristics of patients with HCM, as well as to assess the accuracy of current ECG criteria for LV hypertrophy used as indicators of HCM.

Material and Methods

This retrospective observational study was performed using hospital medical records of 42 patients with the diagnosis of HCM. The collected data included demographic characteristics, medical history, clinical and laboratory findings, ECG and echocardiographic findings. The diagnosis of HCM was established using echocardiography with LV wall thickness of ≥ 15 mm, in the absence of other cardiac or systemic diseases which could contribute to its development, such as aortic stenosis or arterial hypertension.

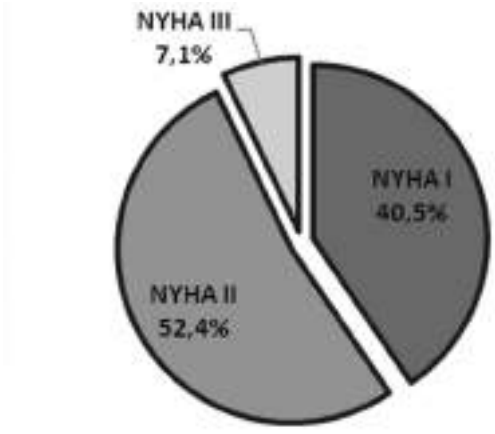
All the participants underwent a 12-lead ECG in supine position with standard calibration (voltage 0.1 mV/mm; paper speed 25 mm/s). Detailed ECG analysis, apart from all the usual parameters, involved calculation of the criteria used for diagnosing LV hypertrophy including Sokolow aVL, Cornell voltage, Cornell product, and Sokolow-Lyon index. These indices were calculated by measuring the voltages of the particular QRS complex components in specific leads. Detailed formulas for the calculation of specific indices and their cut-off values for LV hypertrophy are shown in **Table 1**. Reference values are the same for both sexes, with the exception of Cornell voltage criteria which are sex-specific.

Continuous variables are expressed as mean values ± standard deviation, while categorical variables are presented as absolute numbers and percentages. Acquired results were statistically analyzed using Pearson's linear correlation coefficient and independent samples t-test. Statistical significance was set at $p < 0.05$.

Results

The study included 42 patients of whom 69.0% (29/42) were male and 31.0% (13/42) were female. The patients were 61.7 (± 10.4) years old with a mean body mass index (BMI) of 29.5 (± 4.1) kg/m^2. There were 19.1% (8/42) of patients in the normal (healthy) weight, 38.1% (16/42) of patients were overweight, 35.7% (15/42) of patients were with grade I and 7.1% (3/43) of patients with grade II obesity.

In regard to clinical characteristics, 61.9% (26/42) of patients felt fatigue, 33.3% (14/42) felt chest pain, and 33.3% (14/42) had shortness of breath. There were 23.8% (10/42) of asymptomatic patients. According to the New York Heart Association (NYHA)



Graph 1. Distribution of HCM patients according to the NYHA classification
Grafikon 1. *Distribucija pacijenata sa hipertrofičnom kardiomiopatijom prema New York Heart Association klasifikaciji*

Table 1. Formulas used for calculation of the ECG criteria for LV hypertrophy
Tabela 1. *Formule korišćene za izračunavanje elektrokardiografskog kriterijuma hipertrofije leve komore*

Name *Naziv*	Calculation formula *Formula za izračunavanje*	Cut-off value for LV hypertrophy *Granična vrednost za hipertrofiju LV*
Sokolow augmented vector left	R (aVL)	> 11 mm
Cornell voltage	R (aVL) + S (V3)	> 28 mm (male/*muškarci*) > 20 mm (female/*žene*)
Cornell product	(R (aVL) + S (V3)) x QRS	> 2436 mm·ms
Sokolow-Lyon	S (V1) + R (V5/V6)	> 35 mm

Table 2. General characteristics of the HCM patients' cohort
Tabela 2. *Opšte karakteristike kohorte pacijenata sa hipertrofičnom kardiomiopatijom*

Females/*Žene*	13 (30.9%)
Age/*Starost* (years/*godine*)	61.7 (±10.4)
Body mass index/*Indeks telesne mase* (kg/m^2)	29.5 (±4.1)
Symptoms/*Simptomi*	
Fatigue/*Zamaranje*	26 (61.9%)
Dyspnea/*Gušenje*	14 (33.3%)
Chest pain/*Bol u grudima*	14 (33.3%)
Asymptomatic/*Bez simptoma*	10 (23.8%)
NYHA classification/*New York Heart Association klasifikacija*	
I	17 (40.5%)
II	22 (52.4%)
III	3 (7.1%)
Comorbidities/*Komorbiditeti*	
Diabetes mellitus/*Dijabetes melitus*	6 (14.3%)
Chronic obstructive pulmonary disease/*Hronična opstruktivna bolest pluća*	4 (9.5%)
Thyroid disease/*Bolest štitaste žlezde*	2 (4.8%)
Renal dysfunction/*Bubrežna disfunkcija*	2 (4.8%)

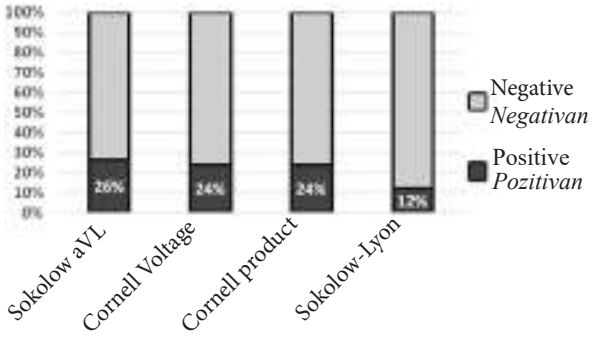
classification for heart failure **(Figure 1)** 40.5% (17/42) of patients had class I symptoms, 52.4% (22/42) of them had class II, while 7.1% (3/42) had class III symptoms. There were no patients with NYHA class IV. Demographic data and general characteristics of HCM patients are shown in **Table 2**.

Family history of HCM was positive in 19.0% (8/42) of patients, while only one patient (2.4%) had a positive family history of dilated cardiomyopathy. The most common comorbidity was diabetes mellitus, in 14.3% (6/42) of patients, followed by chronic obstructive pulmonary disease in 9.5% (4/42), renal insufficiency in 4.8% (2/42) and thyroid gland disease in 4.8% (2/42) of patients.

Mean systolic blood pressure was 136.7 (± 20.4) mmHg and diastolic 77.6 (± 10.0) mmHg. Sinus rhythm

Table 3. Correlation between ECG indices for LV hypertrophy and echocardiographically measured LV wall thickness
Tabela 3. *Korelacija između elektrokardiografskog indeksa za hipertrofiju leve komore i ehokardiografski merene debljine zidova leve komore*

	Interventricular septum thickness *Debljina interventrikularnog septuma*		Posterolateral wall thickness *Debljina posterolateralnog zida*	
	r	p	r	p
Sokolow augmented vector left	-0.143	0.366	0.475	0.001*
Cornell voltage	0.094	0.556	0.368	0.016*
Cornell product	-0.013	0.934	0.290	0.063
Sokolow-Lyon	0.206	0.191	0.042	0.793



Graph 2. Percentage of HCM patients with positive and negative criteria for LV hypertrophy
Grafikon 2. *Broj pacijenata sa hipertrofičnom kardiomiopatijom sa pozitivnim i negativnim kriterijumima za hipertrofiju leve komore*

was recorded in 95.2% (40/42) of patients, while atrial fibrillation was found in 4.8% (2/42). Normal heart axis was found in 42.8% (18/42) of patients, left heart axis was found in 52.4% (22/42), and right heart axis was observed in 4.8% (2/42) of cases.

Regarding the Sokolow aVL index, its mean value was 7.1 (± 5.8) mm, with 26.2% (11/42) of patients measuring over the reference value of 11 mm, and 73.8% (31/42) of them were below 11 mm. Mean Cornell voltage index in males was 18.1 (± 8.9) mm, and in females 20.0 (± 7.7) mm. There were 23.8% (10/42) of patients with a value over the reference limit, while 76.2% (32/42) were below it. Mean Cornell product index was 2161.8 (± 1662.4) mm·ms. In 23.8% (10/42) of patients, the value was over the reference limit (2436 mm·ms), and in 76.2% (32/42) of patients it was below it. Mean Sokolow-Lyon index was 20.5 (± 10.9) mm. There were 11.9% (5/42) of patients with a value over the reference limit, which is 35 mm, and in 88.1% (37/42) it was below it. **Figure 2** shows the distribution of HCM patients with various LV hypertrophy criteria.

The LV myocardial thickness was measured at posterolateral wall and interventricular septum. Both measures were increased, so the mean interventricular septum thickness was 17.4 (± 4.3) mm, and posterolateral wall thickness was 15.5 (± 3.4) mm. Mean LV ejection fraction was preserved 61.9 % (± 9.7).

All of the above-mentioned ECG criteria for LV hypertrophy were correlated with the true echocardiographically measured wall thickness **(Table 3)**. The obtained results showed a mild positive correlation between the Sokolow aVL index and posterolateral wall thickness ($r = 0.475$; $p < 0.05$), and also between the Cornell voltage index and posterolateral wall thickness ($r = 0.368$; $p < 0.05$). Borderline correlation was found between the Cornell product index and posterolateral wall thickness ($r = 0.290$; $p = 0.063$). Interventricular septum thick-

Table 4. Comparison of LV wall thickness on echocardiography between patients with positive and negative ECG indices for LV hypertrophy
Tabela 4. *Poređenje debljinve zidova leve komore na ehokardiografiji između pacijenata sa pozitivnim i negativnim elektrokardiografskim indeksima za hipertrofiju leve komore*

		N *Br*	Mean *Prosečna vrednost*	Standard deviation *Standardna devijacija*	p-value *p-vrednost*
Sokolow augmented vector left					
Interventricular septum *Interventrikularni septum*	Negative/*Negativan*	31	17.23	4.60	0.702
	Positive/*Pozitivan*	11	17.82	3.63	
Posterolateral wall *Posterolateralni zid*	Negative/*Negativan*	31	14.81	2.30	0.033*
	Positive/*Pozitivan*	11	17.36	5.26	
Cornell voltage					
Interventricular septum *Interventrikularni septum*	Negative/*Negativan*	32	17.44	4.41	0.882
	Positive/*Pozitivan*	10	17.20	4.29	
Posterolateral wall *Posterolateralni zid*	Negative/*Negativan*	32	14.84	2.30	0.032*
	Positive/*Pozitivan*	10	17.50	5.48	
Cornell product					
Interventricular septum *Interventrikularni septum*	Negative/*Negativan*	32	17.38	4.61	0.988
	Positive/*Pozitivan*	10	17.40	3.53	
Posterolateral wall *Posterolateralni zid*	Negative/*Negativan*	32	14.88	2.34	0.042*
	Positive/*Pozitivan*	10	17.40	5.48	
Sokolow-Lyon					
Interventricular septum *Interventrikularni septum*	Negative/*Negativan*	37	17.16	4.31	0.380
	Positive/*Pozitivan*	5	19.00	4.64	
Posterolateral wall *Posterolateralni zid*	Negative/*Negativan*	37	15.19	3.50	0.145
	Positive/*Pozitivan*	5	17.60	2.30	

ness showed no significant correlation with any of the ECG indices of LV hypertrophy.

The difference in the LV wall thickness between patients with positive and negative results was analyzed for each of the ECG index **(Table 4)**. A significantly higher posterolateral wall thickness was found in patients with positive compared to those with negative Sokolow aVL index (17.4 vs. 14.8 mm, $p < 0.05$), as well as for Cornell voltage (17.5 vs. 14.8 mm, $p < 0.05$) and Cornell product (17.4 vs. 14.9 mm, $p < 0.05$). No statistically significant difference was found in interventricular septum thickness in patients with positive and negative results for any of the studied ECG indices.

Discussion

This study investigated the value of classical ECG criteria in the detection of LV hypertrophy in patients with HCM. The main findings revealed a generally low reliability of all examined ECG criteria, especially regarding the interventricular septum thickness. Nevertheless, Sokolow aVL and Cornell voltage criteria can be used as solid indicators of posterolateral wall thickness in HCM patients.

In our cohort, there was a 2:1 male predominance, which confirmed the literature data that males account for 55 – 75% of patients with the diagnosis of HCM [12, 14, 15]. This uneven gender distribution is attributed to reduced disease penetrance in women, as well as slower progression of myocardial hypertrophy that could be related to protective role of female sex hormones. Moreover, the lack of gender specific HCM diagnostic criteria means that women require relatively higher level of myocardial hypertrophy in order to reach the diagnostic threshold of wall thickness > 15 mm, because on average they have smaller hearts than men. This translates into increased relative wall thickness compared with lower LV cavity sizes in females, leading to more severe symptoms and higher NYHA functional class [16].

The BMI analysis showed that the majority of HCM patients in this study were overweight and obese, which is outlined as a predominant risk factor for symptoms and negative outcome in HCM, as well as for the development of LV outflow tract obstruction [17].

The most common clinical symptom in our study was fatigue, which has not been suggested as a specific symptom of HCM in the literature [1]. Our results showed that the majority of patients had mild symptoms and were in NYHA class I and II; nearly one quarter of patients were completely asymptomatic, which correlates with the literature data [12]. Mild clinical presentation of HCM does not necessarily mean good prognosis, since these patients can be at high risk of sudden cardiac death regardless of the symptoms and their intensity.

Sinus rhythm was registered in the majority of patients. Only a small fraction of patients had atrial fibrillation, which is consistent with the literature [12, 18, 19]. The development of atrial fibrillation in patients with HCM is associated with other markers of more severe disease expression such as left atrial enlargement, higher LV wall thickness, and LV outflow tract obstruction [20]. Examination of cardiac axis in ECG showed that more than half of HCM patients had left heart axis that can be attributed to increase in LV myocardial mass, which is similar to the results of other studies [12, 21].

Examination of ECG criteria for determining LV hypertrophy showed that none of the ECG criteria positively indicate interventricular septum hypertrophy, while Sokolow aVL, Cornell voltage and Cornel product may point to posterolateral wall hypertrophy, because the thickness of posterolateral wall is significantly increased in those with positive compared to those with negative findings. All four of the examined ECG criteria for LV hypertrophy were positive in about one quarter of patients, which means that, according to our results, as much as three quarters of patients with HCM present without ECG symptoms of LV hypertrophy. Such results are consistent with the literature data, and confirm the low sensitivity of ECG in regard to screening and diagnosis of HCM [12].

Correlation analysis showed that Sokolow aVL and Cornell voltage indices show a positive correlation to LV posterolateral wall thickness, and therefore, the grater the correlation, the greater the posterolateral wall hypertrophy. This is consistent with the results of Monzo et al. [12] who also concluded that these two ECG criteria best correspond to the maximal LV wall thickness. While the calculated Cornell voltage index in our study positively correlated with posterolateral wall thickness, results of other studies showed its correspondence with LV anterior wall and interventricular septum. On the other hand, none of the analyzed criteria in our study showed significant correlation with the interventricular septum thickness.

Although ECG criteria for determining LV hypertrophy have been thoroughly examined in various disorders that cause or contribute to myocardial hypertrophy, such as arterial hypertension and stenosis of the aortic valve, there are scarce data on their use in genetically transmitted HCM. Our study is one of the few across the literature that analyzed the properties and value of these ECG criteria in patients with HCM, and certainly the first one performed in the HCM population of Serbia.

While the present study focused on diagnostic performance of ECG in recognition of LV hypertrophy in patients with HCM, the interest of the scientific community is currently aimed at HCM disease progression and prognosis. In that light, our ongoing multicenter SILICOFCM trial [22, 23] seeks to provide novel data on whether the complementary addition of either sacubitril/valsartan or lifestyle intervention to the optimal standard therapy improves cardiovascular performance in patients with non-obstructive HCM as well as their clinical phenotypic characteristics, injury and stretch activation markers, habitual physical activity, and quality of life.

Conclusion

The electrocardiography characteristics of patients in our study showed that the majority of patients with hypertrophic cardiomyopathy were in sinus rhythm, while the most common arrhythmia was atrial fibrillation. Left heart axis was present in most hypertrophic cardiomyopathy patients due to left ventricular hypertrophy. The values of Sokolow augmented vector left and Cornell voltage criteria best indicated the level of posterolateral wall hypertrophy, whereas none of the examined criteria correlated well with the interventricular septum thickness.

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SABER-SHEATH TRACHEA – A RARE OR UNDERDIAGNOSED MORPHOLOGICAL VARIETY OF THE TRACHEA

TRAHEJA OBLIKA KORICA SABLJE – REDAK ILI NEDOVOLJNO DIJAGNOSTIKOVAN MORFOLOŠKI VARIJETET TRAHEJE

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Summary

Introduction. The saber-sheath trachea is characterized by widened anteroposterior and narrowed laterolateral tracheal diameter. It is usually found in patients with chronic obstructive pulmonary disease. The aim of this study was to determine the incidence of this disorder, as well as to gain insight into the basic socio-demographic characteristics of patients and the clinical features of this tracheal deformity. **Material and Methods**. Endoscopic findings of patients undergoing bronchoscopy at the Institute for Pulmonary Diseases of Vojvodina in the period January 1, 2013 – January 1, 2021 were analyzed. Individual socio-demographic data of patients with the diagnosis of saber-sheath trachea were collected and clinical parameters were analyzed. **Results**. The analysis of 15.381 bronchoscopic findings showed an incidence of 0.56%, most often in the elderly, predominantly in men, and those who were active or former smokers. The most common comorbidities were chronic obstructive pulmonary disease, cardiovascular diseases, and diabetes. In all cases, this deformity was found as an incidental finding during bronchoscopy that was mostly done for the diagnosis of primary carcinoma of the bronchus. Accordingly, the most common pre-bronchoscopic symptoms were shortness of breath, cough, and chest pain. No significant narrowing of the tracheal lumen was found in any of the patients, nor did this anatomical variety of the trachea affect the length of survival. **Conclusion**. Although this is a rare disorder, its recognition can guide the clinician to apply additional procedures in order to establish a diagnosis of chronic obstructive pulmonary disease, but also significantly contribute to avoiding potential complications in need of endotracheal intubation.

Key words: Trachea; Cartilage; Congenital Abnormalities; Pulmonary Disease, Chronic Obstructive; Bronchoscopy; Intubation, Intratracheal; Sociodemographic Factors; Signs and Symptoms

Sažetak

Uvod. Traheju oblika korica sablje karakteriše proširenje anteroposteriornog prečnika, uz istovremeno suženje laterolateralnog prečnika dušnika. Najčešće se javlja kod obolelih od hronične opstruktivne bolesti pluća. Cilj ovog istraživanja bio je da se utvrdi učestalost pomenutog poremećaja, kao i da se stekne uvid u osnovne sociodemografske karakteristike obolelih, kao i kliničke karakteristike ovog deformiteta traheje. **Materijal i metode**. Analizirani su endoskopski nalazi bronhoskopiranih bolesnika u Institutu za plućne bolesti Vojvodine u periodu 1. 1. 2013–1. 1. 2021. godine. Za bolesnike kod kojih je potvrđeno postojanje *saber-sheath* traheje, prikupljeni su pojedini sociodemografski podaci i analizirane kliničke karakteristike obolelih. **Rezultati.** Analizom 15.381 bronhoskopskog nalaza, utvrđeno je da je učestalost traheje oblika korica sablje u ispitivanoj populaciji bila 0,56%; najčešće se javlja kod osoba starijeg životnog doba, i to predominantno kod muškaraca i onih koji su aktivni ili bivši pušači. Najčešće pridružene bolesti bile su hronična opstruktivna bolest pluća, kardiovaskularne i šećerna bolest. U svim slučajevima, deformitet traheje je uočen kao slučajan nalaz prilikom bronhoskopije, indikovane najčešće zbog primarnog karcinoma bronha. U skladu s tim, najčešći prebronhoskopski simptomi bili su otežano disanje, kašalj i bolovi u grudima. Istovremeno, ni kod jednog bolesnika nije utvrđeno značajnije sužavanje lumena dušnika, niti je ovaj anatomski varijetet traheje uticao na dužinu preživljavanja. **Zaključak.** Iako se radi o retkom poremećaju, njegovo prepoznavanje može usmeriti kliničara na primenu dodatnih procedura u cilju postavljanja dijagnoze hronične opstruktivne bolesti pluća, ali i značajno doprineti izbegavanju potencijalnih komplikacija u slučaju potrebe za izvođenjem endotrahealne intubacije.

Ključne reči: traheja; hrskavica; kongenitalne anomalije; hronična opstruktivna bolest pluća; bronhoskopija; endotrahealna intubacija; sociodemografski faktori; znaci i simptomi

Introduction

Saber-sheath trachea is a fixed deformity characterized by an acquired widened anteroposterior and a substantially narrowed laterolateral tracheal diameter [1]. According to previous research, tracheal narrowing occurs only in the intrathoracic airway, while the extrathoracic part of the trachea remains normal [2]. Unlike tracheomalacia, tracheal rings are thicker and the trachea is not collapsible.

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Abbreviations

COPD – chronic obstructive pulmonary disease
CT – computed tomography
IPDV – Institute for Pulmonary Diseases of Vojvodina

The exact cause of this anatomical variation of trachea is unknown. It is most frequent in patients with chronic obstructive pulmonary disease (COPD) [3]. Studies show that diagnosing COPD in patients with saber-sheath trachea has a high specificity (92.9%), but a low sensitivity (39.1%) [4]. The diagnosis of saber-sheath trachea is most commonly made as a coincidental finding on chest X-ray or chest computed tomography (CT) [5], or as an incidental finding during bronchoscopy.

In the vast majority of cases, this condition does not result in a significant narrowing of the tracheal lumen and therefore it does not require any specific treatment. However, several cases of difficult endotracheal intubation have been reported in the literature [6, 7]. Therefore, it is important to consider this disorder in the preoperative patient preparation for surgeries requiring general anesthesia.

The aim of the present study was to determine the incidence of saber-sheath trachea based on descriptions of endoscopic findings in patients undergoing bronchoscopy for various reasons, as well as to gain insight into basic socio-demographic characteristics of patients and clinical manifestations of the disorder. We also wanted to provide additional information about this rare, but important condition.

Material and Methods

In this retrospective observational study, 15.381 bronchoscopic findings of patients who underwent bronchoscopy at the Institute for Pulmonary Diseases of Vojvodina (IPDV), Sremska Kamenica, Serbia, from January 1, 2013 to January 1, 2021, were evaluated. The final analysis included findings of 82 patients who met the inclusion criterion of the study: diagnosis of saber-sheath trachea (trachea with a narrowed laterolateral and increased anteroposterior diameter) confirmed by bronchoscopy. Even though all patients who underwent bronchoscopy had previously performed chest X-ray and/or CT scan, radiological confirmation of saber-sheath trachea was not a mandatory criterion for inclusion in this study. Data on gender, age, smoking habits, comorbidities, past surgeries requiring general anesthesia, symptoms reported by patients prior to bronchoscopy, lung function parameters, and disease outcome after a period of follow-up, were extracted from the patients' medical records.

The informed consent of the patients included in the study was not necessary, because the study was based on retrospective analysis of medical data. However, all subjects signed an informed consent for bronchoscopy. Statistical data processing was performed using the software packages IBM Statistical Package for the Social Sciences Statistics v. 23 and Microsoft Office 2013. The central tendency of numerical features is shown by the arithmetic mean, and the minimum, maximum, and range of values. Attributive features are shown using absolute and relative frequencies.

Results

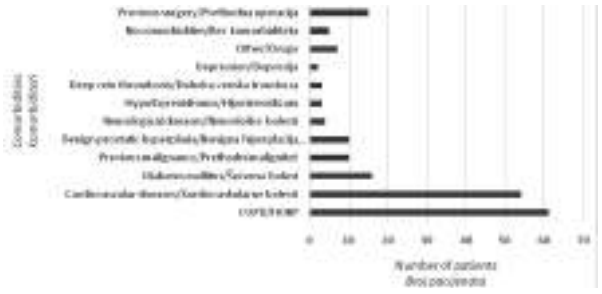
During the eight-year study period, 15.381 bronchoscopies were performed at the IPDV including 14.545 patients undergoing bronchoscopy (some patients underwent bronchoscopy two or more times). Saber-sheath trachea was confirmed by endoscopy in 82 patients, accounting for 0.56% of the overall sample.

Males dominated in our sample (78 respondents, i.e. 95.1%). The average age of patients was 68.3 years, ranging from 53 to 87 years, indicating that this condition was found primarily in elderly.

Every patient in this study had previously smoked cigarettes. A total of 52 patients (63.4%) were current smokers, consuming 65.4 packs per year (ranging from 30 to 140) on average, while 30 patients (36.6%) had previously smoked 50.8 packs per year (ranging from 30 to 100) on average, but were presently nonsmokers. **Table 1** shows the main socio-demographic characteristics of our sample.

The COPD was the most common comorbidity in the sample, having been previously identified in 49 patients (59.7%). Spirometry results were available for another 12 of the remaining 33 individuals who had not been previously diagnosed with COPD, and all 12 patients had lung volumes and capacities, as well as bronchodilator reversibility test values that indicated COPD. Thus, COPD was found to be practically present in 61 patients in the study population (74.4%). However, it must be noted that 21 patients (25.6%) were not diagnosed with COPD prior to bronchoscopy and had not undergone spirometry, which is the reason why this part of the research group could not be assessed for the presence of COPD. Of the other comorbidities, the most common were cardiovascular diseases – arteriosclerosis, cardiomyopathy, and ischemic heart disease (54 patients, i.e. 65.8%), followed by diabetes mellitus (16 patients, i.e. 19.5%), history of previously treated malignant diseases, and benign prostatic hyperplasia (10 patients in both cases, i.e. 12.2%). Only five participants (6%) were found to have no comorbidities. A total of 15 patients (18.3%) had previously undergone surgery under general anesthesia, but no data on possible difficulties during endotracheal intubation were found in any of the subjects' medical records. **Graph 1** shows findings on patients' comorbidities.

The most prevalent complaints prior to bronchoscopy were (in descending order): shortness of breath (54 patients, i.e. 65.8%), cough (52 patients, i.e. 63.4%), chest pain (22 patients, i.e. 26.8%), expectoration of blood (10 patients, i.e. 12.2%), fever (8 patients, i.e. 8.5%), while only 3 patients had no symptoms (3.6%). The majority of patients underwent bronchoscopy due to lung carcinoma (66 patients, i.e. 80.5%), whereas 11 patients (13.4%) had



Graph 1. The incidence of comorbidities
Grafikon 1. *Učestalost komorbiditeta pacijenata*

inflammatory processes in the lungs. Bronchoscopy did not reveal the cause of alterations in the lung parenchyma in the remaining 5 patients (6.1%).

Disease outcome data were available for a total of 65 patients. The average follow-up period after bronchoscopy was 10.7 months (ranging from 1 to 60), and the follow-up shows that most patients with bronchial cancer died with an average survival time of 8.2 months (38 patients; data were unavailable for the remaining 28 patients). In those with inflammatory changes in the lung parenchyma, the symptoms completely resolved after the application of an adequate therapeutic protocol, and patients were asymptomatic during the follow-up period. Considering this, it can be concluded that this anatomical variation did not result in a substantial degree of tracheal constriction and subsequent clinically significant problems.

Discussion

Our research shows that the prevalence of saber-sheath trachea in the general population is low (0.56%), that it mostly affects the elderly, predominantly men, and those who are active or former smokers. The most prevalent comorbidity is COPD, followed by cardiovascular diseases and diabetes mellitus. In most cases, saber-sheath trachea is observed as an accidental finding during bronchoscopy, which is performed most usually due to bronchial cancer. Accordingly, the most common symptoms prior to bronchoscopy are shortness of breath, cough, and chest pain. Patients with this tracheal deformity present with no significant narrowing of the tracheal lumen, nor does this anatomical variety affect the survival time of these patients.

The data in the literature vary greatly when it comes to the prevalence of this acquired anatomical variety of the trachea. However, everyday clinical practice shows that this variety often remains unrecognized, which indicates that its prevalence is probably significantly higher than reported in the literature. Considering the general population, one study reported the prevalence of saber-sheath trachea of 5% [8], while in our study it was significantly lower – 0.56%. When it comes to studies including only patients with COPD, Green et al. [9] found that 95% of patients with saber-sheath trachea also had COPD, while in Gupta's study [10] the prevalence of this anatomical variety of the trachea in patients with COPD was 35%. Previous research indicated that the tracheal index was negatively related to the duration of COPD, length of cigarette smoking, dyspnea scale scores, and spiroplethysmographic scores [11–13]. Therefore, it is possible that this tracheal variety occurs in the early stages of COPD, as well as that the laterolateral diameter of the trachea narrows more and more over time. It is possible that this disorder is a consequence of changes in the value of intrathoracic pressure and air trapped in the lungs, with hitherto unrelated concomitant action of associated factors, such as environmental exposure or genetics [1, 12]. Other mechanisms are likely to play a role in the development of this disorder, since not all patients with COPD have saber-sheath trachea, nor all patients with saber-sheath trachea have COPD. It has been hypothesized that this tracheal disorder may also occur due to degenerative changes and ossification of tracheal rings, which may be caused by chronic extensive cough [7].

Several research, including our own, found that this disorder is more common in men [1, 9] and those over the age of 50 [6], the latter supporting the thesis of acquisition of the disorder. However, other investigations have found no variations in prevalence depending on gender or age [12].

The diagnosis of saber-sheath trachea is usually made based on the results of radiological imaging methods (chest radiography or CT scan) or endoscopy with bronchoscopy. The tracheal index, which shows the ratio between the transverse and anteroposterior diameters of the trachea, can be assessed on chest X-ray and is usually measured 1 cm above the aor-

Table 1. Socio-demographic characteristics of the analyzed sample
Tabela 1. *Socio-demografske karakteristike analiziranog uzorka*

	No/*Br*	%
Prevalence/*Prevalencija*	82	0.56
Gender/*Pol*		
Male/*Muški*	78	95.1
Female/*Ženski*	4	4.9
Smoking status/*Pušački status*		
Smokers/*Pušači*	52	63.4
Ex-smokers/*Bivši pušači*	30	36.6
Non-smokers/*Nepušači*	0	0

tic arch [13]. In this tracheal anatomical variant, the tracheal index is smaller than 0.67 (2 : 3) [12]. Chest CT scans provide substantially more reliable information because the trachea's rotation does not impact the thoracic index measurement, which is not the case with chest X-ray. Despite the fact that saber-sheath trachea is usually easily detected on a chest X-ray, literature data indicate that this condition is frequently overlooked in everyday clinical practice [1]. The diagnosis is made most commonly as an accidental endoscopic finding during bronchoscopy performed for various reasons. During bronchoscopy, the degree of tracheal collapse during expiration can be also assessed [15].

When diagnosing this condition, it is important to rule out other disorders where the trachea can take on the appearance of the saber-sheath. These include mediastinal mass (malignant or benign tumor, metastasis), iatrogenic or post-inflammatory tracheal stenosis, ankylosing spondylitis, and granulomatous disorders such as amyloidosis, sarcoidosis, and granulomatosis with polyangiitis [16].

Shortness of breath, cough, chest pain, hemoptysis, and fever were among the most common symptoms experienced by the patients in our study prior to bronchoscopy. All of these symptoms, however, could be related to the underlying disease (bronchial cancer in the majority of cases, or less frequently, pulmonary inflammation), rather than saber-sheath trachea. It is considered that this anatomical tracheal deformity is usually not associated with any symptoms. Investigations over the last century have shown that it is necessary to reduce the tracheal lumen by 70% to significantly reduce airflow through it, and thus to cause clinically significant symptoms [17]. However, as a result of this malformation, endotracheal intubation can be challenging at times. In such cases, it is recommended to use an endotracheal tube with a smaller diameter than expected [6], or flexible bronchoscopy with the tip of the endotracheal tube introduced to the narrowest part of the trachea, because further advancement of the endotracheal tube may cause tracheal mucosa injuries [12]. Although saber-sheath trachea is normally smaller in diameter than the normal trachea, saber-sheath trachea lumen enlargement has been reported in the literature. Because the circle-shaped tube balloon cannot approach the sagittal elongated tracheal walls in such cases, air leakage during endotracheal insertion is possible [7]. In such circumstances, a laryngeal mask or other supraglottic agents provide an alternate option for providing appropriate mechanical ventilation [5, 18].

There is no recommended therapeutic protocol for the treatment of patients with saber-sheath trachea [19]. External tracheal fixation with artificial materials during thoracotomy, as well as suturing an absorbable mesh on the anterior tracheal wall during anterothoracic tracheoplasty, have both been described in the literature, and led to the reduction of airflow obstruction [20]. Temporary stents can also be used to expand the tracheal lumen, though this comes with the risk of injury to the airway mucosa due to stent migration and/or fracture. However, such procedures have yet to be standardized, and the literature only contains descriptions of specific cases [21]. The advantage of this study is that the cohort of patients with saber-sheath trachea from the territory of the Republic of Serbia was analyzed for the first time and that the obtained data increase the total amount of knowledge about this rare and understudied disorder. The limitation of the study is that for a large number of patients data were not available during the follow-up period (survival time, above all), since bronchoscopy was performed on an outpatient basis in the IPDV and patients were further treated in regional health facilities. In addition, in the last ten years a limited number of literature sources describing any aspect of the diagnosis and/or treatment of this malformation is available, which significantly limits the interpretation of the obtained results.

Conclusion

In the general population, saber-sheath trachea is a rather uncommon acquired morphological tracheal abnormality, but it is a relatively common radiological and endoscopic finding in chronic obstructive pulmonary disease patients. As a result, in those who have not previously been diagnosed with chronic obstructive pulmonary disease, it may be considered as a major radiological sign suggesting the necessity for additional diagnostic procedures to diagnose chronic obstructive pulmonary disease. Although narrowing of the tracheal lumen often does not result in significant airflow obstruction or clinical manifestations, physicians of various specialties (primarily pulmonologists, radiologists, and anesthesiologists) should consider this anatomical malformation of the trachea during the preoperative assessment of patients who are planned for general anesthesia or mechanical ventilation for any indication, since this disorder may cause significant difficulties during endotracheal intubation.

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CASE REPORTS
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Case report
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MINIPUBERTY IN EXTREMELY PREMATURE FEMALE INFANTS – A REPORT OF TWO CASES

MINIPUBERTET KOD EKSTREMNO PREVREMENO ROĐENIH ŽENSKIH ODOJČADI – PRIKAZ DVA SLUČAJA

Đurđina STANKOVIĆ[1] and Ivana VORGUČIN[1, 2]

Summary

Introduction. Minipuberty occurs during the first months of life after the activation of the hypothalamic-pituitary-gonadal axis which causes an increase in gonadotropic and sex hormones. Usually, it does not induce clinically evident physical changes. Studies have shown that minipuberty in extremely premature infants is more pronounced and lasts longer, leading to higher levels of sex hormones induce climically evident in physical changes. **Case Report**. We present two extremely premature female infants, born at 25 weeks of gestation, with clinically evident physical changes during minipuberty. The first infant presented with vaginal bleeding at the age of 4 months, corrected age of 2 weeks. The vaginal bleeding lasted for two days and stopped spontaneously. The infant also had small glandular breast buds of 1 cm bilaterally, swelling in the pubic region, swollen vulva and clitoris. The second infant presented with swelling in the genital region, suprapubic area and the anterior part of thighs, at the age of 4 months, corrected age of 2 weeks. Both infants had ovarian cysts. In both cases, laboratory tests were consistent with minipuberty. The described changes disappeared gradually and spontaneously. **Conclusion**. In order to avoid unnecessary testing, clinicians should be aware of possible physical changes during minipuberty in extremely premature infants. Clinical monitoring of these infants is recommended until regression of newly developed physical characteristics.

Key words: Hypothalamo-Hypophyseal System; Puberty; Infant, Extremely Premature; Ovarian Cysts; Uterine Hemorrhage; Genitalia, Female; Diagnosis; Signs and Symptoms

Sažetak

Uvod. Minipubertet se odvija u prvim mesecima života nakon aktivacije hipotalamo-hipofizno-gonadalne osovine koja uzrokuje porast gonadotropina i polnih hormona. Uobičajeno, plima hormona minipuberteta ne dovodi do klinički evidentnih fizičkih promena. Studije su pokazale da je minipubertet kod ekstremno prevremeno rođene odojčadi izraženiji i traje duže, što dovodi do viših nivoa polnih hormona koji uzrokuju klinički evidentne fizičke promene. **Prikaz slučaja.** Prikazujemo dva odojčeta ženskog pola, koji su ekstremno prevremeno rođeni u 25. nedelji gestacije, kod kojih su evidentirane fizičke promene tokom minipuberteta. Prvo odojče je razvilo vaginalno krvarenje u uzrastu od četiri meseca, korigovani uzrast dve nedelje. Vaginalno krvarenje je trajalo dva dana i sponatno je prestalo. Odojče je imalo i pupoljke grudi promera 1 cm obostrano, otok u predelu pubične regije, vulvarni otok i otok klitorisa. Kod drugog odojčeta, u uzrastu od četiri meseca, korigovani uzrast dve nedelje, došlo je do pojave otoka genitalne regije, suprapubične regije i prednjeg dela butina. Oba odojčeta su imala ovarijalne ciste. Laboratorijski nalazi su ukazivali na minipubertet. Opisane promene su se spontano postepeno povukle. **Zaključak.** Kliničari bi trebalo da budu svesni mogućih fizičkih promena tokom minipuberteta kod ekstremeno prevremeno rođenih novorođenčadi, što bi sprečilo nepotrebna ispitivanja. Savetovano je kliničko praćenje ove dece do povlačenja novonastalih fizičkih karakteristika.

Ključne reči: hipotalamo-hipofizealna osovina; pubertet; ekstremno prevremeno rođeno dete; ovarijalna cista; krvarenje iz materice; ženski genitalni organi; dijagnoza; znaci i simptomi

Introduction

Activation of the hypothalamic-pituitary-gonadal (HPG) axis, which causes gender-specific elevation in the gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and sex steroid hormones (testosterone in males and estradiol in females), occurs three times during lifetime. These three events can be described as "endocrine puberties". The first endocrine puberty happens during intrauterine life. The second endocrine puberty, that occurs during the first months of life, is called minipuberty. The third puberty occurs in adolescence [1].

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Abbreviations
HPG – hypothalamic-pituitary-gonadal
LH – luteinizing hormone
FSH – follicle-stimulating hormone

Ordinarily, minipuberty does not induce clinically evident physical changes, but it is evident in serum hormone changes [2]. Data of minipuberty in extremely premature infants are scarce. Here, we report two cases of minipuberty in extremely premature female infants with elevated hormone levels that resulted in clinically evident physical manifestations of hormonal changes.

Case 1

At the age of 4 months, corrected age of 2 weeks, a female infant presented with vaginal bleeding that lasted for two days. She was born at 25 1/7 weeks of gestation and had a prolonged hospital stay due to bronchopulmonary dysplasia, patent ductus arteriosus and gastroesophageal reflux. The infant presented with small glandular breast buds of 1 cm bilaterally, swelling in the pubic region, swollen vulva and clitoris. The laboratory tests showed normal complete blood count, coagulation mechanism and albumin level. Elevated levels of LH, FSH and estradiol were detected and they were in the pubertal range **(Table 1).** Further laboratory tests showed normal levels of 17-OH progesterone, alpha-fetoprotein and beta-human chorionic gonadotropin, as well as normal female karyotype. Pelvic ultrasonography revealed a multiseptated 16 mm cyst on the right ovary. Vaginal bleeding spontaneously resolved. The infant did not experience another episode of uterine bleeding. Laboratory surveillance revealed decreasing levels of estradiol and gonadotropins that returned to the prepubertal range, and by that time, breast buds and genital swelling had regressed entirely. The ovarian cyst resolved spontaneously.

Case 2

At the age of 4 months, corrected age of 2 weeks, a female infant presented with swelling in the genital region, suprapubic area and the anterior part of thighs. She was born at 25 4/7 weeks of gestation and had a prolonged hospital stay due to bronchopulmonary dysplasia, intracerebral hemorrhage, patent foramen ovale and retinopathy of prematurity. Elevated levels of gonadotropins and estradiol were detected, also 17-OH progesterone was elevated - 7.34 ng/mL (reference levels < 3.1) **(Table 1)**. Laboratory test results were in reference range for blood glucose, sodium, potassium, albumin, testosterone, and she had a normal female karyotype. Pelvic ultrasonography revealed cysts on both ovaries, up to 37 mm. The swelling gradually subsided and the levels of estradiol, 17-OH progesterone and gonadotropins decreased. The cysts remained on both ovaries. This patient needs further monitoring.

Discussion

Minipuberty is the second transient activation of the HPG axis. It develops after birth as placental sex steroid hormones stop suppressing the HPG axis. A surge in gonadotropin secretion leads to sex-specific hormone changes. The FSH levels are higher in girls than in boys and remain high until the age of 3 - 4 years, compared to boys in whom they decline by the age of 6 months. According to FSH levels, estradiol levels are elevated in girls, with average levels that are same as those seen in Tanner stage 4 girls. Estradiol levels fluctuate with rises and falls of estradiol concentration [3, 4]. Serial urine estradiol measurements show vast inter-individual differences [4]. The LH levels are higher in boys than in girls, and they decline by the age of 4 – 6 months. It stimulates testosterone secretion and it peaks in the second and third months of life [5].

In general, the transient rise of sex hormones is not followed by clinically visible physical changes [2]. Its effects are visible through ultrasound evaluation of testicular size in boys and monitoring the speed of penile growth [6, 7]. In girls, a positive correlation of the mammary gland diameter and estradiol was described at the age of 3 months [8].

The onset of minipuberty in extremely premature infants is similar to that in full-term infants. Still, HPG axis activation in the extremely premature infants is increased and prolonged, resulting in higher sex steroid hormone concentrations in preterm babies [2]. Reference data based on serum measured in 82 preterm babies for LH and FSH showed higher levels of gonadotropins in preterm infants than in full-term infants [9]. This was confirmed in a study that showed that estradiol levels were significantly higher in preterm than in full-term girls. This study also proved the effect of estradiol level on target tissue, showing that mammary glandular diameter and uterine length were significantly larger in preterm than in full-term girls [4].

Vogiatzi et al. described two cases of menstrual bleeding as a manifestation of minipuberty, debat-

Table 1. Hormone levels
Tabela 1. *Nivoi hormona*

	Estradiol pg/mL/*Estradiol pg/mL*		LH IU/mL		FSH IU/mL	
	2 weeks *2 nedelje*	7 months *7 meseci*	2 weeks *2 nedelje*	7 months *7 meseci*	2 weeks *2 nedelje*	7 months *7 meseci*
Case 1 *Slučaj 1*	246	54	13	1.27	6.61	5.38
Case 2 *Slučaj 2*	276	7.43	21.13	3.72	7.2	6.35

Legend/*Legenda*: LH – Luteinizing hormone/*Luteinizirajući hormon* FSH –Follicle-stimulating hormone/*Folikulostimulišući hormon*

ing that estrogen level was high enough to cause uterine maturation and shedding. Their two cases were similar to our first case and showed two preterm babies born at 25 weeks of gestation with one episode of vaginal bleeding accompanied by breast buds. Vaginal bleeding spontaneously stopped and did not recur. Laboratory findings were consistent with minipuberty. The LH, FSH, and estradiol levels were higher than those seen in full-term girls and spontaneously returned to normal levels [10]. Another case report showed a preterm girl with vaginal bleeding that was primarily understood as precocious puberty and was treated as such. Later, the treatment was withheld; there were no signs of puberty, the former diagnosis was rejected and the vaginal bleeding was attributed to minipuberty [11].

The swelling in the genital area, described in both our cases, occurs due to the increase of estrogen level. Similar manifestations are described in preterm ovarian hyperstimulation syndrome [12]. It has also been established that high vascular endothelial factor levels contribute to genital swelling. Elevated vascular endothelial factor is predominantly the consequence of LH surge [13]. Clitoromegaly, which may be seen as part of swelling in the genital region, could lead to further unnecessary examinations.

Ovarian cysts form due to HPG axis activation and should gradually reduce in size and disappear after HPG axis recovers. Therefore, it is essential to monitor ovarian cysts due to the possibility of surgical complications, i. e. ovarian torsion, rupture or hemorrhage, which is most common if the cysts are large (> 4 – 5 cm) [14].

Physical manifestations of minipuberty were also noted in extremely preterm male infants. A clinical study revealed higher testosterone levels in preterm boys than in full-terms boys, resulting in significantly faster testicular and penile growth [15]. However, these physical manifestations have not been reported as the reason for inpatient visits, probably since these changes are less noticeable than those in girls.

Conclusion

Minipuberty in extremely preterm female infants may have physical manifestations ranging from mammary glandular tissue swelling, swelling in the genital area, to uterine bleeding. Since the neonatal care is constantly improving, the number of extremely preterm infants who survive increases. Clinicians should be aware of possible physical manifestations of minipuberty in premature infants in order to avoid further examinations and unnecessary treatment of this physiological condition. Instead, monitoring of these changes only through inpatient visits is advised to prove their transitory nature.

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1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

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

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

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

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

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

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

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

Materijal i metode

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

Rezultati

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

Diskusija

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

Zaključak

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

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

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

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

Manuscript submission should be made on the web address:

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

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

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

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

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

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

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

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

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

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

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

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

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

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

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

3. The text of the paper.

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

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

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

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

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

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

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

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *A computer file*

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

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

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

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

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

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

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

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

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

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

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

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.