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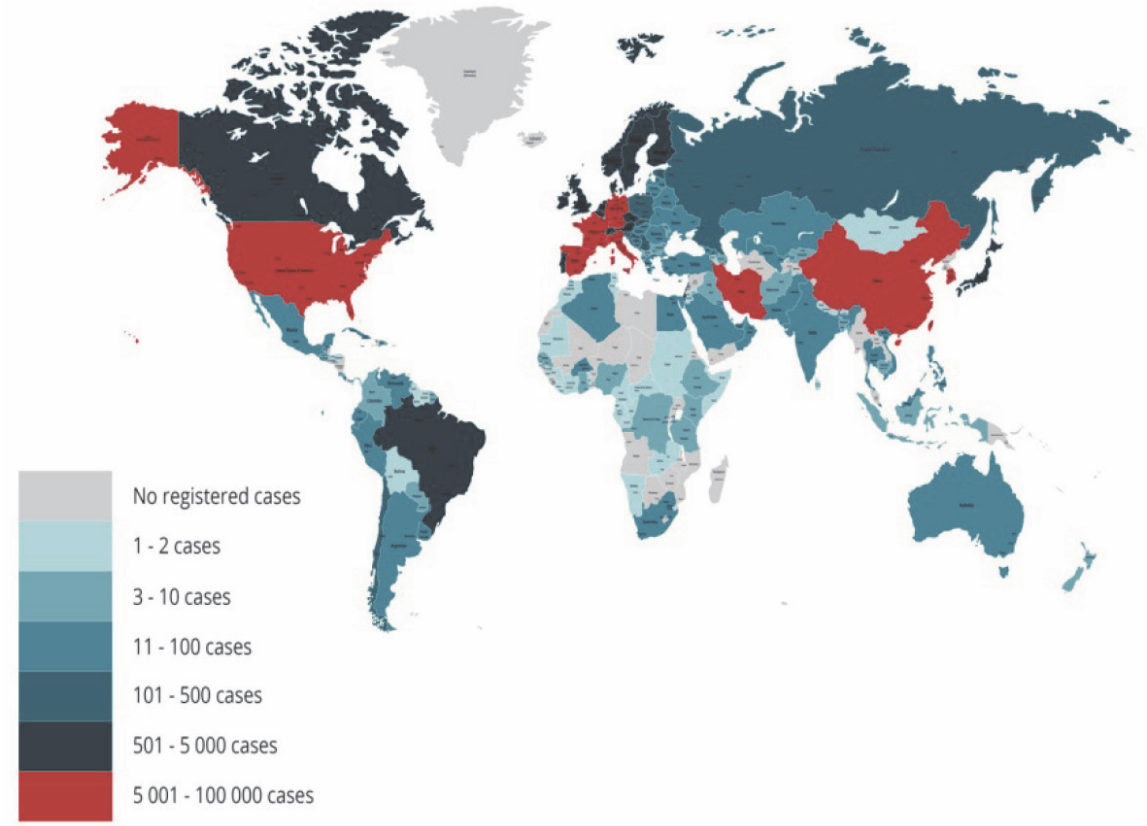

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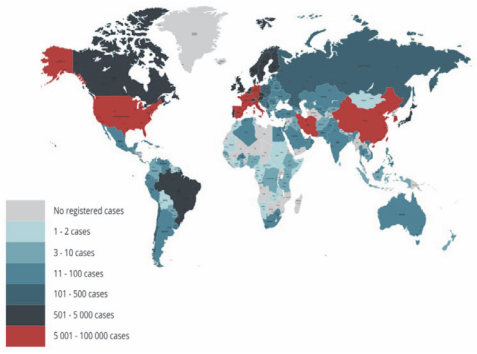


COVID-19 around the world on 23 March 2020.
Concerns about the spread of coronavirus continue to grow around the world which is why all countries, including Serbia, are taking rigorous measures to prevent transmission of the virus and stop its fatal consequences. The Editorial Board of the *Vojnosnitetski Pregled* invites its readers, authors, reviewers and other contributors to take all measures of personal and collective protection to contribute to joint fight against COVID-19.

COVID-19 širom sveta 23. marta 2020.
I dalje raste velika zabrinutost širom sveta zbog širenja koronavirusa, zbog čega sve zemlje, uključujući i Srbiju, preduzimaju rigorozne mere za sprečavanje prenosa virusa i zaustavljanje njegovih pogubnih posledica. Uredništvo "Vojnosanitetskog pregleda" poziva svoje čitaoce, autore, recenzente i ostale saradnike da preduzimaju sve mere lične i kolektivne zaštite kako bi doprineli zajedničkoj borbi protiv COVID-19.

ORIGINAL ARTICLES

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# Salivary and plasma inflammatory mediators and secretory status in preterm delivery women with periodontitis – a cross sectional study

## Salivarni i inflamatorni medijatori plazme i sekretorni status prevremeno porođenih žena sa periodontitisom – studija preseka

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**Abstract**

**Bacground/Aim.** Preterm birth is defined as a delivery prior to the completed 37th week of gestation. Literature data suggested that periodontal processes may influence to the feto-placental unit and induce preterm delivery. The degree of the periodontal disease is influenced by secretor status. Pro-inflammatory cytokines are involved in periodontitis as well as in delivery. The combined influence of these factors on the risk of preterm birth has not been explored. The aim of our study was to investigate the associations between periodontal diseases, secretor status, and interleukin-1-β (IL1-ß) and prostaglandine E2 (PGE2) levels in women delivered preterm. **Methods.** The study included 56 preterm delivery women and 56 women delivered at term as a control group, aged between 17 and 41 years. Periodontal examination, blood and saliva sampling were performed within 48 hours following delivery. Secretor phenotype was determined by hemagglutination inhibition method. The concentrations of IL1-ß and PGE2 were measured by high sensitivity Enzyme-linked Immunosorbent Assay (ELISA). **Results.** In the pre-term birth group there were 66.1% of women with periodontitis, while in the control one there were 12.5% ($p < 0.01$). Concentrations of IL1-ß and PGE2 in plasma were significantly higher in the non-secretor group of women who gave birth pre-term and had periodontitis comparing to other groups. There was a significant correlation between salivary and plasma levels of PGE2 and IL1-ß in the preterm birth group ($R = 0.416$, $p = 0.017$ and $R = -0.592$, $p < 0.001$, respectively). There were no such correlations in women who delivered at term. **Conclusion.** Our results support the hypothesis that non-secretor phenotype and periodontitis are at least in part responsible for pathogenesis of preterm birth. This probability of negative impact of non-secretor status cannot be ignored. These findings support the need for additional research into the biology of human parturition.

**Key words:**
**premature birth; periodontitis; interleukin-1beta; dinoprostone; saliva; plasma.**

**Apstrakt**

**Uvod/Cilj.** Prevremeni porođaj se definiše kao porođaj pre navršene 37 nedelje gestacije. Podaci iz literature govore u prilog tome da periodontalni procesi mogu uticati na feto-placentalnu jedinicu i indukovati preterminski porođaj. Sekretorni status može uticati na stepen periodontalne bolesti. Proinflamatorni citokini imaju uticaj na periodontitis kao i na porođaj. Kombinovani uticaj ovih faktora rizika za prevremeni porođaj nije dovoljno istražen. Cilj ove studije je bio da istraži povezanost između periodontalne bolesti, sekretornog statusa, nivoa interleukina 1-β (IL1-ß) i prostaglandina E2 (PGE2) kod žena koje su imale prevremeni porođaj. **Metode.** Studijom je bilo obuhvaćeno 56 žena, koje su imale prevremen porođaj i 56 žena u kontrolnoj grupi koje su se porodile u terminu, starosti između 17 i 41 godine. Periodontalni pregled, uzorkovanje krvi i salive je izvršeno u prvih 48 sati po porođaju. Sekretorni status je

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određen metodom inhibicije hemaglutinacije. Koncentracije IL1-ß i PGE2 su merene visoko senzivinim *Enzyme-linked Immunosorbent Assay* (ELISA) testom. **Rezultati.** U grupi prevremenih porođaja bilo je 66,1% žena sa periodontitisom, a u kontrolnoj grupi 12,5% ($p < 0.01$). Prevremeno porođene žena, nesekretori sa periodontitisom imale su u plazmi značajno više vrednosti IL 1-ß i PGE 2 u odnosu na ostale grupe ($p < 0,01$). U grupi prevremeno porođenih žena postojala je značajna korelacija između salivarnih i plazmatskih koncentracija PGE2 i IL1-ß ($R = 0.416$, $p = 0.017$ i $R = -0,592$, $p < 0,001$, redom). Ove korelacije nisu postojale kod žena koje su imale terminski porođaj. **Zaključak.** Naši rezultati podržavaju hipotezu da su sekretorni status i periodontitis, bar delimično, odgovorni za patogenezu preterminskog porođaja. Verovatnoća negativnog uticaja nesekretornog statusa se ne sme ignorisati. Ovi zaključci ukazuju na potrebu za dodatnim istraživanjima porođaja.

**Ključne reči:**
**porođaj, prevremeni; periodontitis; interleukin-1beta; dinoproston; pljuvačka; plazma.**

## Introduction

Preterm birth (PTB) is defined as a delivery prior to the completed 37th week of gestation [1]. Two-thirds of PTBs are spontaneous. The global prevalence rate of preterm birth is ranging from 5% to 13.3% [2]. PTB is the leading cause of perinatal morbidity and mortality. PTB is associated with multiple pathological processes such as medical conditions of the mother or fetus, multiple pregnancies, genetic influences, male fetus, environmental exposure, infertility treatments, behavioral and socioeconomic factors, and iatrogenic prematurity [3]. Intra-amniotic infection has been causally linked to PTB. Intra-amniotic infection induces the production of pro-inflammatory cytokines involved in term delivery, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8, IL-6, IL-1β, and prostaglandine E2 (PGE2). In some cases of PTB microorganisms cannot be detected by cultivation and other microbiology techniques despite high levels of cytokines in amniotic fluid. Literature data suggest that in cases of PTB with sterile intra-amniotic inflammation, cytokines are produced in distant part of the body due to infection and inflammation, cross the placental barrier, and when they reach appropriate quantities stimulate labor [4]. This statement is in accordance with Miller's focal infection theory published in 1891 [5].

Microbiological, immunological and animal model studies suggested that periodontal processes may influence to the feto-placental unit and induce preterm delivery (PTD) [6].

### *Periodontal diseases*

Periodontal diseases are infectious diseases that result in the inflammation of the specialized tissues that both surround and support the teeth. Diseases are multifactorial and they are initiated by bacterial colonization of the dentogingival environment, sustained by the presence of dental biofilm and host immune defense [7]. According to the Armitage [8], there are two major categories of periodontal diseases: gingivitis – non-destructive and reversible gingival inflammation, and periodontitis – destructive inflammation of teeth supporting tissues [8].

### *Gingivitis*

Gingivitis is a reversible and nondestructive gingival inflammation related to a non-specific bacterial challenge. Dental plaque is the principal etiologic factor in gingivitis. It is characterized by inflammation, edema, erythema and bleeding of the gingival marginal portion. Studies reported prevalence of gingivitis in around 80% children and adolescents. Gingivitis is, therefore, the form of periodontal disease most commonly found. Among pregnant women incidence of gingivitis is even greater. Based on clinical observation, the frequency of gingivitis in pregnant women ranges from 35% to 100%. This variation may be a reflection of both the population studied and the clinical parameters used [9]. Hormonal changes during pregnancy influence periodontal tissues through different mechanisms and alter maternal immune response [7]. Increased circulating levels of progesterone in pregnancy can cause dilatation of gingival capillaries, increased capillary permeability, and gingival exudate. The onset of increased gingival inflammation observed in the second month of gestation, peaks in the eighth month, and coincides with an increase in the circulating levels of hormones. Prostaglandin concentration within the gingiva and gingival fluid also increases dramatically with the occurrence of gingival inflammation. When gingivitis is persistent, it can further leading to periodontitis [7, 9].

### *Periodontitis*

Periodontitis (PD) is a destructive inflammatory disease of the supporting tissues of the teeth initiated by polymicrobial biofilm. PD is a result of a chronic immune and inflammatory response following infection with a complex microbiome [10]. Typical for the disease is formation of periodontal pockets and a chronic destructive inflammation which impacts the whole organism. Synergistic relationship between periodontal pathogens and their endotoxins induces chronic oral infection; enhance humoral immune response and production of inflammatory markers [10]. Pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and prostaglandins (prostaglandine 1 – PGE1 and PGE2) are produced in response to infection [11]. Vascular permeability is also increased – allowing the diffusion of cytokines into the blood flow which may have systemic effects on the host. During the second and third trimester of pregnancy, the gingival/periodontal inflammation often becomes more severe [9]. Published data show that cytokines produced in periodontal tissues are enabling to promote inflammation in feto-placental unit [12]. Analysis of amniotic fluid obtained at the time of preterm

birth without chorio-amnionitis shows elevated levels of inflammatory cytokines [13]. Maternal periodontal infections provide a chronic reservoir of inflammatory mediators and cytokines (TNF-α, IL-1, IL-6, and PGE2) that could adversely affect pregnancy outcome [14].

According to literature data, degree of periodontal inflammation correlates with cytokines levels [15]. Furthermore, it has been shown that the degree of the periodontal disease is influenced by secretor status [16, 17].

*Secretor status*

The secretor status is regulated by the fucosyltransferase2 (FUT2) gene. Individuals who express blood group antigens on cells surface, and in the saliva and other body fluids are termed secretors. Blood group antigens in non-secretors are present on cells surface but not in body fluids. Blood group antigens are oligosaccharides. Blood group substances in secretors body fluids (A, B, H, Lewis b, Lewis y) are glycoproteins. In saliva the blood reactive antigens are found primarily on mucins. Blood type antigens and other oligosaccharides act as receptors for bacterial adhesion and regulate the oral bacteria – oral microbiome. Binding of pathogens to these receptors activates a distinct signaling pathway that shapes the immune response. Therefore, secretor/non-secretor phenotypes are associated with some metabolic and infectious diseases. Recent evidence suggests that non-secretors are at increased risk of carrying some pathogenic microorganisms in their body [18]. In accordance with these data, non-secretors are at increased risk of inflammatory diseases, pre-cancerous and cancerous lesions along with periodontitis [19].

Overall, the study findings are inconsistent and the combined influence of these factors on the risk of PTB has not been explored.

Given the established link between periodontitis and secretor status as well as the association between inflammatory mediators, periodontitis, and preterm birth we hypothesized that in some instances PTB risk could be associated with the co-occurrence of increased cytokine levels and secretor status in women with periodontitis. More specifically, elevated levels of IL-1 ß and PGE2 occur in combination with non-secretor status.

Therefore, the purpose of our study was to investigate the associations between periodontal diseases, secretor status, and IL-1 ß and PGE2 levels and risk of PTB.

## Methods

This study included 112 women (56 preterm delivery women and 56 women that delivered at term as a control) aged between 17 and 41 years. Women were enrolled from August 2012 to March 2014. All women had their delivery in the Clinic for Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade.

The study was conducted after obtaining approval from the Ethical Committee of the Faculty of Medicine, University of Belgrade and the Clinical Center of Serbia. Written informed consent from all the participants were obtained in accordance with the Helsinki Declaration, revised in 2000.

Blood sampling, saliva sampling, and periodontal examination were performed within 48 hours following delivery. Random sample of control mothers was selected from the birth register simultaneously as the cases. Only mothers with a singleton gestation were included in the study. Data for mothers and newborns were collected from medical records.

Gestational age was estimated by the last menstrual period and ultrasound examination.

Delivery prior to complete 37 weeks of gestation was considered as PTB.

Exclusion criteria included the following: multiple pregnancies, assisted reproductive technique, fetal congenital disease, diabetes, preeclampsia, intra-amniotic infection during pregnancy and clinical signs of infection (body temperature over 38°C).

Blood and saliva sampling were performed just before periodontal examinations.

*Blood sampling*

A 4-mL of venous blood samples from antecubital fossa were collected in EDTA Vacutainer® tube (Becton Dickinson, UK). Blood samples were centrifuged at 3000 rpm for 15 min and the plasma was aliquoted. The plasma samples were frozen at -70°C until further analysis. The remaining content of tube was used for blood group determination.

*Salivary sampling*

Unstimulated whole saliva was collected in 10 mL glass tubes. No antiseptic mouth rinse was used prior to collection. Collected saliva samples were centrifuged within 1 hour of collection at 3,500 rpm for 20 min at 4°C to obtain a cleared supernatant. Two thirds of supernatant were aliquoted, and stored at -70°C for further ELISA testing. The remaining amount of saliva samples was incubated in boiling water bath during 10 min, centrifuged (at 3,500 rpm for 10 min), and supernatant was separated and stored at -20°C for further secretor status testing.

*Periodontal examination*

The full-mouth periodontal measurements were performed in six sites per tooth by one same experienced examiner. Periodontal measurements include following periodontal clinical parameters: Probing Depth (PD), Clinical Attachment Level (CAL), Bleeding on Probing (BOP), Visible Plaque Accumulation (PI). According to the classification of periodontal diseases, periodontal status was defined as: healthy periodontium, gingivitis, and periodontitis [20].

*Determination of blood group and secretor status*

ABO and Rh blood groups were determined in fresh blood ethylenediaminetetracetic acid (EDTA)-samples by standard hemagglutination methods [21]. Red blood cells were suspended in a 2–3% (v/v) saline solution; 50 uL of this suspension was mixed in tubes with 50 uL of specific antisera, then incubated

for 10 min at room temperature, and the results were read by naked eye after centrifugation at 2,000 rpm for 1 min.

Secretor and non-secretor phenotypes were evaluated by boiled saliva samples using the Hemagglutination Inhibition Assay test [21]. For each patient 3 tubes were prepared; 50 uL of boiled saliva samples was mixed with 50 uL diluted commercial antisera (anti-A, anti-B, and anti-H); tubes were incubated for 10 minutes at room temperature, after that, 50 μL of corresponding erythrocytes (A, B, O) were added to the test mixture and all the test tubes were agitated and left at room temperature for another 10 minutes. Results were read by naked eye after centrifugation at 2,000 rpm for 1 min. Negative reaction for agglutination is interpreted as positive for secretor status. Positive reaction for agglutination means a negative test which has proven that the person is non-secretor.

The commercial antisera used for determination of blood groups and secretor status were the following: 1) monoclonal anti-A, anti-B, anti-AB, and anti-D (Lorne, UK) and 2) Anti-H lectin (CE Immunodiagnostics, Germany). All assays included appropriate known controls. Each aliquot of saliva and blood samples was used only once in an assay, and then discarded.

*Determination of IL-1ß and PGE2*

The concentrations of IL-1ß and PGE2 were measured by commercially available high sensitivity enzyme-linked immunosorbent assay (ELISA) eBioscience kits, Vienna, Austria, and EIA kit Enzo Life Science, Germany. The microplates were read according to the manufacturer's recommended time frame using an automated plate reader: Sunrise, Tecan Dorset, UK.

*Statistical analysis*

Numerical data were presented as mean ± standard deviation (SD) for normaly distributed data or median with interquartile range for non-normaly distributed data, while categorical variables were presented as frequencies or percentages. Distribution of periodontal status among PTB and FTB groups was assessed using Fisher's exact test. Inter-group comparisons of age, and biochemical parameters was performed using Mann-Whitney test. Depending on the data types, differences between independent samples were assessed using $\chi^2$-test, Fisher test, Student's *t*-test, Kruskal-Wallis, Mann-Whitney *U* test, while differences between the related groups were examined by Wilcoxon test. The correlations between clinical parameters and laboratory parameters as well as between saliva and plasma levels of biomarkers amongst PTB and FTB were tested with the Spearman's rank correlation test. The statistical analysis was performed using commercial software SPSS 20.0, Inc., Chicago, IL; $p$ values < 0.05 were considered to be significant.

## Results

The demographic and clinical characteristics of patients who gave preterm birth and full term birth are displayed in Table 1.

**Table 1**

**Demographic and clinical characteristics of patients with preterm and term delivery**

| Parameter | Preterm birth n = 56 | Term birth n = 56 | *p* |
|---|---|---|---|
| Maternal age (years), mean ± SD | 30.7 ± 5.5 | 27.0 ± 3.9 | ns |
| Maternal (ABO) blood type, n (%): | | | |
| O | 16 (28.6) | 14 (25.0) | ns |
| A | 24 (42.8) | 28 (50.0) | ns |
| B | 14 (25.0) | 12 (21.4) | ns |
| AB | 2 (3.6) | 2 (3.6) | ns |
| Maternal RhD factor, n (%): | | | |
| positive | 46 (82.1) | 50 (89.3) | ns |
| negative | 10(17.9) | 6 (10.7) | ns |
| Maternal secretory status, n (%): | | | |
| secretor | 44 (78.6) | 44 (78.6) | ns |
| non-secretor | 12 (21.4) | 12 (21.4) | ns |
| Gingival status, n (%): | | | |
| healthy periodontium | 4 (7.1) | 31 (55.3) | < 0.001 |
| gingivitis | 15 (26.8) | 18 (32.1) | ns |
| periodontitis | 37 (66.1) | 7 (12.5) | < 0.001 |
| Infant gender, n (%): | | | |
| female | 24 (42.8) | 34 (60.7) | < 0.05 |
| male | 32 (57.2) | 22 (39.3) | < 0.01 |
| Infant birth length (cm) mean ± SD | 44.96 ± 3.1 | 50.68 ± 1.2 | < 0.05 |
| Infant birth weight (g) mean ± SD | 1862 ± 381 | 3300 ± 208 | < 0.01 |
| Apgar score, mean ± SD | 8.02 ± 1.5 | 9.02 ± 0.1 | < 0.01 |
| Apgar score 9–10/1 min | 32 (57.2) | 56 (100) | < 0.01 |
| Apgar score < 7/1 min | 5 (8.9) | 0 (0) | < 0.01 |
| Parity | | | |
| primiparous | 35 (62.5) | 34 (60.7) | ns |
| multiparous | 21 (37.5) | 22 (39.3) | ns |

**SD – standard derviation; ns – non significant.**

**Table 2**

**Number of secretors and non-secretors women according to their periodontal status**

| Secretor status | Periodontal status, n (%) | | |
|---|---|---|---|
| | healthy periodontium | gingivitis | periodontitis |
| Non-secretor | 4 (11.4) | 6 (18.2) | 14 (31.8)* |
| Secretor | 31 (88.6) | 27 (81.8) | 30 (68.2)* |

***Chi-square test, $\chi^2 = 5.00$; $p < 0.05$.**

**Table 3**

**Number of subjects in the preterm birth (PTB) and full term birth (FTB) groups according to the periodontal and secretory status**

| Periodontal status | PTB secretors n (%) | PTB non-secretors n (%) | FTB secretors n (%) | FTB non-secretors n (%) |
|---|---|---|---|---|
| Periodontitis | 27 (61.4) | 10 (83,3)* | 3 (6.8) | 4 (33.3) |
| Gingivitis | 14 (29.5) | 2 (16.7) | 14 (31.8) | 4 (33.3) |
| Healthy periodontium | 4 (9.1) | 0 (0.0) | 27 (61.4)* | 4 (33.3) |

***Chi-square test, $\chi^2 = 43.6$; $p < 0.001$.**

**Table 4**

**Inflammation markers and basic hematological parameters in the preterm and term birth subgroups**

| Parameter | Preterm birth Median (25th–75th percentile) | Full term birth Median (25th–75th percentile) | *p* |
|---|---|---|---|
| IL-1ß, sal (pg/mL) | 10.837 (9.882–11.570) | 11.778 (5.690–12.094) | ns |
| IL-1ß, pl (pg/mL) | 0.0125 (0.0115–0.0141) | 0.0099 (0.0075–0.0133) | < 0.01 |
| PGE2, sal (pg/mL) | 279 (62–567) | 327 (215–423) | ns |
| PGE2, pl (pg/mL) | 967 (107–1267) | 461(36.1–1600) | < 0.01 |
| hsCRP, pl (mg/L) | 20.4 (7.43–36.7) | 19.0 (8.4–47.6) | ns |
| WBCx$10^9$/L | 15.2 (13.4–16.8) | 15.6 (12.7–18.2) | ns |
| Hb (g/L) | 112 (95.6–118) | 111 (101–122) | ns |
| Plt x$10^9$/L | 204 (202–265) | 218 (201–293) | ns |

**sal – saliva; pl – plasma; WBC – white blood cells; Hb – hemoglobin; Plt** – p**latelets;**
**IL-1β – interleukin-1β; PFE2 – prostaglandine E2; hsCRP – high sensitivity C-reactive protein; ns – non significant.**

Tested groups were homogenous comparing to age and parity. ABO and Rh representation as well as secretor status did not show the difference between the tested group and the control.

In the entire research group there were 44/112 (39.3%) women with periodontitis. It was noted that the prevalence of periodontitis was significantly higher ($p < 0.001$) in the PTB group in comparison to the control group of women delivered at term (66.1% and 12.5%, respectively). The prevalence of gingivitis in the PTB and the FTB group did not show a statistically significant difference ($p > 0.05$).

Analysis of the periodontal status and the secretory status showed that there were only 11.4% of non-secretors in the group with a healthy periodontium, while in the group with periodontitis there were 31.8% of non-secretors which was a statistically significant difference (Table 2).

At baseline, there was no difference between number of preterm and term birth subjects according to their secretory status (Table 1). However, there was significantly greater number (83.3%) of non-secretors preterm birth subjects with periodontitis compared to other periodontal disease categories. In the PTB non-secretors mothers there were no subjects with healthy periodontium. Also, full term birth secretors had the highest number of subjects (61.4%) with healthy periodontium ($p < 0.001$) (Table 3).

When we compared inflammation markers in plasma of women who gave preterm birth with women with term birth we found significantly higher IL-1ß and PGE2 values in plasma of the preterm birth group compared to the FTB group. Differences in other parameters in blood, as well as in saliva did not reach statistical significance (Table 4).

According to Spearman's correlation, in the PTB group there was a significant association between salivary levels of PGE2 and IL-1ß ($R = 0.416$, $p = 0.017$). The significant negative/inverse correlation was identified between plasma concentrations of IL-1ß and PGE2 ($R = -0.592$, $p < 0.001$) in the PTB group. These correlations were not found in women who delivered at term. In the FTB group there were significant correlation between salivary and plasma levels of PGE2

and maternal age (R = 0.428, $p = 0.0009$ and R = -0.289, $p = 0.03$, respectively) (Table 5).

**Table 5**
**Correlation of laboratory parameters in the PTB and FTB groups**

| Parameters | PTB (R, *p*) | FTB (R, *p*) |
|---|---|---|
| sal IL-1b – sal PGE2 | R = 0.416 | R = 0.336 |
| | $p = 0.017$ | $p = 0.09$ |
| pl IL-1b – pl PGE2 | R = -0.592 | R = 0.138 |
| | $p < 0.001$ | $p = 0.48$ |
| sal PGE2 – Age | R = 0.131 | R = 0.428 |
| | $p = 0.34$ | $p = 0.0009$ |
| pl PGE2 – Age | R = -0.265 | R = -0.289 |
| | $p = 0.06$ | $p = 0.03$ |

**R – Spearman's correlation coefficient; PTB – preterm birth; FTB – full term birth; sal IL-b – saliva interleukin-1b; pl IL-1b – plasma interleukin-1b; sal PGE2 – saliva prostaglandine E2; pl PGE2 – plasma prostaglandine E2**

At baseline, the mean IL-1ß and PGE2 values in the two subsets of patients (secretors and non-secretors) were not significantly different (Table 6). However, mean IL-1ß level in the non-secretor PTB subgroup with periodontitis was significantly higher than in other groups ($p < 0.001$) (Figure 1).

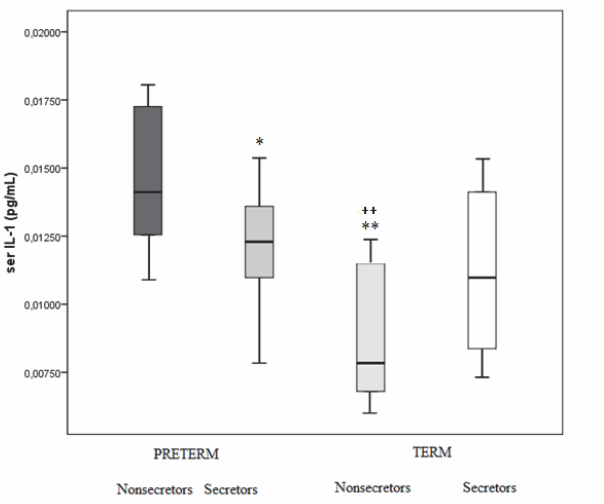


**Fig. 1 – Quantitative comparison of median blood interleukin-1b (IL-1b) concentration in the study group with periodontitis according to birth term and secretor status.**
***,** $p < 0.05$, 0.01, respectively vs. preterm non-secretor group; †† $p < 0.01$ vs. preterm secretor group according to Kruskal-Wallis and subsequent Mann-Whitney *U* test [the box represents the first and third quartiles (rectangular boxes); the line within the box is the median, and vertical bars show the 95% confidence interval].**
**Values obtained from the preterm birth (PTB) non-secretor group differed significantly from other groups at level $p < 0.01$.**

**Table 6**
**Inflammation markers and basic hematological parameters in subgroups according to the secretory status**

| Parameter | Non-secretor | Secretor | *p* |
|---|---|---|---|
| Age (years), median (range) | 35 (33–37) | 29.5 (23–31) | ns |
| IL-1ß, sal, pg/mL | 11.203 (10.834–11953) | 10.598 (5.690–11.953) | ns |
| IL-1ß, pl, pg/mL | 0.0149 (0.0126–0.0173) | 0.0126 (0.0089–0.0137) | 0.081 |
| PGE2.sal, pg/mL | 506 (279–733) | 327 (62–514) | ns |
| PGE2, pl, pg/mL | 454 (107–800) | 1117 (142–1433) | ns |
| hsCRP, pl, mg/L | 22.6 (8.4–46.7) | 19.0 (7.3–40.6) | ns |
| WBC $\times 10^9$/L | 12.5 (9.8–15.2) | 16.0 (13.4–19.1) | ns |
| Hb, g/L | 104.8 (95.6–114.0) | 111.5 (98.0–123.2) | ns |
| Plt $\times 10^9$/L | 183 (162–204) | 220 (204–279) | 0.040 |

**Note: Results are expressed as medians (25th – 75th percentile); sal – saliva; pl – plasma, WBC – white blood cells; Hb – hemoglobin; Plt – platelets; IL 1β – interleukin-1β; hsCRP – high sensitivity C-reactive protein; ns – non significant.**

## Discussion

Consistent with the hypothesis, we found an increased amount of IL1-ß and PGE2 in plasma samples obtained from non-secretor preterm delivery women. In addition, there was a strong correlation between IL1-ß and PGE2 levels in the PTB group compared with the control (FTB) subjects. These data support the hypothesis that non-secretor phenotype and periodontitis are at least in part responsible for pathogenesis of PTB and the probability of negative impact of non-secretory status cannot be ignored.

There are various risk factors for preterm birth, out of which a previous preterm birth is one of the most important (odds ratio 4.5–7.1). This risk factor likely reflects persistent genetic and epigenetic components. Nullparity and prior cesarean birth are important risk factors for spontaneous preterm birth, but with small associations (odds ratio 1.4–2.4). The gender of the unborn baby also seems to play a role in the process of being born prematurely with low risk odds ratio [22].

Numerous cohort/cross-sectional studies have been shown more and less strong association between PTB and periodontitis [23, 24].

There are a few proposed pathways by which periodontitis might affect pre-term birth [25]: 1) directly when periopathogens invade the fetal-placental unit subsequently stimulating local inflammation; 2) indirectly when inflammatory mediators circulate from periodontal burden and synergistically increase local inflammation; 3) by fetal inflammatory re-

sponse to mother's oral pathogens [26]; 4) by mother's enhanced antigraft response [27]; 5) genetically, by heritable factors [28].

Periodontitis was diagnosed in 66.1% of women in the PTB group. Similar to our findings Dörtbudak et al. [29] have reported that periodontitis was diagnosed in 20% of normal cases and in 83% of preterm birth ones. These differences could be attributed partially to inconsistent definitions of periodontal disease and different definitions of adverse pregnancy outcomes. The data of Jarjoura et al. [30] on 83 PTB cases and 120 controls support the notion that periodontitis is independently associated with PTB and low birth weight.

The representation of periodontitis in both groups of our patients was 39.3% which is in accordance with data presented by Lieff et al. [31] who found that in the population of pregnant women there were around 40% cases of periodontitis.

However, some cohort and case-control studies did not find a significant association between PTB and periodontitis. In the prospective study of 273 women performed by Soucy-Giguère et al. [32] there was no significant association between disease of the periodontium and preterm birth but the study could not exclude an association between periodontal disease and intra-amniotic inflammation.

Preterm birth, as well as periodontitis, is characterized by increased levels of inflammatory markers and among them IL-1ß and PGE2 [4, 15].

IL-1ß is a pro-inflammatory cytokine and is expressed by many cells including macrophage, natural killer cell (NK) cells, monocytes, and neutrophils. It belongs to the IL-1 family cluster that includes IL-1a, and IL1-RN genes. IL-1α and IL-1ß participate in the regulation of immune response, inflammatory reactions, and haematopoiesis. During systemic inflammation IL-1 induction in the hypothalamus may regulate neuroendocrine functions. The inactive precursor IL1-ß has to be processed into mature bioactive form of IL1-ß and is usually proteolitically mediated by inflammatory cysteine protease caspase-1 [33]. IL-1ß is one of cytokines in the inflammatory cascade resulting in increased production of cyclooxygenase-2 (COX-2) and prostaglandins [10, 33]. Prostaglandins act as long-term mediators of inflammation. IL-1ß and PGE2 are involved in biochemical processes in inflammation along with delivery [10, 11].

Spontaneous delivery at term is characterized by the expression of inflammasome components, which may participate in the activation of caspase-1 and lead to the cleavage and release of mature IL-1β by the chorio-amniotic membranes. These results support the participation of the inflammasome in the mechanisms responsible for spontaneous parturition at term [34]. IL-1ß will activate an inflammatory cascade that leads to increased concentrations of PGE2 that are required for onset of delivery. Prostaglandins are the most effective mediators for cervical dilatation in women and stimulation of labor. In the myometrium prostaglandins contribute to increased uterine contractions and in cervix cause degradation of the extracellular matrix resulting in effacement and dilatation. PGE2 has been shown to be a key step for the activation of labor [33].

Intrauterine infection induces an intra-amniotic inflammatory response involving the activation of a number of cytokines among them IL-1ß and PGE2 which, in turn, may trigger preterm contractions, cervical ripening and rupture of the membranes, and induce PTB . Zhumakanovaet al. [35] reported that increasing of level IL-1β, IL- 6 and TNF-α in serum during pregnancy can be used as a nonspecific marker in women at risk of preterm birth.

IL-1ß and PGE2 are increased in infections of the periodontium tissue. Many authors have shown that salivary IL-1b levels in subjects with periodontitis were significantly greater than those detected in healthy controls. Studies performed by Kinney et al. [36], and Rathnayake et al. [15], showed that levels of IL-1ß correlated with periodontium status. Moreover, salivary IL-1b of IL1-ß correlated significantly with clinical degree of periodontal inflammation. Certain number of research papers show that in patients with periodontitis there is a faster deterioration and rejection of allografts [23].

It has been demonstrated that production of IL-1ß and prostaglandins is increased during rejection and that these molecules are able to interfere with graft function [37].

Published data has shown that IL-1ß enhances the host antigraft adaptive response and suggests that IL1-ß may have an inherited condition that causes a hyperactivity, which in turn may be responsible for PTB [38].

Vamvakopoulos et al. [39] have demonstrated an association between IL-1ß and chronic rejection at the genetic level in heart graft recipients. The risk of rejection was 20-fold increased in patients with both the IL-1ß (π3953) C allele and the IL1RN1 allele.

Medawar [40] first posed the theory of the fetus-as-allograft nearly 60 years ago explaining the normal course of pregnancy by maternal-fetal interface, antigenic inertness of the fetus and maternal immune tolerance of foreign tissue. That tolerance is compromised in PTB.

Many authors reported that levels of IL-1ß, IL-6 and PGE2 in the blood samples were higher in the preterm delivery women than in the healthy control group and that IL-1ß and PGE2 levels in maternal blood were higher among those with severe disease of the periodontium in the PTD group. According to Kedzierska-Markowicz et al. [41] the level of IL-1β concentration is an independent predictor of preterm delivery in patients with threatened preterm labor.

Consistently with the reported results, in our study groups plasma levels of IL-1ß and PGE2 were significantly higher in the PTB group in comparison to the FTB group.

Differences in plasma levels of C-reactive protein (CRP) between the PTB patients and the FTB ones did not reach statistical significance. This data is in accordance with results of Michalowicz et al. [42] who have suggested that in pregnant women levels of CRP were not associated with infant birth weight or a risk for preterm birth.

PGE2 level in saliva of PTB mothers showed a significant positive correlation with IL-1ß in saliva, while plasma level of PGE2 showed a significant negative correlation to the plasma level of IL-1ß. It may be related to the fact that the half-life of IL-1ß is very short (3–4 h), therefore it will show a positive correlation only if tested on the place of its secretion and not in plasma where it is distant from the in-

flammatory lesion. Here the correlation becomes negative because PGE2 is a long-term activator of the inflammatory pathway and it remains high even after its descend in plasma, but the levels of IL-1ß decrease due to its short half-life [43].

Higher levels of IL-1ß and PGE2 in the PTB group could be explained by the effect of inflammation in the periodontal tissue, hyperactive IL-1ß [36], reaction of the rejection of allograft [23, 39] or hereditary genetic factors [28, 37].

The recognition that heritable factors play a role in PTB [37] is compatible with the notion that extent of periodontitis is influenced by secretory status which is to a large extent inherited and stable [17, 28].

Secretory status of an individual is genetically determined by a pair of allomorphic genes: Se and se with Se dominant over se. Approximately 80% of the population has the secretor (Se) gene [18].

In secretors salivary blood group antigens agglutinate oral pathogens and thus enable multiple functions of saliva such as rinsing, bacterial clearance and antimicrobial defense [19]. In non-secretors there are no soluble blood group substances, oral pathogens recognize histo-blood group antigens on cells surface as attachment factors, form polymicrobial gingival/subgingival biofilm, and cause gingival infection and induction of periodontitis resulting in an increased level of TNF-alpha, IL-1ß, IL-6, and PGE2 [10, 15, 18].

Blood group oligosaccharide structures are also important for blastocyst adhesion and resistance to microbial invasion. Recent studies suggest intrauterine selection against non-secretor embryo carried by a secretor mother. This data could have practical importance in assessing the risk of infertility and success of assisted reproductive techniques [44].

Furthermore, oligosaccharides, glycans, found in the breast milk of secretor mothers protect newborns from pathogens and play important role in development of the neonatal immune system. In the preterm infant they show protective effect against gut immaturity. Low salivary blood group oligosaccharides were associated with 10-fold increased odds of necrotizing enterocolitis deaths in newborns [45].

According to literature data it is conceivable that non-secretors with a lower level of iso-antibodies and immunoglobulins may have a lower resistance to infection and thereafter higher rate of periodontitis [19]. Results of Rocha et al. [16] suggest an active role of mucin glycoproteins in the innate immune regulation of periodontal bacterial colonization and disease progression. Tabasum and Nayak [46] reported 22.2% non-secretors in the chronic periodontitis group. The higher results in our group could be attributed to placental hormones that might affect the clinical and biological features of periodontal infections during pregnancy [7, 9].

Despite much accumulated knowledge on individual etiological factors, the interactions among risk factors and the pathophysiology of preterm birth remain unclear and there is no biologic explanation for 2/3 of all preterm births [22].

## Conclusion

Afore mentioned risk factors are surely a surrogate for genetic and epigenetic causes of preterm birth, and support the need for additional research into the biology of human parturition. The etiology of spontaneous PTB is still unknown because PTB is a complex syndrome with different co-factors, involving a complex interaction between genetic, immunological and environmental factors. We believe that the identification of genomic and proteomic markers may represent an added value in the further investigation of the association among periodontitis, secretory status and adverse pregnancy outcomes. A randomized clinical trial will be necessary to appropriately test our hypothesis and conclude whether non-secretory status have impact on adverse pregnancy outcome.

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## REFERENCES

1. *Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R*, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379(9832): 2162–72.
2. *Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH,* et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 2010; 88(1): 31–8.
3. *Shiozaki A, Yoneda S, Nakabayashi M, Takeda Y, Takeda S, Sugimura M,* et al. Multiple pregnancy, short cervix, part-time worker, steroid use, low educational level and male fetus are risk factors for preterm birth in Japan: a multicenter, prospective study. J Obstet Gynaecol Res 2014; 40(1): 53–61.
4. *Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z*, et al. Sterile and microbialassociated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2015; 28: 1394–409.
5. *Miller WD*. The human mouth as a focus of infection. Dent Cosmos 1891; 33(9): 689–713.
6. *Stadelmann PF, Eick S, Salvi GE, Surbek D, Mohr S, Bürgin W,* et al. Increased periodontal inflammation in women with preterm premature rupture of membranes. Clin Oral Investig 2015; 19(6): 1537–46.
7. *Oliveira Costa F, Soares Dutra Oliveira AM, Miranda Cota LO*. Interrelation Between Periodontal Disease and Preterm Birth, In: *Erez O,* editor. Preterm Birth. Rijeka: InTech; 2013; p. 3–39.
8. *Armitage GC*. Periodontal disease and pregnancy: discussion, conclusions and recommendations. Ann Periodontol 2001; 6(1): 189–92.
9. *Robinson P, Schmerman M*. Influence of Pregnancy on the Oral Cavity. Glob libr women's med 2015; ISSN: 1756–2228; [about 5 p]. Available from: https://www.glowm.com [cited 2017 Jun 19].
10. *Chukkapalli SS, Rivera-Kweh MF, Velsko IM, Chen H, Zheng D, Bhattacharyya I*, et al. Chronic oral infection with major periodontal bacteria Tannerella forsythia modulates systemic ather-

osclerosis risk factors and inflammatory markers. Pathog Dis 2015; 73(3): pii: ftv009.
11. *Roth-Isigkeit A, Hasselbach L, Ocklitz E, Brückner S, Ros A, Gehring H,* et al. Inter-individual differences in cytokine release in patients undergoing cardiac surgery with cardiopulmonary bypass. Clin Exp Immunol 2001; 125(1): 80–8.
12. *Abati S, Villa A, Cetin I.* Oral environment, inflammation and preterm birth. J Pediatr Neonat Individual Med 2015; 4(2): e040239.
13. *Saini R, Saini S, Saini SR.* Periodontitis: A risk for delivery of premature labor and low-birth-weight infants. J Nat Sci Biol Med 2010; 1(1): 40–2.
14. *Chaemsaithong P, Romero R, Korzeniewski SJ, Dong Z, Yeo L, Hassan SS*, et al. A point of care test for the determination of amniotic fluid interleukin-6 and the chemokine CXCL-10/IP-10. J Matern Fetal Neonatal Med 2015; 28(13): 1510–9.
15. *Rathnayake N, Akerman S, Klinge B, Lundegren N, Jansson H, Tryselius Y,* et al. Salivary biomarkers of oral health: a cross-sectional study. J Clin Periodontol 2013; 40(2): 140–7.
16. *Rocha Dde M, Zenóbio EG, Van Dyke T, Silva KS, Costa FO, Soares RV.* Differential expression of salivary glycoproteins in aggressive and chronic periodontitis. J Appl Oral Sci 2012; 20(2): 180–5.
17. *Nikolić Lj, Janković Lj, Čakić S, Movsesijan A.* Secretory status and periodontal disease. Bilt Transf 1996; 42(1): 23–6. (Serbian)
18. *Nurjadi D, Lependu J, Kremsner PG, Zanger P.* Staphylococcus aureus throat carriage is associated with ABO-/secretor status. J Infect 2012; 65(4): 310–7.
19. *Gunput ST, Ligtenberg AJ, Terlouw B, Brouwer M, Veerman EC, Wouters D.* Complement activation by salivary agglutinin is secretor status dependent. Biol Chem 2015; 396(1): 35–43.
20. *Armitage GC.* Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4(1): 1–6.
21. Blood grouping and typing. In: *Walker RH*, editor. Technical manual. 11th ed. Bethesda: American Association of Blood Banks; 1993. p. 621–38.
22. *Ferrero DM, Larson J, Jacobsson B, Di Renzo GC, Norman JE, Martin JN Jr,* et al. Cross-Country Individual Participant Analysis of 4.1 Million Singleton Births in 5 Countries with Very High Human Development Index Confirms Known Associations but Provides No Biologic Explanation for 2/3 of All Preterm Births. PLoS One. 2016; 11(9): e0162506.
23. *Gümüş P, Öztürk VÖ, Bozkurt E, Emingil G.* Evaluation of the gingival inflammation in pregnancy and postpartum via 25-hydroxy-vitamin D3, prostaglandin E2 and TNF-α levels in saliva. Arch Oral Biol 2016; 63: 1–6.
24. *Corbella S, Taschieri S, Del Fabbro M, Francetti L, Weinstein R, Ferrazzi E.* Adverse pregnancy outcomes and periodontitis: A systematic review and meta-analysis exploring potential association. Quintessence Int 2016; 47(3): 193–204.
25. *Walia M, Saini N.*Relationship between periodontal diseases and preterm birth: Recent epidemiological and biological data. Int J Appl Basic Med Res 2015; 5(1): 2–6.
26. *Boggess KA, Moss K, Madianos P, Murtha AP, Beck J, Offenbacher S.* Fetal immune response to oral pathogens and risk of preterm birth. Am J Obstet Gynecol 2005; 193(3 Pt 2): 1121–6.
27. *Madianos PN, Bobetsis YA, Offenbacher S.* Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. J Periodontol 2013; 84(4 Suppl): S170–80.
28. *Kamei H, Ishihara Y, Fuma D, Niwa T, Kamiya Y, Yokoi T,* et al. Interleukin-1 receptor gene variants are associated with aggressive periodontitis in the Japanese. Arch Oral Biol 2014; 59(7): 756–63.
29. *Dörtbudak O, Eberhardt R, Ulm M, Persson GR.* Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol 2005; 32(1): 45–52.
30. *Jarjoura K, Devine PC, Prez-Delboy A, Herrera-Abreu M, D'Alton M, Papapanou PN.* Markers of periodontal infection and preterm birth. Am J Obstet Gynecol 2005; 192(2): 513–9.
31. *Lieff S, Boggess KA, Murtha AP, Jared H, Madianos PN, Moss K,* et al. The oral conditions and pregnancy study: periodontal status of a cohort of pregnant women. J Periodontol 2004; 75(1): 116–26.
32. *Soucy-Giguère L, Tétu A, Gauthier S, Morand M, Chandad F, Giguère Y,* et al. Periodontal Disease and Adverse Pregnancy Outcomes: A Prospective Study in a Low-Risk Population. J Obstet Gynaecol Can 2016; 38(4): 346–50.
33. *Gringhuis SI, Kaptein TM, Wevers BA, Theelen B, van der Vlist M, Boekhout T,* et al. Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. Nat Immunol 2012; 13(3): 246–54.
34. *Romero R, Xu Y, Plazyo O, Chaemsaithong P, Chaiworapongsa T, Unkel R,* et al. A Role for the Inflammasome in Spontaneous Labor at Term. Am J Reprod Immunol 2018; 79(6): e124403
35. *Zhumakanova K, Abeuova B, Kuzgibekova A, Kenzhebayeva K, Eremicheva G.* Some clinical and immunological aspects of preterm birth. Georgian Med News 2016; (253): 17–25. (Russian)
36. *Kinney JS, Morelli T, Braun T, Ramseier CA, Herr AE, Sugai JV,* et al. Saliva/pathogen biomarker signatures and periodontal disease progression. J Dent Res 2011; 90(6): 752–8.
37. *Karjalainen MK, Huusko JM, Ulvila J, Sotkasiira J, Luukkonen A, Teramo K,* et al. A Potential Novel Spontaneous Preterm Birth Gene, AR, Identified by Linkage and Association Analysis of X Chromosomal Markers, PLoS One 2012; 7(12): e51378.
38. *Amirisetty R, Patel RP, Das S, Saraf J, Jyothy A, Munshi A.* Interleukin 1β (+3954, -511 and -31) polymorphism in chronic periodontitis patients from North India. Acta Odontol Scand 2015; 73(5): 343–7.
39. *Vamvakopoulos JE, Taylor CJ, Green C, McNeil K, Wallwork J, Goodman R,* et al. Interleukin 1 and chronic rejection: possible genetic links in human heart allografts. Am J Transplant 2002; 2(1): 76–83.
40. *Medawar PB.* Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 1953; 7: 320–38.
41. *Kedzierska-Markowicz A, Krekora M, Biesiada L, Głowacka E, Krasomski G.* Evaluation of the correlation between IL-1β, IL-8, IFN-γ cytokine concentration in cervico-vaginal fluid and the risk of preterm delivery. Ginekol Pol 2015; 86(11): 821–6. (Polish)
42. *Michalowicz BS, Novak MJ, Hodges JS, DiAngelis A, Buchanan W, Papapanou PN,* et al. Serum inflammatory mediators in pregnancy: changes after periodontal treatment and association with pregnancy outcomes. J Periodontol 2009; 80(11): 1731–41.
43. *Lopez-Castejon G, Brough D.* Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev 2011; 22(4): 189–95.
44. *Gloria-Bottini F, Magrini A, Cozzoli E, Neri A, Pietroiusti A, Amante A,* et al. ABH secretor genetic polymorphism: evidence of intrauterine selection. Eur J Obstet Gynecol Reprod Biol 2011; 154(1): 20–3.
45. *Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG,* et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants, Microbiome 2015; 3: 13.
46. *Tabasum ST, Nayak RP.* Salivary blood group antigens and microbial flora. Int J Dent Hyg 2011; 9(2): 117–21.

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# Predictive value of extremely low PAPP-A, free βhCG and extremely high mean uterine artery pulsatility index in the first trimester for fetal growth restriction

## Prediktivna vrednost izuzetno niskih nivoa PAPP-A, slobodnog βhCG i izuzetno visokog srednjeg pulzatornog indeksa uterinih arterija u prvom trimestru trudnoće u proceni nastanka intrauterusnog zastoja u rastu ploda

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**Abstract**

**Background/Aim.** Adverse pregnancy outcomes such as preeclampsia (PE), placental abruption (PA), fetal intrauterine growth restriction (IUGR) and stillbirth could be recognized by prenatal screening. The objective of this study was to predict IUGR by using first-trimester extremely low pregnancy-associated plasma protein-A (PAPP-A), extremely low free beta-human chorionic gonadotropin (free βhCG) levels, and extremely high Pulsatility-index (PI) of uterine arteries, as single and combined predictors for IUGR development. **Methods.** This was a prospective first-trimester study analyzing singleton pregnancies at 11–13+6 weeks' gestation who underwent routine first-trimester screening at the Department of High Risk Pregnancy of the Clinic for Gynecology and Obstetrics „Narodni front“, University of Belgrade, Serbia. First-trimester screening for PAPP-A, free βhCG, and PI was performed in nulliparous, normotensive women with extremely low PAPP-A (PAPP-A ≤ 0.52 unit multiple of median – MoM) and/or extremely low free βhCG (free βhCG ≤ 0.56 MoM) and/or extremely high PI (PI ≥ 2.52). **Results.** Of 85 pregnant women included in the final analysis, 14 (16.5%) developed IUGR. PAPP-A ≤ 0.52 MoM and PI ≥ 2.52, as single categorical variables, found to be with high predictable values for IUGR development (odds ratio – OR = 3.064, 95% confidence interval – CI= 0.634-14.810, $p = 0.046$, and OR = 2.129, 95% CI = 0.449–10.713, $p = 0.021$, respectively). Furthermore, the receiver operating characteristic (ROC curve identified PAPP-A and PI as continuous variables to be significant predictors of IUGR (area under curve – AUC = 0.671, 95% CI = 0.521−0.820, $p = 0.045$, and AUC = 0.744, 95% CI = 0.587−0.902, $p = 0.004$, respectively). **Conclusion.** The present study suggests that the first trimester extremely low PAPP-A and increased Doppler-PI levels are single predictors of IUGR. Described model could be used in a routine daily clinical practice in resource limited settings where other parameters are not available for the prediction of IUGR development.

**Key words:**
**pregnancy trimester, first; fetal growth retardation; ultrasonography, doppler, color; pregnancy-associated plasma protein-a; chorionic gonadotropin, beta subunit, human.**

**Apstrakt**

**Uvod/Cilj.** Neželjeni ishodi trudnoće kao što su preeklampsija, abrupcija placente i zaostajanje rasta fetusa i mrtvorođenost mogu biti prepoznati u okvirima prenatalnog skrininga. Cilj ove studije bio je ispitati mogućnost predviđanja pojave intrauterusnog zastoja u rastu ploda (IUGR) pojedinačnom i kombinovanom upotrebom izuzetno niskih vrednosti plazma proteina A povezanog sa trudnoćom (PAPP-A), izuzetno niskih vrednosti slobodne beta subjedinice humanog horionskog gonadotropina (free βhCG), kao i ekstremno visokih srednjih vrednosti doplerskog pulsatilnog indeksa (PI) uterinih arterija u prvom trimestru trudnoće. **Metode.** Prospektivnom studijom analizirane su jednoplodne trudnoće starosti 11–13+6 nedelja gestacije u okviru rutinskog skrininga na Daunov sindrom. Studija je rađena na Odeljenju za visoko rizične trudnoće, univerzitetske Ginekološko-akušerske klinike

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„Narodni front“ u Beogradu, Srbija. Skrining prvog trimestra je obuhvatao analizu: PAPP-A, *free* βhCG i PI kod normotenzivnih nulipara, kod kojih su zabeležene ekstremno niske vrednosti PAPP-A (PAPP-A ≤ 0,52 *unit multiple of median* – MoM) i/ili ekstremno niske vrednosti *free* βhCG (*free* βhCG ≤ 0,56 MoM) i/ili ekstremno visoke vrednosti PI (PI ≥ 2,52). **Resultati.** Kod 85 trudnica uključenih u konačnu analizu, kod njih 14 (16,5 %) je zabeležena pojava IUGR ploda. PAPP-A ≤ 0,52 MoM i PI ≥ 2,52, kao pojedinačne kategorijske varijable, su prepoznate kao varijable sa visokim prediktivnim značajem za pojavu IUGR (*odds ratio* – OR = 3,064, 95% *confidence* interval – CI = 0,634 – 14,810, $p$ = 0,046 i OR = 2,129, 95% CI = 0,449 – 10,713, $p$ = 0,021). Daljom analizom, *receiver operating characteristic* (ROC) – kriva je identifikovala PAPP-A i PI, kao kontinualne varijable, koje su značajni prediktori za pojavu IUGR ploda (*area under the curve* – AUC = 0,671, 95% CI = 0,521 – 0,820, $p$ = 0,045 i AUC = 0,744, 95% CI = 0,587 – 0,902, $p$ = 0,004). **Zaključak.** Ova studija sugeriše da u prvom trimestru trudnoće ekstremno niske vrednosti PAPP-A i ekstremno visoke srednje vrednosti Doppler-PI uterinih arterija mogu biti značajni parametri za predviđanje pojave IUGR ploda. Opisani model bi mogao da se primeni u svakodnevnoj kliničkoj praksi u zemljama sa ograničenim mogućnostima, kada ostali parametri za predviđanje pojave IUGR ploda nisu dostupni.

**Ključne reči:**
**trudnoća, prvi trimestar; fetus, zaostajanje u rastu; ultrasonografija, dopler kolor; plazma, protein a, udružen sa trudnoćom; horionski gonadotropin, beta subjedinica, humani.**

## Introduction

In the recent studies it has been shown that the placenta is a new field of prenatal screening and diagnosis. Adverse pregnancy outcomes such as preeclampsia (PE), placental abruption (PA), fetal growth restriction (IUGR) and stillbirth could be recognized by prenatal screening. Mentioned adverse pregnancy outcomes occur in 5% to 10% of pregnancies worldwide [1].

The function of the placenta is based on an adequate trophoblastic invasion into the maternal circulation during the first and second trimester. Aberrations of this invasion may lead to a high resistance vascularization, hypo-perfusion of placental and chorionic villi, deposition of fibrin and increased apoptosis process [2]. Histological characteristics of the placenta changes correspond with the appearance of the adverse pregnancy outcomes, thus decrease in placental function could also decrease production of pregnancy-associated plasma protein A (PAPP-A) [3–5]. On the other hand, impaired placental perfusion in pregnancies with PE, PA, IUGR and/or stillbirth has been provided by Doppler studies where pulsatility index (PI) of the uterine arteries were increased [6–8].

Since there are no currently recommended models for prediction of IUGR, our primary objective was to evaluate extremely low levels of maternal serum PAPP-A, extremely low levels of free βhCG and extremely high levels of mean Doppler uterine PI in the first trimester, as an independent single or combined predictors for development of IUGR.

## Methods

This prospective, first-trimester study analyzed singleton pregnancies during 11–13+6 gestation weeks. The study was performed at the Department of High Risk Pregnancy of the Clinic of Gynecologyand Obstetrics «Narodni front», University of Belgrade, Serbia. Written consent was obtained from all participants and the study was approved by the Ethics Committee, Faculty of Medicine, University of Belgrade, Serbia.

### *General inclusion criteria*

All pregnancies underwent routine first-trimester screening. Combined first-trimester screening comprised a combination of maternal age, fetal nuchat translucency (NT) thickness, maternal serum free ßhCG and PAPP-A in a single point of time [9–12]. The pregnancy was dated according to the last menstrual period. In the case that the date was uncertain or the estimated gestation by crown–rump length (CRL) was discordant by more than 7 days from the estimated gestation, the CRL was used to date the pregnancy.

Inclusion criteria in the study were following: all nulliparous, normotensive pregnant women with singleton pregnancy. Included subjects were routinely screened for Down syndrome and they were screened for ultrasound markers such as: fetal crown-rump length and nuchal translucency, as well as for maternal first trimester biochemistry analysis: PAPP-A and free βhCG. Only subjects with all mentioned parameters were included in the final analysis.

Exclusion criteria were following: multiparous women, women with multiple gestations and pregnancies with a major fetal chromosomal or structural anomaly. Pregnancies with no fetal abnormality findings at the 11–13 weeks scan and/or the 20–23 weeks scan which resulted in termination, miscarriage or stillbirth, as well as those lost to follow-up were also excluded.

In order to be included in a final analysis, besides fulfilling all general inclusion criteria, patients had to have at least one out of three following criteria: extremely low maternal serum PAPP-A levels, extremely low free βhCG levels and/or extremely high mean uterine artery PI index. Maternal serum samples for PAPP-A and free βhCG were assayed with the Kryptor software package analyzer and results were expressed in the multiple of the median (moM) unit, while the uterine artery mean PI measurements were performed by transabdominal ultrasound (US) with 3–7.5 MHz curvilinear transducers by the same experienced fetal medicine specialists. In order to do so, transabdominal US (e.g. Voluson 730 Expert series, GE Healthcare, Kretzttechnik, Zipf, Austria) was

performed to measure fetal crown rump lenght (CRL) and NT thickness. Pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel with angle of insonation was less than 30°. When three similar consecutive waveforms were obtained of the PI measurement, the mean PI of the right and left arteries was calculated [13]. In each pregnancy the mean uterine artery PI were expressed as a multiple of the expected median calculated from The Fetal Medicine Foundation's reference limits for singleton pregnancies after correction for maternal ethnicity, body mass index (BMI) and gestational age [14, 15]. The extreme PAPP-A, free βhCG and mean PI index cut-off values were as follows: first-trimester PAPP-A ≤ 0.52 MoM [16], first-trimester free βhCG ≤ 0.56 MoM [13] and mean PI ≥ 2.52 [13–15], respectively. All patients who fullfield inclusion criteria were followed by intensive antenatal care such as: growth scans, blood pressure measurements and biochemical analysis at 24, 28, 32 and 36 gestational weeks.

*Maternal history*

Patients were asked to complete a questionnaire about maternal age, ethnicity, smoking status during pregnancy, spontaneous or assisted conception such as usage of ovulation drugs and/or *in vitro* fertilization. Patients were also asked about their medical history, in particular about diabetes mellitus, antiphospholipid syndrome, trombophilia, human immuno-deficiency virus infection and sickle cell disease. They were also asked about concomitant medication usage of antidepressant, antiepileptic, anti-inflammatory drugs, aspirin, beta-mimetic, insulin and thyroxin. One part of the questionnaire was focused on parity (multiparous or nulliparous), obstetric history if any and family history (mother).

*Outcome measure*

In patients included in the final analysis, patients were followed-up until the end of pregnancy and data on pregnancy outcome were recorded, including adverse pregnancy outcomes such as PE, PA and IUGR. For this particular study we were focused only on pregnancies with IUGR outcome. IUGR was defined as an estimated weight ≤ 10th centile of a given population at the same gestational age [17].

*Statistical analysis*

Data were presented as numer (percentage) or mean ± SD, depending on the data type. Pearson's $\chi^2$ or Fisher's exact tests were used for the comparison of categorical variables and Student's *t*-tests were used for continuous variables. Univariate analysis was initially performed to determine variables with a significant association with IUGR. Backward stepwise logistic regression was used to determine the combined prediction model for IUGR. Receiver operating characteristic (ROC) analysis was performed to assess cut-off values of markers that best predict adverse event in pregnancy. Defined level of significance was $p < 0.05$. Statistical analyses were performed using SPSS 20.0 (IBM Corporation) software package.

## Results

The 11,326 singleton pregnancies underwent routine first trimester screening at 11–13+6 weeks of gestation. They comprised 9,944 pregnancies ending in a live birth without fetal abnormality, 1,030 pregnancies with abnormal fetuses (566 chromosomal abnormalities and 464 structural defects) and 354 pregnancies were lost to follow-up (Figure 1).

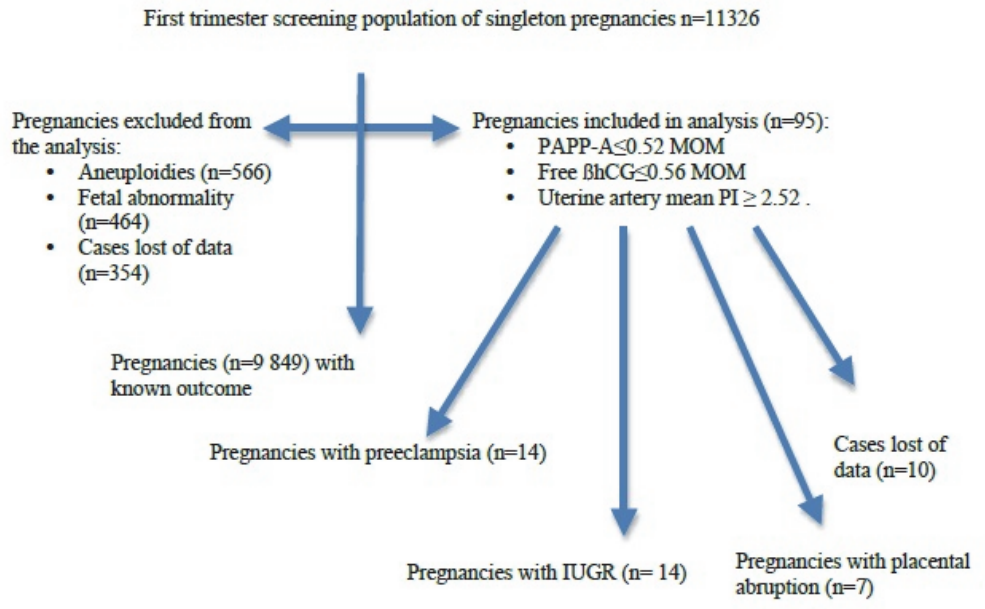


**Fig. 1 – Flow-chart of the study population.**

**PAPP-A – pregnancy-associated plasma protein A; fβhCG – free beta-human chorionic gonadotropin; PI – pulsatility index; MoM – unit multiple of median; IUGR – intrauterine growth restriction.**

In the final analysis 85 patients, all Caucasians, all normotensive, were included in the study. IUGR as an adverse pregnancy outcome, has been diagnosed in 14 (16.47%) patients. Baseline patients' characteristics are shown in Table 1.

Association between first trimester maternal parameters (PAPP-A, free-βHCG and uterine artery Doppler PI) using various thresholds for all three variables are shown in Table 2. Overall percentage of correctly classified data was between 81.2% and 85.9%. PAPP-A, as continuous variable, tended to be significantly associated with IUGR ($p = 0.060$), which was not a case with PAPP-A < 0.52 MoM, as categorical variable ($p = 0.164$). However, PI, as continuous variable, was not significantly associated with IUGR development ($p = 0.108$), with high odds ratio (OR) for development of IUGR. Also, PI ≥ 2.52 MoM, as a categorical variable, was also not found to be significantly associated with IUGR ($p = 0.332$). There was no statistically significant association between free βHCG either as continuous or categorical variable with IUGR across all defined thresholds ($p = 0.357$ and $p = 0.494$, respectively).

Backward stepwise logistic regression used to determine the combined prediction model including categorical variables PAPP-A ≤ 0.52 MoM together with PI ≥ 2.52 was found not to be significantly associated with IUGR development ($p = 0.224$) (Table 3). Overall percentage of correctly classified data was 83.5%.

**Table 1**

**Baseline patient's characteristics**

| Characteristics | IUGR development | | *p* |
|---|---|---|---|
| | Yes (n = 14) | No (n = 71) | |
| Maternal age (year), mean ± SD | 31 ± 3.74 | 31.14 ± 4.33 | 0.910 |
| BMI (kg/m$^2$), mean ± SD | 29.95 ± 3.35 | 26.70 ± 4.34 | < 0.01 |
| Smoking, n (%) | | | |
| yes | 9 (37.5) | 15 (62.5) | < 0.01 |
| no | 5 (8.2) | 56 (91.8) | |
| DM, n (%) | | | |
| yes | 2 (33.3) | 4 (66.7) | 0.256 |
| no | 12 (15.2) | 67 (84.8) | |
| IVF, n (%) | | | |
| yes | 1 (25) | 3 (75) | 1.000 |
| no | 13 (16) | 68 (84) | |
| PAPP-A (MoM), mean ± SD | 0.44 ± 0.16 | 0.66 ± 0.42 | < 0.05 |
| PI, mean ± SD | 2.56 ± 0.25 | 2.41 ± 0.30 | < 0.01 |
| free βHCG (MoM) , mean ± SD | 0.51 ± 0.25 | 0.64 ± 0.47 | 0.141 |

**IUGR – intrauterine growth restriction; BMI – body mass index; SD – standard deviation; DM – diabetes mellitus; IVF – *in vitro* fertilisation; PAPP-A –pregnancy-associated plasma protein-A; PI – pulsatility index; free βHCG – free beta-human-chorionic-gonadotropin; MoM – unit multiple of median.**

**Table 2**

**Univariate logistic regression analysis of the first-trimester biomarkers and uterine artery Doppler findings and their association with IUGR**

| Parameters | *p* | OR (95% CI) | $R^2$ |
|---|---|---|---|
| PAPP-A | 0.060 | 0.043 (0.002–1.138) | 0.117 |
| PAPP-A < 0.52 MoM | 0.164 | 3.064 (0.634–14.810) | 0.046 |
| PI | 0.108 | 13.765 (0.564–33.668) | 0.075 |
| PI ≥ 2.52 | 0.332 | 2.192 (0.449–10.713) | 0.021 |
| Free-βHCG | 0.357 | 0.361 (0.041–3.156) | 0.024 |
| Free βHCG ≤ 0.56 MoM | 0.494 | 1.745 (0.353–8.620) | 0.010 |

**IUGR – intrauterine growth restriction; PAPP-A – pregnancy-associated plasma protein-A; PI – Pulsatility index; βHCG – beta-human chorionic-gonadotropin; MoM – unit multiple of median; OR – odds ratio; CI – confidence interval.**

**Table 3**

**Backward stepwise logistic regression of combined first-trimester PAPP-A and uterine artery Doppler findings and their association with IUGR**

| Parameters | *p* | OR (95% CI) | $R^2$ |
|---|---|---|---|
| PAPP-A ≤ 0.52 MoM + PI ≥ 2.52 | 0.224 | 2.171 (0.622–7.576) | 0.031 |

**PAPP-A – pregnancy-associated plasma protein-A; PI – Pulsatility index; IUGR – intrauterine growth restriction; OR – odds ratio; CI – confidence interval; MoM – unit multiple of median.**

Area under curve (AUC) of the ROC curve was performed to assess cut-off value of studied variables that best predicts IUGR, as adverse pregnancy outcome. The model identified first- trimester PAPP-A [AUC = 0.671, 95% confidence interval (CI) = 0.521–0.820; $p = 0.045$) and PI (AUC = 0.744, 95% CI = 0.587–0.902; $p = 0.04$) to be significant predictors for IUGR development, and first trimester free βHCG (AUC = 0.375, 95% CI = 0.215–0.536; $p = 0.142$) to be a non-significant predictor for IUGR (Figure 2).

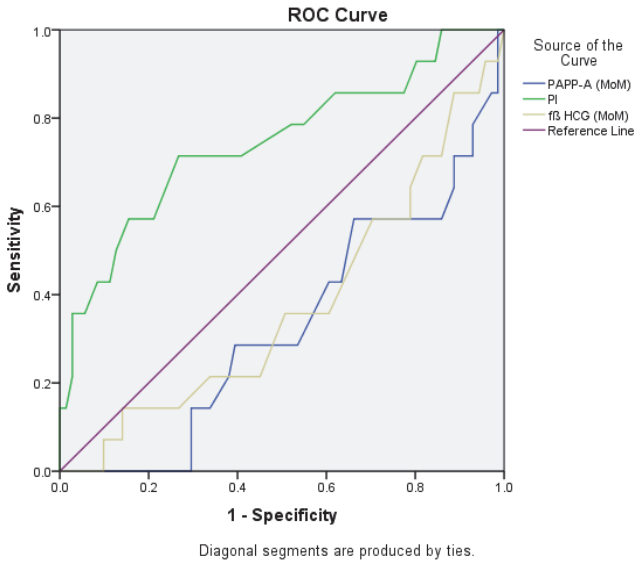


**Fig. 2 – Receiver operating characteristic (ROC) curve for the prediction of IUGR using first- trimester PAPP-A, PI of uterine arteries and free βHCG.**

**IUGR – intrauterine growth restriction; PAPP-A – pregnancy associated plasma protein-A; PI – pulsatility index; fβHcg – free beta-human chorionic gonadotropin; MoM – unit multiple of median.**

## Discussion

The etiology of IUGR has not been completely understood. However, impaired trophoblastic invasion of spiral arteries and the lack of placentation are highly marked in pregnancies destined to develop IUGR [12]. It has been also shown that low levels of PAPP-A, which is a protease for insulin-like growth factor (IGF) binding protein-4 (IGFBP-4), are associated with higher IGFBP-4 and lower IGF [16]. Therefore, IGF influences trophoblast invasion and may have a role in PE as well as IUGR development [18].

The findings of this prospective study in nulliparous women with extreme levels of at least one out of three risk parameters for fetal IUGR have demonstrated that the first trimester low levels of maternal serum PAPP-A and first trimester high levels of uterine Doppler PI are associated with an increased risk for subsequent development of fetal IUGR. Our study also demonstrated no association between low levels of maternal serum free β-HCG and development of fetal IUGR. The same results were presented in several other studies [13, 18–21].

Our results are also consistent with the results of the FASTER Trial Research Consortium study which demonstrated that women with low first-trimester PAPP-A levels were at significantly increased risk for obstetric complications and consequently adverse obstetric outcomes such as fetal IUGR [19]. Low first-trimester PAPP-A levels used in the predictive model for detection of fetal IUGR without PE, had detection rate of 73% with false positive rate of 10% [20]. In addition, a significant increase in the relative risk of fetal IUGR development is associated with a further decline of PAPP-A [21].

Important evidence for impaired placental perfusion in pregnancies destined to develop fetal IUGR has been provided by Doppler studies of increased pulsatility index (PI) of uterine arteries in first trimester. A number of first trimester studies using single abnormal uterine artery Doppler PI high levels demonstrated an overall sensitivity of 25% for the prediction of IUGR improving to about 60% for its development [20–24]. In our study extremely high levels of mean uterine artery Doppler evaluation (e.g. PI ≥ 2.52), as a single predictor, showed a high relative risk for fetal IUGR development, even though with no statistical significance. First-trimester mean uterine artery Doppler PI, was found to be significantly associated with IUGR development [23]. Recently, Cruz-Lemini et al. [25] demonstrated a significant increase in the first-trimester uterine artery Doppler PI in patients who developed both early and late-onset preeclampsia compared to controls.

In our study, multivariate prediction model combining the first-trimester extremely high levels of mean uterine artery Doppler PI together with the first-trimester extremely low levels of PAPP-A significantly improve predictive capability and efficiency for IUGR. Single addition of above mentioned first- trimester maternal biochemical and hemodynamic markers could improve the prediction of IUGR, but limitation according to low sensitivity and specificity still exist.

While waiting for the specific predictive model for fetal IUGR development, alternative strategies could possibly focus on the early identification of high-risk pregnancies and undertaking of the necessary measures to improve placentation. Algorithms combining maternal characteristics and biophysical and biochemical tests at 11–13 weeks could identify most pregnancies delivering preterm IUGR neonates in the presence or absence of PE [20, 24].

This study has the limitation of being a single-center study with a small sample size. The important aspect of this study was to demonstrate the capability of extremely low levels of maternal serum PAPP-A and free βhCG used for Down syndrome screening in the first trimester, and extremely high levels of Doppler uterine PI in the first trimester, as a single and combined predictors for IUGR development. Evaluation of high-risk pregnant women for PE and/or IUGR as a part of a routine perinatology clinical practice in the first trimester by using maternal serum PAPP-A and uterine artery PI is efficient model with no additional costs, which might be important in the resource limited settings.

## Conclusion

The present study suggests that the first-trimester extremely low levels of maternal serum PAPP-A and Doppler PI are single predictors of IUGR. Described model could be used in a routine daily clinical practice in resource limited settings where other parameters are not available for the prediction of IUGR development.

## R E F E R E N C E S


1. *Hossain N, Paidas MJ*. Adverse pregnancy outcome, the uteroplacental interface and preventive strategies. Semin Perinatol 2007; 31(4): 208–12.
2. *Frøen JF, Cacciatore J, McClure EM, Kuti O, Jokhio AH, Islam M,* et al.Lancet's Stillbirths Series Steering Committee: Stillbirths: why they matter. Lancet 2011; 377(9774): 1353–66.
3. *Brosens I, Pijnenborg R, Vercruysse L, Romero R*. The "Great Obstetrical Syndrome" are associated with disorders of deep placentation. Am J Obstet Gynecol 2011; 204(3): 193–201.
4. *Cnattingius S, Stephansson O*. The challenges of reducing risk factorsfor stillbirths. Lancet 2012; 377(9774): 1294–5.
5. *Costa SL, Proctor L, Dodd JM, Toal M, Okun N, Johnson JA*, et al. Screening for placental insufficiency in high-risk pregnancies: is earlier better? Placenta 2008; 29(12): 1034–40.
6. *Tikkanen M*. Etiology, clinical manifestations and prediction of placental abruption. Acta Obstet Gynecol Scand 2010; 89(6): 732–40.
7. *Poon LC, Volpe N, Muto B, Yu CK, Syngelaki A, Nicolaides KH*. Second-Trimester Uterine Artery Doppler in the Prediction of Stillbirths. Fetal Diagn Ther 2013; 33(1): 28–35.
8. *Sarmiento A, Casasbuenas A, Rodriguez N, Angarita AM, Sarmiento P, Sepulveda W*. First-trimester uterine artery Doppler velocimetry in the prediction of birth weight in a low-risk population. Prenat Diagn 2013; 33(1): 21–4.
9. *Borrell A, Grande M, Bennasar M, Borobio V, Jimenez JM, Stergiotou I*, et al. First-trimester detection of major cardiac defects with the use of ductus venosus blood flow. Ultrasound Obstet Gynecol 2013; 42(1): 51–7.
10. *Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH*. One stop clinic for assessment of risk for fetal anomalies: a report of the first year of prospective screening for chromosomal anomalies in the first trimester. Br J Obstet Gynecol 2003; 107(10): 1271–5.
11. *Karadzov-Orlic N, Egic A, Milovanovic Z, Marinkovic M, Damnjanovic-Pazin B, Lukic R,* et al. Improved diagnostic accuracy by using secondary ultrasound markers in the first-trimester screening for trisomies 21, 18 and 13 and Turner syndrome. Prenat Diagn 2012; 32(7): 638–43.
12. *Poon LC, Nicolaides KH*. First-trimester maternal factors andbiomarker screening for preeclampsia. Prenat Diagn 2014; 34(7): 618–27.
13. *Goetzinger KR, Singla A, Gerkowicz S, Dicke JM, Gray DL, Odibo AO*. Predicting the risk of pre-eclampsia between 11 and 13 weeks' gestation by combining maternal characteristics and serum analytes, PAPP-A and free β-hCG. Prenat Diagn 2010; 30(12–13): 1138–42.
14. *Poon LC, Staboulidou I, Maiz N, Plasencia W, Nicolaides KH*. Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11–13 weeks. Ultrasound Obstet Gynecol 2009; 34(2): 142–8.
15. *Resnik R*. Intrauterine growth restriction. Obstet Gynecol 2002; 99(3): 490–6.
16. *Pilalis A, Souka AP, Antsaklis P, Daskalakis G, Papantoniou N, Mesogitis S*, et al. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11-14 weeks' gestation. Ultrasound Obstet Gynecol 2007; 29(2): 135–40.
17. *Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH*. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther 2012; 32(3): 171–8.
18. *Irwin JC, Suen LF, Martina N, Mark SP, Giudice LC*. Role of the IGF system in trophoblast invasion and pre-eclampsia. Hum Reprod 1999; 14 Suppl 2: 90–6.
19. *Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH*, et al.First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol 2004, 191(4): 1446–51.
20. *Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH*. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11–13 weeks. Fetal Diagn Ther 2011; 29(2): 148–54.
21. *Kirkegaard I, Uldbjerg N, Henriksen TB*. PAPP-A and free ß HCG in relation to admission to neonatal invasive care unit and neonatal disease. Prenat Diagn 2011; 31(12): 1169–75.
22. *Martin AM, Bindra R,Curcio P, Cicero, Nicolaides KH*. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2001; 18(6): 583–6.
23. *Parra M, Rodrigo R, Barja P, Bosco C, Fernández V, Muñoz H*, et al. Screening test for preeclampsia through assessment of uteroplacental blood flow and biochemical markers of oxidative stress and endothelial dysfunction. Am J Obstet Gynecol 2005; 193(4): 1486–91.
24. *Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S,* et al. Prevention of preeclampsia and intrauterine growth restriction with aspirinstarted in early pregnancy: a meta-analysis. Obstet Gynecol 2010; 116(2 Pt 1): 402–14.
25. *Cruz-Lemini M, Crispi F, Van Mieghem T, Pedraza D, Cruz-Martínez R, Acosta-Rojas R*, et al. Risk of perinatal death in early-onset intrauterine growth restriction according to gestational age and cardiovascular Doppler indices: a multicenter study. Fetal Diagn Ther 2012; 32(1–2): 116–22.


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# Reverse modeling of the human mandible 3D geometric model

## Reverzno modeliranje 3D geometrijskog modela donje ljudske vilice

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**Abstract**

**Background/Aim.** The geometry of each bone in the human skeletal system is unique. The aim of this research was to present application of a new method, method of anatomical features (MAF), for the creation of the geometrical model (surface and solid) of the human mandible. **Methods**. The method was based on Referential Geometrical Entities (RGEs) which have been defined on mandible polygonal model in accordance with anatomical properties of the mandible. Polygonal model was created over the input data (anatomical landmarks of the mandible) acquired from computed tomography scans. For the creation of computer-aided design (CAD) models in CATIA software, referential geometrical entities were defined according to the bone geometry and morphology features. **Results.** Definition of B-spline curves was performed on the body and on the ramus of the mandible. In this way, it was possible to create the geometrically accurate and anatomically correct three-dimensional geometric (surface and solid) models. The accuracy of the obtained surface model was tested through comparison with the geometry of the original bone model. **Conclusion.** Compared to the previously applied methods for creating geometric models, MAF provides more satisfactory results, and in some cases even better.

**Key words:**
**mandible; methods; models, anatomic; printing, three-dimensional; technology, medical; tomography, x-ray computed.**

**Apstrakt**

**Uvod/Cilj.** Geometrija svake kosti skeletnog sistema čoveka je jedinstvena. Cilj ovog istraživanja bio je da se prezentuje primena nove metode, metode anatomsikih entiteta (MAF) za kreiranje geometrijskog modela (površinskog i zapreminskog) ljudske donje vilice. **Metode**. Metod je baziran na referentnim geometrijskim entitetima (RGEs) koji se definišu na poligonalnom modelu mandibule, u skladu sa anatomskim osobinama mandibule. Poligonalni model je kreiran preko ulaznih podataka (anatomskih orjentira donje vilice) dobijenih na osnovu podataka sa snimaka kompjuterizovanom tomografijom. Za kreiranje kompjuterski podržanim dizajnom (CAD) modela u programu CATIA, referentni geometrijski entiteti su bili definisani u skladu sa geometrijskim i morfološkim odlikama mandibule. **Rezultati**. Definisanje B-*spline* krivih vršeno je na telu i granama mandibule. Na taj način je bilo moguće kreirati geometrijski tačan i anatomski korektan trodimenzioni geometrijski (površinski i zapreminski) model. Tačnost dobijenih površinskih modela je testirana upoređivanjem sa geometrijom originalnog modela kosti. **Zaključak.** U odnosu na ranije primenjene metode za kreiranje geometrijskih modela, MAF daje zadovoljavajuće rezultate, u nekim slučajevima čak i bolje.

**Ključne reči:**
**mandibula; metodi; modeli, anatomski; štampanje, trodimenzionalno; tehnologija, medicinska; tomografija, kompjuterizovana, rendgenska.**

### Introduction

The development of computed tomography (CT) and three-dimensional (3D) reconstruction brought a revolution in diagnostic radiology. 3D reconstruction algorithms were more optimized and three-dimensional image reformatting of standard two-dimensional (2D) CT data became an often used tool to provide the radiologist and surgeon with readily recognizable images of the complex anatomic structures [1].

The creation of the 3D digital models with the help of reverse engineering and geometric morphometric methods (GMM) create the fundamental part of a new field of the "virtual anthropology" (VA) [2]. VA, or "virtual morphology" enables working with virtual copies of specimens and analyzes of shape and size based on a comprehensive quantitative basis that can capture the complete geometry. In VA, advance statistical methods, computer graphics, informational technologies are used, which enables us to

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obtain geometrical, topological and morphological data on the observed object [2].

An anatomically correct and geometrically accurate model of a human bone (in this case the human mandible) is necessary in the computer-based preoperative planning especially in orthodontics, prosthetics, and maxillofacial surgery. Such model allows the creation of the patient-adapted bony implants, and different devices such as the fixators improving the preparation and simulation of surgical interventions [3].

"The mandible is more susceptible to trauma compared to other body parts due to its localization and anatomy" [4]. A surgical reconstruction of some mandibular congenital deformities is a very complex procedure, difficult for patients. Sometimes it is accompanied with distinct craniofacial deformities or missing bony structures. The main reasons for such pathological bony features are: bony fractures, malformations, tumors and another injuries. Creation the 3D geometrical model of the human mandible is a challenge, because the mandible has a very complex shape, structure and geometry [5].

There are two general approaches to the generation of 3D geometrical models of human bones [6].

The first method for creating a geometric model is based on the use of volumetric scanning methods. Volumetric scanning methods (CT or magnetic resonance imaging – MRI) allow the creation of 3D data sets that can be transformed into an adequate model (e.g. polygonal) suitable for further processing. Data processing is usually performed in specialized software solutions [7, 8], such as, for example, Mimics [9], 3D Doctor [10] which at the same time enable the conversion between various formats of geometrical models.

The second method for creating a geometric model of the bone is based on the bone shape prediction. Predictive models are models whose geometry and topology can be adjusted to a specific patient, based on specific parameters [6]. Morphometric parameters are measurable dimensions, and can be obtained based on 2D images (X-ray) or volumetric models obtained by a volumetric scanning method (CT, MRI). Models obtained by this method are very accurate if the number of parameters are adequate and model structure itself is well-chosen. Benazzi et al. [11] and Higgins et al. [12] propose the mathematical-statistical GMM to create predictive (statistical) model.

The mandible, because of its characteristic shape, structure and geometry represents a real challenge for geometric modeling. The accuracy of the model geometry plays a crucial role in a variety of research and analysis. Stavness et al. [13] developed a biomechanical model of the human jaw and laryngeal structures. The process of creating the model geometry is realized on high resolution CT scans. Software application Rhino [14] is used to fit nurbs surfaces to the segmented data. Biomechanical models provide information on the model geometry and provide an important platform during the appliance and efficiency of the simulation of the human system. Therefore, correct and precise geometry of a model is necessary. Model of the human masticatory system which can be used to simulate the action of simple bites, is presented by Essen et al. [15]. The geometry of the skull and jaw model was created using high order cubic Hermite elements. The authors have chosen these items because such model: preserves continuity between the derivative element boundaries allowing for a mesh that accurately represents the geometry using a far smaller number of elements. The accuracy of the model geometry plays a crucial role, because of analyzes carried out on the model.

Mandible shape modeling using the second eigenfunction of the Laplace-Beltrami operator is presented by Seo et al. [16]. The method described in the previously mentioned paper is based on the centreline, anatomical landmarks to quantify mandibular shape. The centreline (passing through the middle of mandible), provides a framework for modeling and assessment of the development of the mandible jaw between the ages from birth to 20 years. This approach provides additional information about the anatomical marks and morphometric measurements, model or poor geometric accuracy.

Application of method of anatomical features (MAF) is initially used for the creation of geometrical models of the human long bones (polygonal, surface and solid) [6]. The main challenge for the author of this study was to change the MAF for creating geometric models and other types of bones.

MAF is based on referential geometrical entities (RGEs). RGEs (lines, planes, curves, points, axes) are defined on polygonal model. The process of creating RGEs for the creation of an appropriate geometric model of the bone is based on the anatomical and morphological characteristics of the bone. In this way, the estimated models maximally suits the real bone of the patient. A quality of the created geometric model allows to an easier and more precise preoperative preparation in the surgery, implementation of the implants, placement of the fixators etc.

The main aim of MAF application is to generate the 3D geometrical models of the bones (polygonal, surface and solid) and parametric models [17] (predictive bone models), with high geometrical accuracy and anatomical precision. Geometrically accurate and anatomically correct 3D model of the bones, created by using application of MAF, also allows the creation of the patient-adapted (personalized) bone fixators [18] and implants [19].

In this paper application of MAF for the creation of 3D surface model of human mandible is presented. The accuracy of obtained 3D surface was tested by two analysis: one for the surface deviations and the second one for the analysis of morphometric parameters. The results of analyses were satisfactory.

## Methods

In this research, geometrical analysis of the human mandible was based on input data by using CT scans. 64-slice – multislice CT (MSCT) scanner was used (Aquillion 64, Toshiba, Japan), and standard protocol for recording was applied: voltage of 120 kVp, tube current of 150 mA, rotation time of 0.5 s, and thickness of 0.5 mm. The

mandible samples came from Serbian adults males aged from 50–70 years. The raw data, coordinates of points of scanned tissue, were imported into the appropriate computer-aided design (CAD) software for reverse modeling. Reverse modeling of the human bone's geometry using CAD software to create 3D digital model of human bones was primarily based on radiological images (X-ray, CT, MRI). In this research, CATIA V5 R21 [20], CAD software were used.

The steps that used to create surface model of the mandible were described by Vitković et al. [7] and they were: creation of the anatomical model; preparatory processes (importing and sorting out the cloud of points, creating a polygonal model); definition of the RGE of the human mandible; definition of the anatomical points and creation of the spline curves; creation of the surface model of human mandible.

*Creation of the anatomical model*

The anatomical model was based on anatomical landmarks of the certain human bones. Anatomical landmarks were presented on anatomical pictures in anatomical atlases, but according to the complex morphology of the human bones, their best definition had to do experts, anatomists or anthropologists. Anatomical model of the bone "described" the relations between anatomical landmarks and their position on the polygonal model.

The mandible (lower jaw, lat. *mandibula*) [21–24], is the biggest and most massive face bone of the viscerocranium, connected with skull by temporomandibular joint. It participates in the construction of the temporomandibular joint by its condilar process. The temporomandibular joint is only movable joint of the head. The main parts of the mandible are the body and the ramus. Between these parts is a mandibular angle.

The body of the mandible (lat. *corpus mandibulae*) has a horseshoe shape and represents its horizontal part. Its upper part called alveolar part, made inferior dental arch. The body of the mandible has two sides (external and internal) and two margins, the upper one which matches the dental arc (lat. *arcus alveolaris*) and a lower edge or the basis of the mandible (lat. *basis mandibulae*).

The ramus of the mandible has approximately rectangular shape. It is located upward and backward in relation to the mandibular body forming an angle of 90°–140°, most commonly 120°–130°. It has two sides: external and internal, and four edges: upper, lower, anterior and posterior. The upper edge has two processes: anterior or coronoid (lat. *processus coronoideus*) and posterior or condilar (lat. *processus condylaris*). The latest one is composed of two parts: the upper one or head (lat. *caput mandible*) and the lower one or neck (lat. *collum mandible*). The head of the mandible is triangular in shape, flattened in the anteroposterior direction. The neck of the mandible represents the lower, narrow part of the condylar process. Anatomy of human mandible is presented in Figure 1.

*Preparatory processes*

Preparatory processes included: importing and sorting out the cloud of points and creating a polygonal model. After defining the anatomical model, creation of the basic model geometry was introduced. Preparatory processes were presented in the paper Vitković et al. [7] and included the following steps: CT scanning of the part human body (in this case the human mandible), preprocessing of raw data (scans), their transformation into STereoLithography (STL) format, importing the scanned models in STL format into CATIA application. At the end of the preparatory processes [6], the polygonal geometrical bone model was created.

*Definition of the RGE of human mandible*

The coordinate system was created on the polygonal model. Origin of the coordinate system was defined as the middle of the distance between the most lateral points on the right and left condyles. The constructed planes of the object coordinate system (OBC) of mandible were presented on Figure 2 and they were: medio-sagittal plane (MSP), horizontal plane (HP) and coronal plane (CP). MSP was constructed as the plane which contains the Origin of the OBC normal to bicondylar breadth (Con-ConD).

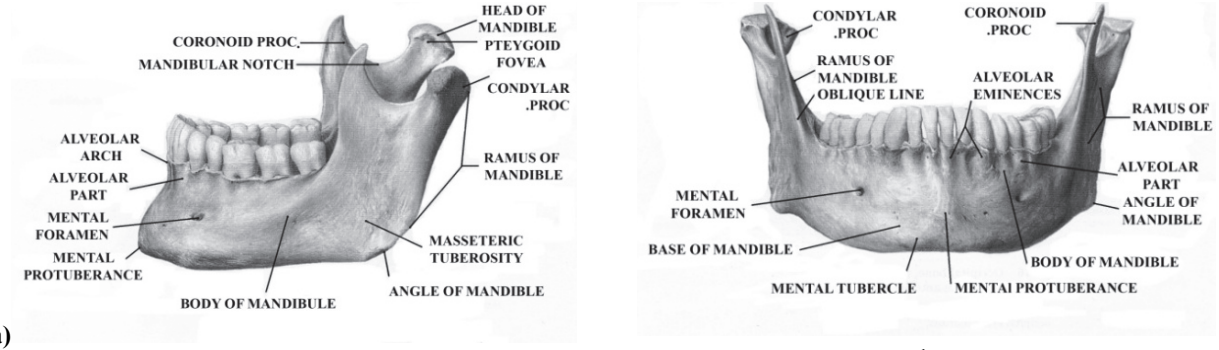


**Fig. 1 –The mandible: a) lateral aspect; b) anterior aspect [1].**

[1]Figure 1 was taken and modified from Atlas of Human Anatomy, Head, Neck, Upper-Limb, Sobbota, 1993.

**Table 1**

**Anatomical landmarks (points)**

| Anatomical landmarks | Definition |
|---|---|
| Mental foramen | One of two foramens located on the anterior surface of the mandibular body |
| Gnathion | The most inferior midline point on the inferior margin of the mandibular body |
| Gonion | The most inferior point on the mandibular angle (bilateral) |
| Condylion | The most prominent point on the condylar process (bilateral) |
| Mandibular notch point | The point in the middle part of the mandibular notchs |

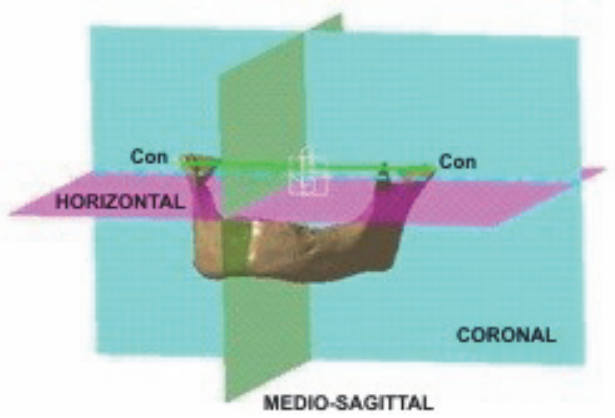


**Fig. 2 – Coordinate system defined on a human mandible polygonal model.**

MSP was a plane which separates human mandible on two halves – left and right. MS contained the gnathion (Gn) anatomical point (the most inferior midline point on the inferior margin of the mandibular body). HP was the plane normal to the MSP and it contained the gonion (the most inferior point on the mandibular angle) anatomical point. To be used as a plane of OBC, this plane was translated to the origin of OBC. CP was a plane which was normal to the HP and divided the mandible on two anatomical sections – anterior and posterior. It was placed at the Origin of OBC. X axis of the OBC was defined as normal to MSP. Y axis was defined as normal to AP plane. Z axis was normal to HP.

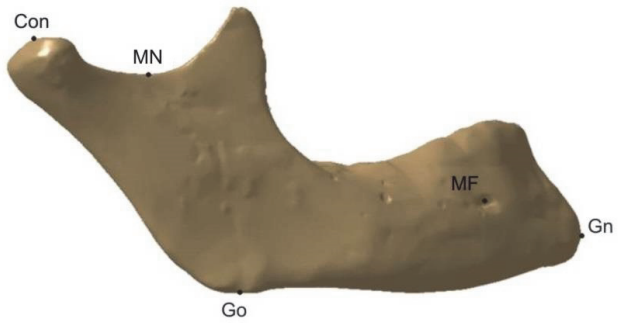


**Fig. 3 – Anatomical landmarks points.**

**Con – condylion; MN – mandibular notch point; MF – mental foramen; Go – fonion; Gn – gnathion.**

Characteristic bony landmarks on the human mandible were defined in the previous publication [25], and in this article they are presented in Table 1 and Figure 3. Definition of the mandibular anatomical landmarks was performed on the polygonal model. Points had to be defined separately for each of the human bone in relation to its anatomical and morphological characteristics. The mandibular notch point (MN) was added by the authors of this research, because it was necessary as a support point for the proper definition of coronoid process geometry.

*Definition of the anatomical points and spline curves*

Definition of the geometric entities was done on the polygonal model of the mandible. Geometrical entities, the B-spline curves, were defined following the bone geometry and its specific morphology, and in accordance to the anatomical bone model of the mandible. B-spline curves were created by the cross section of the adequate planes and polygonal models. The set of B-splines defined over the whole polygonal bone model has been called the skeleton model [7 *]. Definition of B-spline curves were performed on the body and the ramus of mandible. B-spline curves on a polygonal model of the mandible are shown on the Figure 4. The anatomical points on each B-spline were defined manually. B-spline curves were divided into several parts (each part defines the appropriate points).

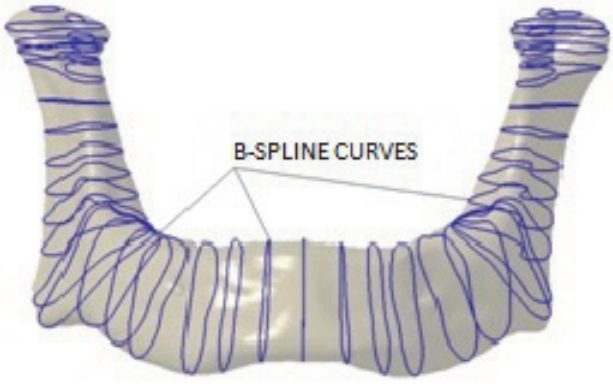


**Fig. 4 – B-spline curves on a polygonal model of the human mandible.**

*Definition of the surface model of human mandible*

Based on the axis of rotation (Z axis) and MSP, it is possible to define rotational planes, which are defined to form a certain angle with the basic plane of the intersection, passing through the axis of rotation. Based on the characte-

* Term *skeleton* has geometrical meaning not anatomical.

ristical anatomical landmarks of the mandible [25] and MSP (rotated to the right angle), sixteen planes were created. Sixteen planes of intersection were defined on the elements of the polygonal model which define some characteristic elements of the bone surface. These planes were used to create cross-sections. At the intersection of these planes with polygonal model, contour curves were generated. These curves were used to create points and spline curves (B-spline) (Figure 5a). Spline curves were used to create surface model of the mandibular body and the ramus (Figure 5b).

A similar procedure, with fourteen planes was used to create a 3D model of the mandibular ramus. Spline curves follow the shape of the ramus, and in that way the morphology (form or shape) of the bone is preserved. B-spline curves created on the ramus polygonal model are presented on the Figure 6a and b, showing that splines follow the external morphology of the bone.

3D surface model of human mandible was created by merging 3D surfaces of the mandible body and ramus. The solid model was obtained by filling the volume of the surface model in CATIA (Figure 7).

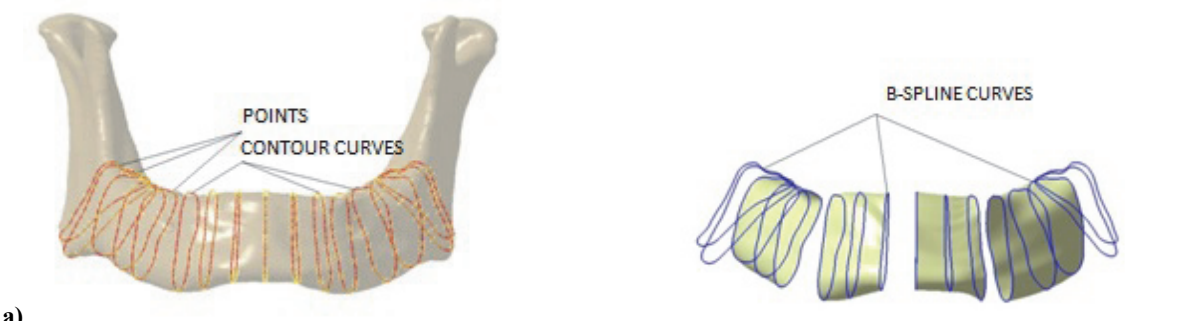


**Fig. 5 – a) Points and contour curves on the body of mandible; b) Creation of the 3D surface model.**

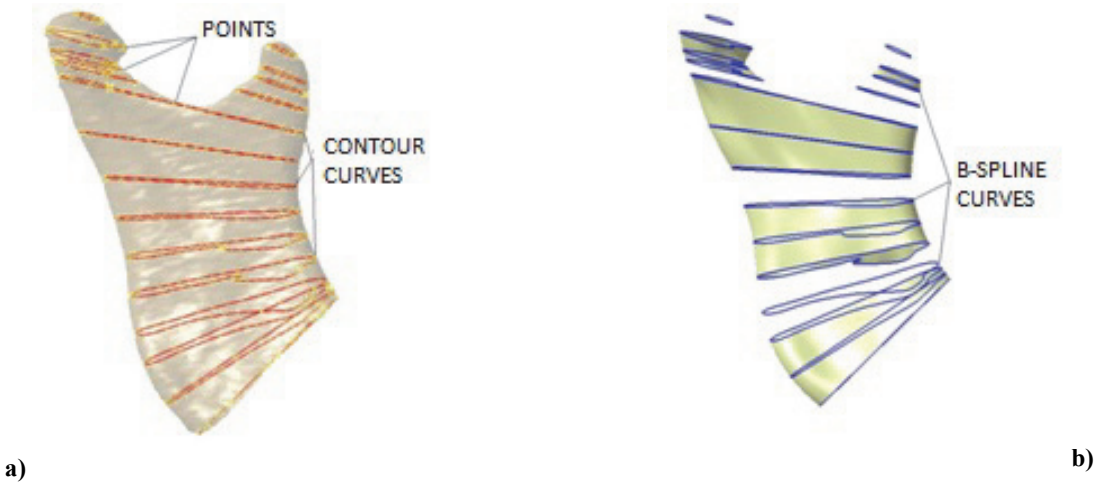


**Fig. 6 – a) Points and contour curves on the ramus of the mandible; b) Creation of the 3D .**

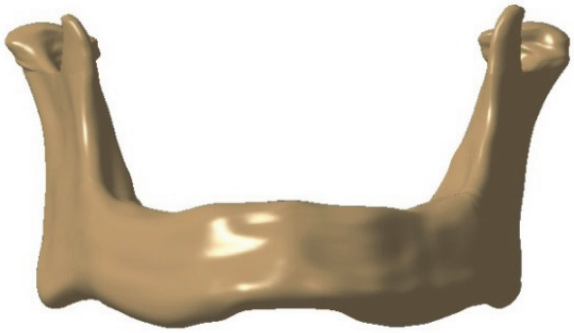

**Fig. 7 – 3D solid model of the human mandible.**

**Results**

Geometrical accuracy of the obtained surface model was tested by the application of the deviations analysis in CATIA software. Two analysis were performed, one for the surface deviations and the second one for the analysis of morphometric parameters.

*Maximum surface deviations*

Maximum surface deviations of the surface model of human mandible created from the input surface models of the original mandible specimens are presented in Figure 8. The surface models were created by using Quick Surface Reconstruction module (QSR) – automatic surface feature in CATIA software.

Deviation values are displayed in different colors. It can be noticed that deviation value was also below the recommended limit. Maximal deviation was 1.66 mm displaying the deviation of common region between two surfaces.

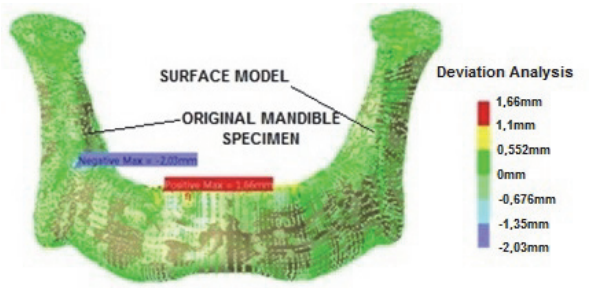


**Fig. 8 – Maximum deviations of the calculated surface model of the human mandible from the input human mandible models.**

*Analysis of morphometric parameters of the human mandible*

Data from the literature [25] (Figures 9–11), indicates that the configuration of the mandible can be accurately perceived by means of ten (10) basic central and bilateral morphometric parameters presented in Table 2 and Figure 12.

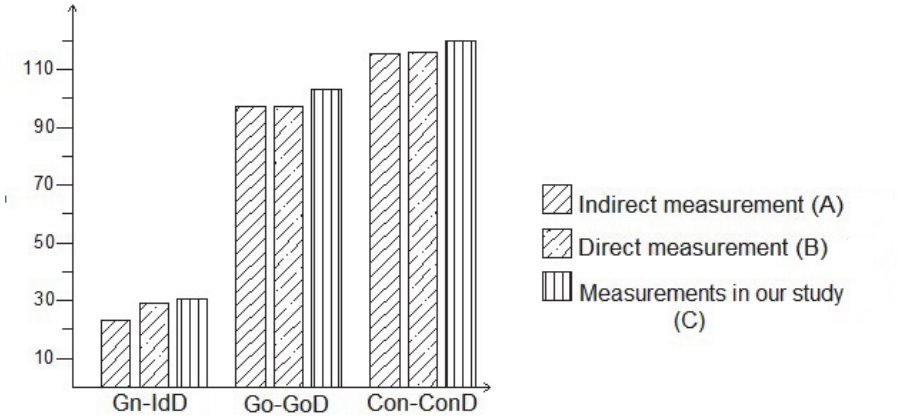


**Fig. 9 – Comparison of our results (C) with those from the study of Arsić et al. [25] (A, B). For abbreviations see in Table 2.**

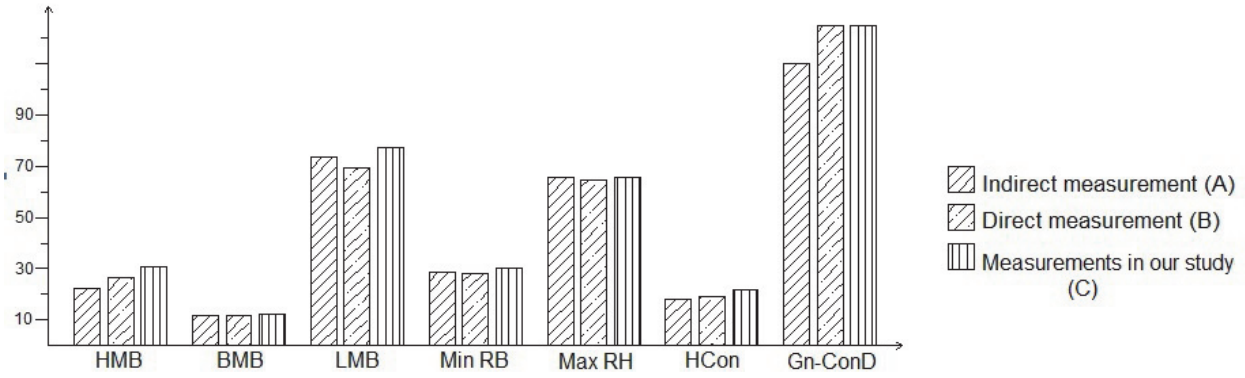


**Fig. 10 – Bilateral morphometric parameters measured on the right half of the mandible: comparison of our results (C) with those from the study of Arsić et al. [25] (A, B) For abbreviations see in Table 2.**

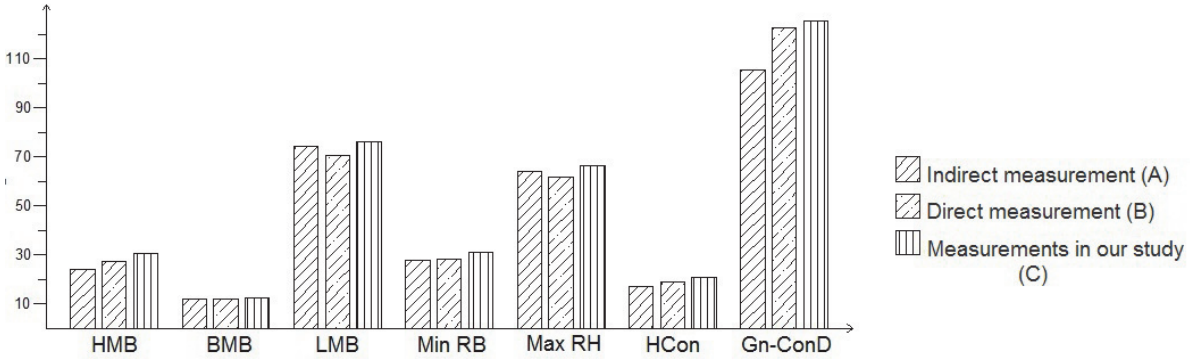


**Fig. 11 – Bilateral morphometric parameters measured on left half of the mandible: comparison of our results (C) with those from the study of Arsić et al. [25] (A, B)**
**For abbreviations see in Table 2.**

**Table 2**

**Central/midline morphometric parameters (MP)**

| MP | Definition |
|---|---|
| Gnathion-interdental distance (Gn-IdD) | Distance from the gnathion (Gn) to the alveolar septum between two incisors |
| Bigonial width (Go-GoD) | Direct distance between right and left gonion (Go) |
| Bicondylar distance (Con-ConD) | Direct distance between the most lateral points on the right and left condyles |
| Height of the mandibular body (HMB) | Distance from the alveolar border to the mandibular base at the level of the mental foramen (MF) |
| Breadth of the mandibular body (BMB) | Maximum breadth measured at the level of the mental foramen perpendiculary to the long axis of the mandible |
| Length of the mandibular body (LMB) | Distance between Go to Gn |
| Minimum ramus breadth (Min RB) | Minimum breadth of the mandibular ramus measured perpendiculary to the plane of the maximal height of the ramus |
| Maximum ramus height (Max RH) | Distance between the highest point on the mandibular condyle (condylion) (Con) to Go |
| Height of the condyle (HCon) | Distance between the Condylion (Con) and axis of the most inferior point of mandibular notch perpendiculary to Max RH |
| Gnathion-condylar distance (Gn-ConD) | Distance between Gnation (Gn) and Condylion (Con) |

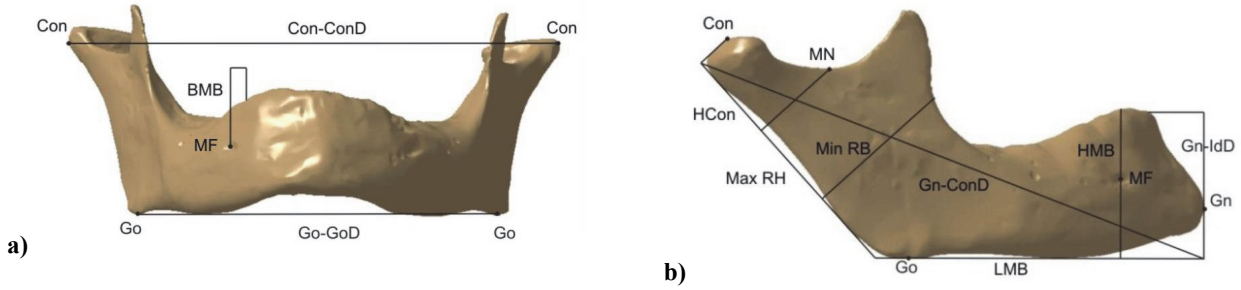


**Fig. 12 – Morfometric parameters and anatomical points presented on polygonal model of the human mandible: a) anterior view; b) lateral view.**
**For abbreviations see in Table 2.**

Position of anatomical point directly affects on values of morphometric parameters. Results of analysis for morphometric parameters are presented for ten (10) surface models created by MAF.

Table 3 shows the mean values of the central/middline morphometric parameters obtained through indirect measured and standard deviation.

Tables 4 show the average values and standard deviations of bilateral morphometric parameters measured on both sides. There was no statistically significant difference between the analyzed measurement values on the left and right sides.

**Table 3**

**Central/midline morphometric parameters (MP)**

| MP | Mean (mm) | SD (mm) |
|---|---|---|
| Gn-IdD | 30.462 | 0.48 |
| Go-GoD | 103.73 | 0.50 |
| Con-ConD | 126.509 | 0.54 |

**Note: MP are defined in Table 2.**
**SD-standard deviation.**

**Table 4**
**Values of the bilateral morphometric parameters (MP) measured on the left and right half of the mandible**

| MP | Left | Right |
|---|---|---|
| | mean ± SD (mm) | mean ± SD (mm) |
| HMB | 30.579 ± 0.45 | 30.648 ± 0.52 |
| BMB | 11.9 ± 0.17 | 12.0 ± 0.16 |
| LMB | 76.957 ± 0.75 | 77.028 ± 0.72 |
| Min RB | 30.937 ± 0.27 | 30.478 ± 0.26 |
| MaxRH | 66.524 ± 0.78 | 65.586 ± 0.59 |
| HCon | 20.974 ± 0.39 | 21.65 ± 0.40 |
| Gn-ConD | 125.653 ± 0.99 | 124.936 ± 1.21 |

**Note: MP are defined in Table 2.**
**SD - standard deviation.**

## Discussion

In the previous published paper [26] two approaches to obtain 3D geometric surface model of the human mandible were presented. The first one was the classical techniques of reverse engineering and the second one was based on the MAF. Maximal deviation value of the surface model created by MAF is 1.66 mm, 22.3% better than on the surface models created by classical techniques of reverse engineering (2.03 mm). This means that the quality of the resulting geometrical model of the mandible has a direct relationship to the precise identification of RGEs. Definition of RGE of the human mandible allows of the significant progress in reverse modeling and defining the precise geometry of the mandible.

The data from the litrerature [25] show that the configuration of the mandible can be accurately defined by means of the ten (10) basic central and bilateral mandibular parameters (Figure 9). Central and bilateral morphometric parameters were obtained by indirect measurement in this research.

In the previous study of Arsić et al. [25], the morphometric parameters were measured directly (using the callipers with precision of 0.05 mm) and indirectly (2D reconstructions of MSCT recording). Our research clearly shows that the indirect measurement of morphometric parameters on 10 mandible surface model, obtained on the basis of CT scan, gives valid results compared to the correspondent values obtained in the mentioned study. Comparison of the measurement results in our study with those in the study of Arsić et al. [25] is shown in Figures 1–3.

In the study of Čutović et al. [27], a radiographic cephalometry analysis of dimensions of condylar processus in persons with mandibular prognathism was done. Comparing the dimensions of condylar instalment in eugnathic people (people who, according to orthodontic current criteria, have a harmonious appearance of the face), has shown that there is a nonexistent statistical significant difference compared to our mean values for HCon (> 2.2 mm) and Max RH (> 1.574 mm). Three-dimensional analysis of the parameters is essential for: creating a statistical model of the mandible [28] and the analysis of craniofacial morphology [29]. Analyzing mean values of parameters Max RH, Gn-ConD and HCon from the above mentioned study [27], it was shown that they are larger (> 5 mm) than our correspondent mean values. Imprecision in the determination of the characteristic anatomical points, directly affects the values of morphometric parameters. For this reason, errors in the definition of the bone geometry, or measurement errors of the morphometric parameters should be kept to a minimum.

Numerous morphometric studies were performed on human mandible suggest their significant variability in relation to gender and ethnic affiliation of population [30–34]. Population shows many differences in the various details of the facial bone morphology [30]. These differences are easily noticeable when comparing individuals of different ethnic backgrounds. Chinese people have more dental protrusion, shorter midfacial length and steeper mandibular plane, compared to British Caucasian counterparts [31]. Huang et al. [32] compared Americans from Africa and Europe, a descent living in Birmingham, and demonstrated greater bidentoalveolar protrusion in the African American sample. Some studies show that differences also exist among populations of the white race [33, 34]. In the feature investigation we can precisely define morphometric parameters according to the anatomical variations, gender, age, ethnic origin and state of dentition, on the 3D model of the human mandible.

## Conclusion

MAF was initially applied for the development of the polygonal, surface and solid model of the human long bones. We presented that it is possible to apply the mention method for creating geometric models and other types of bones (in this case the human mandible). The accuracy of the obtained surface models was tested through the comparison with the original polygonal model geometry, obtained of the CT scans. Two analysis were performed, one for surface deviations and second for the analysis of the morphometric parameters. The created 3D geometrical model of the human mandible obtained by MAF has high geometrical accuracy and anatomical precision.

## Acknowledgement

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## R E F E R E N C E S


1. *Xia J, Ip HH, Samman N, Wang D, Kot CS, Yeung RW,* et al. Computer-assisted three- dimensional surgical planning, and simulation: 3D virtual osteotomy. Int J Oral Max Surg 2000; 29(1): 11–7.
2. *Weber GW*. Virtual Anthropology. Am J Phys Anthropol 2015; 156 Suppl 59: 22–42.
3. *Cohen A, Laviv A, Berman P, Nashef R, Abu-Tair J*. Mandibular reconstruction using stereolithographic 3-dimensional printing modeling technology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(5): 661–6.
4. *Aya M, Kubatb T, Delilbasic C, Ekicid B, Yuzbasioglue H. E, Hartomacioglub S*. 3D Bio- Cad modeling of human mandible and fabrication by rapid-prototyping technology. UUJMS 2013; 2(2): 135–45.
5. *Standring S*. Gray's anatomy, 38th ed. New York: Elsevier; 2005.
6. *Majstorović V, Trajanović M, Vitković N, Stojković M*. Reverse engineering of human bones by using method of anatomical features. CIRP Annals 2013; 62(1): 167–70.
7. *Vitković N, Milovanović J, Korunović N, Trajanović M, Stojković M, Mišić D,* et al. Software system for creation of human femur customized polygonal models. Comput Sci Inf Syst 2013; 10(3): 1473–97.
8. *Mišić D, Manić M, Vitković N, Korunović N*. Toward an integrated information system for the design, manufacturing and application of customized implants. Facta Univ Ser Mechan Engin 2015; 13(3): 307–23.
9. Mimics, software & services for biomedical engineering, [computer program], Available from: http://biomedical.materialise.com/mimics
10. 3D–Doctor, FDA 510K cleared, vector-based 3D imaging, modeling and measurement software, [computer program], Available from: http://www.ablesw.com/3d-doctor/
11. *Benazzi S, Stansfield E, Kullmer O, Fiorenza L, Gruppioni G*. Geometric Morphometric Methods for Bone Reconstruction: The Mandibular Condylar Process of Pico della Mirandola. Anat Rec (Hoboken) 2009; 292(8): 1088–97.
12. *Higgins P, Cobb S. N, Fitton L. C, Gröning F, Phillips R, Liu J,* et al. Combining geometric morphometrics and functional simulation: an emerging toolkit for virtual functional analyses. J Anat 2010; 218(1): 3–15.
13. *Stavness I, Hannam A. G, Lioyd J. E, Fels S*. An integrated dynamic jaw and laryngeal model constructed from CT data. Biomed Simulat 2006; 4072: 169–177.
14. Rhinoceros, design, model, present, analize, realize, [computer program]. Available from: https://www.rhino3d.com/resources/
15. *Essen NL, Anderson IA, Hunter PJ, Carman J, Clarke RD, Pullan AJ*. Anatomically based modeling of the human skull and jaw. Cells Tissues Organs 2005; 180(1): 44–53.
16. *Seo S, Chung MK, Whyms BJ, Vorperian HK*. Mandible shape modeling using the second eigenfunction of the Laplace-Beltrami operator, Proc. SPIE 7962, Medical Imaging 2011: Image Processing, 79620Z (11 March 2011). Available from: https://doi.org/10.1117/12.877537
17. *Vitković N, Mitić J, Manić M, Trajanović M, Husain K, Petrović S, Arsić S*. The Parametric Model of the Human Mandible Coronoid Process Created by Method of Anatomical Features. Comput Math Methods Med. 2015; 2015: 574132.
18. *Manić M, Stamenković Z, Vitković N, Stojković M, Trajanović M, Mitić J, et al.* Customized anatomically adjusted plate for fixation of mandible internal fractures. 2015 IEEE 15th International conference on bioinformatics & bioengineering (BIBE); 2015 November 2–4. Belgrade, University of Belgrade; 2015.
19. *Manić M, Stamenković Z, Mitković M, Stojković M, Shepherd D*. Design of 3D model of customized anatomically adjusted implants. Facta Univ Ser Mechan Eng 2015; 13(3): 269–82.
20. CATIA (Computer Aided Three-dimensional Interactive Application) [computer program] Dassault Systèmes, (France). Available from: http://www.3ds.com/products-services/catia/
21. *Mooore KL*. Clinically oriented anatomy. 3rd ed. Baltimore, MD: Williams & Wilikins;1992.
22. *Sokolović B*. Toothlessness: clinic, diagnosis, treatment. 1st ed. Niš: Prosveta;1997. (Serbian)
23. *Juodzbalys G, Wang HL, Sabalys G*. Anatomy of mandibular vital structures. Part I: mandibular canal and inferior alveolar neurovascular bundle in relation with dental implantology. J Oral Maxillofac Res 2010; 1(1): e2.
24. *Šurdilović S*. Maxillofacial surgery: A traumatology practicum. 1st ed. Niš: Prosveta; 2000. (Serbian)
25. *Arsić S, Perić P, Stojković M, Ilić D, Stojanović M, Ajduković Z,* et al. Comparative analysis of linear morphometric parameters of the humane mandibula obtained by direct and indirect measurement. Vojnosanit Pregl 2010; 67(10): 839–846. (Serbian)
26. *Mitić J, Vitković N, Manić M, Trajanović M, Radovanović Z*. Approaches to geometrical modeling of the human mandible. Educational scientific and professional meeting on measuring and quality control in productive mechanical engineering and environmental protection – ETIKUM 2015, June 19- June 20, 2015; Novi Sad, Srbija.
27. *Čutović T, Pavlović J, Kozomara R*. Radiographic cephalometry analysis of dimensions of condylar processus in persons with mandibular prognathism. Vojnosanit Pregl 2008; 65(7): 513–9. (Serbian)
28. *Park SH, Yu HS, Kim KD, Lee KJ, Baik HS*. A proposal for a new analysis of craniofacial morphology by 3-dimensional computed tomography. Am J Orthod Dentofacial Orthop 2006; 129(5): 600. e23–34.
29. *Kim S, Yi W, Hwang S, Choi S, Lee S, Heo M,* et al. Development of 3D statistical mandible models for cephalometric measurements. Imaging Sci Dent 2012; 42(3): 175–82.
30. *Dietrich M, Kedzior K*. Desing and manufacturing of the human bone endoprostheses using computer-aided system. J Theor App Mech 1999; 37(3): 481–503.
31. *Wu J, Hägg U, Rabie AB*. Chinese norms of McNamara's cephalometric analisis. Angle Orthod 2007; 77(1): 12–20.
32. *Huang WJ, Taylor RW, Dasanayak AP*. Determining cephalometric norms for Caucasians and African Americans in Birmingham. Angle Orthod 1998; 68(6): 503–12 ; discussion 512.
33. *Trenouth MJ, Davies PHJ, Johnson J S*. A statistical comparison of three sets of normative data from which to derive standards for craniofacial measurement. Eur J Orthod 1985; 7(3): 193–200.
34. *El-Batouti A, Bishara S, Oqaard B, Jakobsen J*. Dentofacial changes in Norwegian and Iowan populations between 6 and 18 years of age. Eur J Orthod 1995; 17(3): 241–9.


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# A profile of dementia patients in a Serbian sample – experience from the center for dementia and memory disorders

## Profil bolesnika sa demencijom na uzorku stanovništva Srbije – iskustvo Centra za demenciju i poremećaje pamćenja

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**Abstract**

**Background/Aim.** In accordance with modern trends of organizing specialized service dealing with dementia, the first memory clinic in Serbia – Center for memory disorders and dementia was established in 2008 in Belgrade at Neurology Clinic – Clinical Center of Serbia (CCS) as a university-affiliated outpatient clinic for subjects with cognitive impairment and dementia. The aim of this report was to outline the frequency of diagnosis, sociodemographic and medical characteristics of patients referring to the Center for memory disorders and dementia. **Methods.** The sample consisted of patients registered between 2008 and 2016 who underwent comprehensive and specialized diagnostic procedures in the Center. **Results.** A total of 3,873 visits were made for 2,198 patients, 39.6% of which proceed to annually follow-up visits. The majority of the sample (65.3%) was women. The mean age was 69.8 ± 12.1 years (range 29–89 years) and the average education level was 12.1 ± 3.3 years. Of this total number, at the moment of the first visit, 44.4% of the patients were fulfill criteria for Mild cognitive impairment (MCI), 28.2% had dementia due to Alzheimer's disease (AD), 7.8% had dementia secondary to a vascular pathology (VaD), 7.3% had frontotemporal dementia (FTD), 0.6% had dementia with Lewy bodies (DLB), and 1.7% had dementia due to Parkinson's disease (PDD). The mean Mini Mental test score in the whole sample was 22.6 ± 6.8 points. **Conclusion.** The data collected through the activity of the Center enabled an insight into the demographic and medical characteristics of patients, as well as planning further activities in the health care system. The systemic introduction of more standardized diagnostic practices, establishing and networking of similar centers will improve the accuracy and rate of dementia diagnosis in the Serbian population.

**Key words:**
**dementia; memory disorders; serbia; demography; neuropsychological tests; sensitivity and specificity.**

**Apstrakt**

**Uvod/Cilj.** U skladu sa modernim tendencijama u organizaciji službi specijalizovanih za tretman demencija, 2008. godine u Beogradu, na Klinici za neurologiju Kliničkog centra Srbije (KCS), osnovan je Centar za poremećaje pamćenja i demencije, prvi takve vrste u Srbiji, kao deo zdravstvenog sistema i sistema ustanova Univerziteta, specijalizovan za rad sa ambulantnim bolesnicima sa kognitivnim smetnjama i demencijom. Cilj ovog rada bio je prikaz učestalosti pojedinih dijagnostičkih kategorija, sociodemografskih i medicinskih karakteristika bolesnika upućenih u Centar za poremećaje pamćenja i demencije. **Metode.** Uzorak je uključio registrovane bolesnike na kojima je primenjena obuhvatna i specijalizovana medicinska dijagnostika u Centru, u periodu od 2008. do 2016. godine. **Rezultati.** Ukupno je ostvareno 3 873 poseta koje su obuhvatile 2 198 bolesnika, od kojih je 39,6% nastavilo godišnje praćenje u Centru. Većinu uzorka (65,3%) činile su žene. Prosečna starost ispitanika bila je 69,8 ± 12,1 godinu (29–89 godina), a prosek godina obrazovanja iznosio je 12,1 ± 3,3. Od ukupnog broja bolesnika, u trenutku prve posete Centru, 44,4% ispunjavalo je kriterijume za postavljenje dijagnoze – Blagi kognitivni poremećaj (BKO), 28,2% za dijagnozu demencije u sklopu Alchajmerove bolesti (AB), 7,8% za demenciju u sklopu vaskularne patologije mozga (VaD), 7,3% za frontotemporalnu demenciju (FTD), 0,6% za demenciju sa Levijevim telima (DLT), dok su 1,7% bili bolesnici sa dijagnozom demencije u Parkinsonovoj bolesti (PBD). Prosečan skor na Mini Mental testu, na nivou celokupnog uzorka, iznosio je 22,6 ± 6,8. **Zaključak.** Podaci prikupljeni tokom aktivnosti Centra omogućili su uvid u demografske i medicinske karakteristike bolesnika, kao i planiranje aktivnosti zdravstvenog sistema. Sistema-

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tično uvođenje standardizovanih djagnostičkih procedura, uspostavljanje i umrežavanje sličnih centara će unaprediti tačnost, ali i broj postavljenih dijagnoza u srpskoj populaciji.

**Ključne reči:**
**demencija; pamćenje, poremećaji; srbija; demografija; testovi, neuropsihološki; osetljivost i specifičnost.**

## Introduction

With the aging of the population, dementia is becoming a growing health problem. Inspired by philosophy and practice of the psychogeriatric movement which transformed mental health services for older people in the UK from the late 1960s [1–3] the first memory clinics were described in the 1980s [4]. Recognizing the need for a multidisciplinary approach to a patient with cognitive impairments, in order to provide adequate care and reduce suffering in both patients and caregivers with minimal recourse to mental hospital care, in recent decades there has been a significant increase in the number of memory clinics all over the world [5–24]. They provide early diagnostic assessment, treatment, and follow up of patients with cognitive symptoms and possible dementia in an outpatient setting. But, not all complaints about memory are caused by dementia [25]. Some of them present mild cognitive impairment and/or other symptoms not specific for Alzheimer's disease (AD), and may occur in many other conditions, including potentially reversible conditions. Therefore, and also because of an increasing number of patients, there is a need to create a register of patients covered by the work of the memory clinics.

Accessible, reliable, recent and relevant data are necessary to facilitate prevention, early detection, diagnosis and treatment of dementia. The dementia registries are developing in order to improve the quality of diagnostic work-up, treatment and care of patients with dementia disorders. Data obtained in some countries cannot easily be generalized to other countries. Because local environmental conditions and genetic make-up may be different, prevalence and/or incidence rates reported from the most famous studies in the United Kingdom [25], Sweden [26], Denmark [27] and Spain [27–29] cannot be extrapolated to other countries even in the same region [30].

In accordance with modern trends of organizing specialized services dealing with this complex issue, the first memory clinic in Serbia – Center for memory disorders and dementia was established in 2008 in Belgrade at the Neurology Clinic, Clinical Center of Serbia (CCS) as a university-affiliated outpatient clinic for subjects with cognitive impairment, aimed to improve practice in the identification, investigation, and treatment of memory and other cognitive disorders, including dementia in Serbian patients.

Regarding that the Center covers the majority of Serbian patients, its activities also include, working on constitution of the Serbian Dementia Registry – a population-based epidemiological study that registers all cases of dementia in the Serbian population.

The aim of this study was to report on the frequency of diagnosis, sociodemographic and medical characteristics of the patients referred to the Serbian Center for memory disorders and dementia.

## Methods

The survey was conducted at the Center for dementia and memory disorders at the Neurology Clinic – CCS and included all consecutive patients between March 2008 and December 2016. The local Ethics Committee approved this study. Patients and their relatives were informed of the entry into the Center and had a possibility to decline participation and to have their data removed at any time. Data were de-identified before analysis. Medical and administrative data of outpatients and day clinic patients visiting the Center are routinely recorded by the Center's staff.

The Center contains information on patient demographics, principle and secondary diagnoses, and other admission and discharge data. The principle and secondary diagnoses are determined and coded using the ninth revision of the International Classification of Diseases – Clinical Modification (ICD-9-CM) [30].

### *Subjects and procedures*

Diagnosis of dementia, and its subtypes, was made at a multidisciplinary consensus meeting based on internationally accepted criteria [30–36]. All patients were registered by a neurologist with one of 8 diagnostic category: dementia caused by AD, mixed dementia with AD-vascular dementia – it will be further referred to as Mixed dementia (MD), vascular dementia (VaD), dementia with Lewy bodies (DLB), frontotemporal dementia (FTD), Parkinson's disease with dementia (PDD), unspecified dementia (UD), and other diagnoses (Other). At the first visit, information about their age, gender, education, living condition and quality of self-care and activity of daily living was registered. Global cognitive status was assessed by the Mini-Mental State Examination (MMSE) [36] and its score was recorded. Medical history was obtained *via* self-report and/or family member-report (substantiated through medical records). The presence of risk behavior such as smoking, alcohol abuse, and vascular risk factors such as arterial hypertension (HTN), diabetes mellitus (DM), dyslipidemia and thyroid gland dysfunction was also noted. Head injury with loss of consciousness and, eventually, depression or psychoses symptoms were registered.

All patients received a comprehensive assessment comprised of a standardized diagnostic work-up including neurological examination and several blood tests: complete blood count (CBC), comprehensive metabolic panel (CMP), lipid panel (LP), thyroid gland function tests, vitamin B12 level and a venereal disease reserved laboratory (VDRL) test. All subjects underwent an extensive assessment of cognitive functions which results were presented in the paper and in electronic form. In the Center's clinical practice

neuropsychological evaluation lasts around 1.5 to 2 hours and entails the application of tests which can roughly be divided into two groups – tests intended for general examination of cognition and tests created for assessment individual domains of cognitive functions such as: attention, memory, fluency/executive functions, language, visual and spatial abilities, also known as *domain oriented tests*. In the first group are: Mini-Mental Status Exam (MMSE), Addenbrooke's Cognitive Examination – Revised (ACE-R) [37], Matiss – Dementia Rating Scale (DRS) [38] and Clock Drawing Test (CDT) [39]. The second group includes the following tests: Rey Auditory Verbal Learning Test (RAVLT) [40], Free and Cued Selective Reminding Test (FCSRT) [41, 42], Verbal Fluency – Semantic and Phonemic fluency (SF and FF) [41], Boston Naming Test (BNT) [41–44]. All tests were conducted by a qualified neuropsychologist in a standardized manner consistent across subjects. Applying of the test was adjusted to the overall cognitive ability (MMSE higher than 15), physical ability (lack of visual and/or hearing disability, paresis and/or behavioral difficulties). For the assessment of functional impairment in activities of daily living, we used the Activity of daily living - International Scale (ADL-IS) applied by the Centers' nurse [45]. Patients with young onset dementia (YOD), MCI and dementia diagnosis that were able to undergo neuropsychological assessment were included in an annual follow-up.

Further, patients received additional diagnostic procedures such as ultrasonographic examination of the carotid arteries and computed tomography (CT). Depending on the indication (YOD, differential-diagnosis) approximately 66% of patients underwent a magnetic resonance imaging (MRI) scan, and 38% positron emission tomography (PET) scan, biomarkers and specialized laboratory and genetic analyses. The data on follow-up visits were registered as well.

*Statistics*

Data are expressed as means (M) ± standard deviation (SD) for the continuous variables, and as percentage for the categorical variables. Analysis of variance and *t*-test were utilized to examine group differences in demographic, clinical, and neuropsychological characteristics, and $\chi^2$ test was applied to sets of categorical data. A two-sided $p$ value < 0.05 was considered statistically significant. Data were analyzed using the SPSS 20.0 statistical package (SPSS Inc, Chicago, Illinois, USA).

## Results

*Patients characteristics*

A total of 2,198 patients carried out 3,873 visits between 2008 and 2016 during annual visits which ranged between one to seven visits (Table 1).

The largest number of visits was made by the patients with a diagnosis of MCI (44.9%) and AD (30.8%), while the least common were patients with diagnosis DLB (0.6%) (Table 1 and Figure 1)

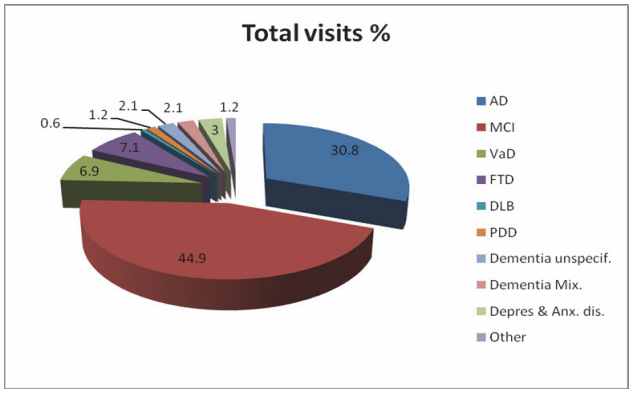


**Fig. 1 – Number of visits according to diagnosis.**
**AD – Alzheimer's dementia; MCI – Mild cognitive impairment; VaD – Vascular dementia; FTD – Frontotemporal dementia; DLB – Dementia with Lewy bodies; PDD – Dementia in Parkinson's disease; Dementia unspecif. – Dementia unspecified; Dementia Mix. – Dementia mixed, Depres & Anx.dis. – Depressive and anxiety disorders.**

At the first visit the majority of participants were female (65.3%) (Figure 2), the average age of the sample was 69.8 ± 12.1 years, male patients were significantly older (70.9 ± 9.4 years) ($t$ = 3.091, $p$ = 0.002), and the average educational level was 12.1 ± 3.3 years.

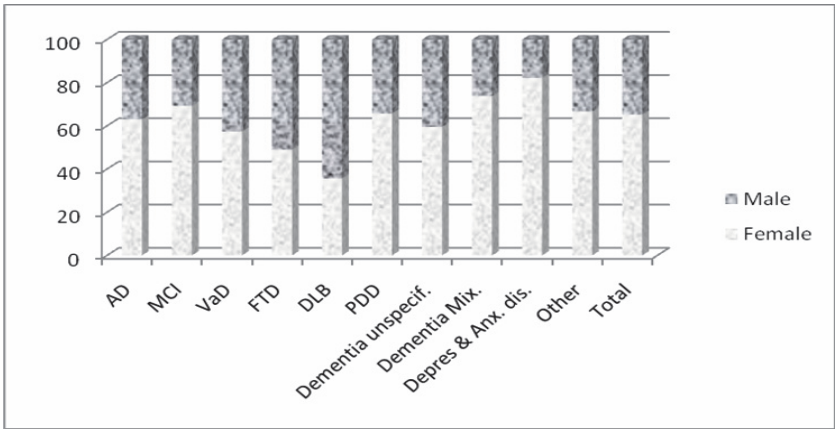


**Fig. 2 – Gender distributions according to diagnosis**
**For abbreviations see under Figure 1.**

**Table 1**

**Frequency of diagnosis after visits to the Center**

| Number of visits | AD | MCI | VaD | FTD | DLB | PDD | Demen. Unspec. | Mix.D | Depres & Anx. dis. | Other | Total n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 619 (100) | 975 (100) | 171 (100) | 161 (100) | 14 (100) | 38 (100) | 52 (100) | 42 (100) | 84 (100) | 42 (100) | 2198 (100) |
| II | 296 (47.8) | 393 (40.4) | 57 (33.3) | 57 (35.4) | 5 (35.7) | 5 (13.2) | 12 (23.1) | 18 (42.9) | 21 (25.0) | 6 (14.3) | 870 (39.6) |
| III | 142 (23.0) | 199 (20.4) | 17 (9.9) | 24 (14.9) | 3 (21.4) | 3 (8.0) | 10 (19.2) | 16 (38.1) | 6 (7.1) | - | 420 (19.1) |
| IV | 79 (12.8) | 100 (10.3) | 14 (8.2) | 19 (11.8) | 1 (7.1) | 2 (5.3) | 4 (7.7) | 6 (14.3) | 4 (4.8) | - | 229 (10.4) |
| V | 35 (5.7) | 54 (5.5) | 10 (5.8) | 9 (5.6) | - | - | 2 (3.8) | - | - | - | 110 (5.0) |
| VI | 17 (2.7) | 17 (1.7) | - | 5 (3.1) | - | - | 1 (1.9) | - | - | - | 40 (1.8) |
| VII | 4 (0.6) | 2 (0.2) | - | - | - | - | - | - | - | - | 6 (0.3) |
| Total | 1192 (30.8) | 1740 (44.9) | 269 (6.9) | 275 (7.1) | 23 (0.6) | 48 (1.2) | 81(2.1) | 82 (2.1) | 115 (3.0) | 48 (1.2) | 3873 (100) |

**Results are given as number (percentage) of patients.**
**For abbreviations see under Figure 1.**

**Table 2**

**Baseline characteristics of the patients**

| Parameters | AD | MCI | VaD | FTD | DLB | PDD | Dementia Unspecif. | Dement. Mix. | Depres & Anx. dis. | Other | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All subjects, n (%) | 619 (28.2) | 975 (44.4) | 171 (7.8) | 161 (7.3) | 14 (0.6) | 38 (1.7) | 52 (2.4) | 42 (1.9) | 84 (3.8) | 42 (1.9) | 2198 (100) |
| Female, n (%) | 390 (63.0) | 677 (69.4) | 98 (57.4) | 79 (49.0) | 5 (35.7) | 25 (65.8) | 31 (59.6) | 31 (73.8) | 69 (82.2) | 31 (73.8) | 1436 (65.3)* |
| Male, n (%) | 229 (37.0) | 298 (30.6) | 73 (42.6) | 82 (51.0) | 9 (64.3) | 13 (34.2) | 21 (40.4) | 11 (26.2) | 15 (17.8) | 11 (26.2) | 762 (34.8) |
| Age (years), mean ± SD (range) | 72.8 ± 8.2 (48–89) | 68.4 ± 9.7 (29–89) | 71.1 ± 9.9 (30–87) | 65.7 ± 10.2 (44–86) | 76.5 ± 7.6 (57–88) | 72.3 ± 7.1 (55–84) | 72.4 ± 9.9 (44–85) | 74.4 ± 5.6 (61–89) | 64.0 ± 8.7 (37–81) | 64.8 ± 10.2 (43–81) | 69.8 ± 12.1** (29–89) |
| Education (years), mean ± SD | 11.6 ± 3.6 | 12.8 ± 3.1 | 10.8 ± 3.2 | 12.1 ± 3.2 | 12.0 ± 3.7 | 12.1 ± 2.8 | 11.1 ± 3.3 | 11.8 ± 2.9 | 11.3 ± 3.0 | 12.8 ± 2.2 | 12.1 ± 3.3** |
| MMSE, range | 0–30 | 3–30 | 0–30 | 1–29 | 11–27 | 9–29 | 0–30 | 8–26 | 16–30 | 14–30 | 0–30 |
| Duration of disease (years), mean ± SD (range) | 3.0 ± 2.0 (0–13) | 2.4 ± 2.2 (0–12) | 2.6 ± 2.6 (0–14) | 3.3 ± 2.6 (0–11) | 2.0 ± 1.6 (0–6) | 3.7 ± 3.2 (0–10) | 2.7 ± 2.4 (0–13) | 2.7 ± 2.1 (0–10) | 2.6 ± 2.9 (0–16) | 3.9 ± 4.6 (0–15) | 2.7 ± 2.3** (0–16) |

**MMSE – mini-mental state examination.**
**For other abbreviations see under Figure 1.**
***p* < 0.05; ** *p* < 0.01.**

**Table 3**

**Applied cognitive tests**

| Tests | AD | MCI | VaD | FTD | DLB | PDD | Dement. Unspecif. | Dementia Mix. | Depres & Anx. disease | Other | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of patients | 619 | 975 | 171 | 161 | 14 | 38 | 52 | 42 | 84 | 42 | 2198 |
| MMSE, n (%) | 595 (96.1) | 944 (96.8) | 160 (93.6) | 156 (96.9) | 14 (100.0) | 33 (86.8) | 48 (92.3) | 42 (100) | 78 (92.9) | 39 (92.9) | 2109 (96.0) |
| CDT, n (%) | 467 (75.5) | 804 (82.5) | 123 (71.9) | 123 (76.4) | 14 (100.0) | 23 (60.5) | 35 (67.3) | 37 (88.1) | 67 (79.8) | 25 (59.5) | 1718 (78.2) |
| RAVLT, n (%) | 358 (57.8) | 768 (78.8) | 103 (60.2) | 104 (64.6) | 9 (64.3) | 30 (78.9) | 20 (38.5) | 24 (57.1) | 58 (69.0) | 25 (59.5) | 1499 (68.2) |
| FCSRT, n (%) | 231(37.3) | 653 (67.0) | 67 (39.2) | 66 (41.0) | 9 (64.3) | 14 (36.8) | 15 (29.8) | 14 (33.3) | 54 (64.3) | 6 (13.3) | 1129 (51.4) |
| Fluency tests, n (%) | 410 (66.2) | 791 (81.1) | 114 (66.7) | 109 (67.7) | 12 (85.7) | 32 (84.2) | 25 (48.1) | 26 (61.9) | 60 (71.4) | 25 (59.5) | 1604 (73.0) |
| BNT, n (%) | 292 (47.2) | 631 (64.7) | 93 (54.4) | 85 (52.8) | 8 (57.1) | 25 (65.8) | 19 (36.5) | 23 (54.8) | 48 (57.1) | 20 (47.6) | 1244 (56.6) |
| MATIS, n (%) | 344 (55.6) | 707 (72.5) | 104 (60.8) | 90 (55.9) | 9 (64.3) | 27 (71.1) | 21 (40.4) | 23 (54.8) | 55 (65.5) | 25 (59.5) | 1405 (63.9) |
| ACE - R, n (%) | 329 (53.2) | 654 (67.1) | 76 (44.4) | 109 (67.7) | 9 (64.3) | 16 (42.1) | 22 (42.3) | 26 (61.9) | 52 (61.9) | 20 (47.6) | 1313 (59.7) |

**AD – Alzheimer's dementia; MCI – Mild cognitive impairment; VaD – Vascular dementia; FTD – Frontotemporal dementia; DLB – Dementia with Lewy bodies; PDD – Dementia in Parkinson's disease; Dementia unspecif. – Dementia unspecified; Dementia Mix. – Dementia mixed, Depres & Anx.dis. – Depressive and anxie ty disorders; MMSE – Mini Mental State Examination; CDT – Clock Drawing Test; RAVLT – Ray Auditory Learning Test; FCSRT – Free and Cued Selective Reminding Test; BNT – Boston Naming Test; ACE -R – Addenbrooke's Cognitive Examination-Revised.**

Average MMSE score was 22.6 ± 6.8, and average duration of disease was 2.7 ± 2.3 years. Around 73.9% of the patients lived in their own home and 59.3% were independent in activity of daily living. The details of the sample at the baseline visit are shown in Tables 2 and 3.

Analysis of variance (ANOVA) was utilized to examine for group differences between different diagnostic groups of age, educational level, MMSE score, duration of disease and duration of HTN; a *t*-test was used to examine for group differences in ages between male and female; a $\chi^2$ test was utilized to examine for group differences in gender and others characteristic (demographic, vascular risk factors and, results of blood tests, performed diagnostic procedures and type of therapy), Tables 3 and 4. Group differences were observed for ages [F (9, 2188) = 12.496; $p = 0.000$], with participants with DLB being the oldest and those who had affective disorder diagnosis (Anxiety & Depression disorder group) being the youngest. Group differences were observed for gender [$\chi^2$ (9) = 26.643; $p = 0.002$]. Namely, in all groups female were more frequent, except in the DLB and the FTD groups. Significant, multiple differences emerged, also in education [F (9, 2188) = 7.983; $p = 0.000$], with the VaD patients being the lowest educated and the MCI ones the highest educated. Multiple group difference was emerged for the MMSE group score [F (9, 2188) = 191.223; $p = 0.000$] with the lowest scores in the group Dementia Unspecified, and the highest in the MCI group. Group differences were observed in the duration of disease [F (9,2188) = 2.184; $p = 0.022$], with patients in the category Other having a diagnosis for the longest period of time, and the DLB the shortest.

Group differences were observed also in: living in their own home [$\chi^2(9) = 9.976$; $p = 0.004$] with difference between the MCI group in comparison to all other subgroups; independence in ADL [$\chi^2(9) = 38.236$; $p = 0.004$] with a difference between patients in the group MCI, the Anxiety & Depression disorders group and the group Other on the one side and other subgroups on the other side; existence of HTN [$\chi^2(9) = 27.438$; $p = 0.037$], with the largest number of the patients with HTN among the VaD and the Mixed Dementia groups compared to other groups; duration of HTN [F (9, 2188) = 2.224; $p = 0.018$] which was the longest in patients with DLB compared to all other subgroups; confirmed CVI [$\chi^2(9) = 84.536$; $p = 0.000$] – the majority was in the subgroup VaD and the Mixed Dementia; CT confirmed vascular lesions [$\chi^2(9) = 38.255$; $p = 0.000$] – the majority was in the subgroup VaD and the Mixed Dementia; brain atrophy [ $\chi^2(9) = 25.997$; $p = 0.002$] with the lowest number in the subgroup Other comparing to the others subgroups; vitamin B12 deficit [$\chi^2(9) = 22.125$; $p = 0.004$] which was significantly the most frequent in the AD patients, FTD and VaD compared to all the other; carotid stenosis on the right [$\chi^2(9) = 31.061$; $p = 0.000$] and on the left [$\chi^2(9) = 43.984$; $p = .000$] which was more often in patients in the VaD, mixed dementia, PDD and AD subgroups compared to other subgroups; LP performed [$\chi^2(9) = 43.147$; $p = 0.000$] which was mostly performed in patients with diagnosis FTD, AD and Unspecified Dementia contrary to the patients with Mixed Dementia, MCI and Other. Finally, group differences were emerged in dementia medication [$\chi^2(9) = 72.975$; $p = 0.000$] with main difference between the FTD, Dementia Unspecified, and AD subgroups contrary to the other subgroups where patients usually did not take drugs for dementia; at the end, group differences were also observed in neuroleptic medication [$\chi^2(9) = 54.111$; $p = 0.000$] – this kind of medication was more often taken by patients with FTD diagnosis in comparison to all the other.

No significant difference emerged in positive hereditary [$\chi^2(18) = 24.69$; $p = 0.134$], head injury [$\chi^2(18) = 17.01$; $p = 0.522$], diabetes mellitus [$\chi^2(18) = 26.05$; $p = 0.099$]; smoking [$\chi^2(18) = 17.01$; $p = 0.522$], slcohol abuse [$\chi^2(9) = 50.51$; $p = 0.757$]; presence of thyroidal disorders [$\chi^2(18) = 17.57$; $p = 0.484$], and VDRL positive blood test [$\chi^2(9) = 32.925$; $p = 0.438$].

The details on the number of performed neuropsychological tests in the baseline across different diagnosis are shown in Table 3.

## Discussion

The main objectives of the Center's practice are to make early diagnosis and treatment; to identify and treat disorders other then dementia that might contribute to patients' problems; to evaluate new therapeutic agents in the treatment of dementia; to reassure people who are worried that they might be losing their memory, when no real deficit is found [4].

Following these principles in every day work during an 8 year period, approximately 4,000 examinations have been conducted on over 2,000 subjects, all being backed up by the most modern diagnostic procedures that are recommended by expert groups, national and international professional associations. Even though primarily profiled for the diagnosis and treatment of dementia, among the professional and general public, the Center is also recognized as a reference institution for the creation of standards and normative criteria on a national but also regional level. In that sense, an important aspect of the Center's activities is the work involving the formation of normative values for neuropsychological tests that are obtained from the results of healthy subjects, and considering the fact that a national dementia registry is not available in Serbia, as well as evidence on morbidity and mortality risks related to dementia in the Serbian population, work on forming its constitution is of utter importance.

Taking into consideration the specificity of an illness such as dementia, the activity of the Center involves the support and advice of caregivers and patients, as well as the expert education provided by professionals that are hired to work with this patient population. Realizing these aims by obeying the principles of good clinical practice, we believe that the Center has given meaning to the reasons for its existance.

All patients complained about memory dysfunction and/or behavioral disturbances and were referred by a general practitioner (51.0%), a neurologist/neuropsychiatrist (30.5%), or a psychiatrist (18.5%) from primary, secondary or tertiary health care. The largest number of patients, (approximately 60%), after performed indicated diagnostic

procedures in the first visit were returned to the doctor or specialist who initially sent them to the Center. Therefore, the majority of the patients that were sent to the Center, already after their first visit, received an adequate answer regarding the problems because of which they were sent to the Center, and thus considering this aspect, the Center justifies the criteria of the tertiary level of healthcare within the scope of the health care system of the Republic of Serbia.

After the baseline assessments almost 40.0% of all patients proceed to annually follow-up visits when all indicative medical procedures are repeated (i.e. a neurological examination, general questionnaire, comprehensive neuropsychological battery with MMSE, blood tests). Significant majority of those patients were patients with diagnosis MCI (40.4%) and AD (47.8%), which is a trend continuing through all annual visits, meaning that these patients were most commonly seen in the Center. Comparing these two subgroups it is notable that the frequency of patients with AD was growing while MCI was decreasing, during the follow-up period, which is expected regarding the progression of the disease, mortality and comorbidity.

Due to cognitive problems, a significant majority of patients that seek help were women (almost 2/3 of the entire sample), in their late seventies when their difficulties were also objectively verified (MMSE = 22.6). Besides, the greater majority of patients in the group that suffer from AD were women, but there were less women patients that suffer from DLB and this is in accordance with the data from other studies [46, 47]. However, our female patients were younger on average than male patients, contrary to explanations that there are more women who suffer from dementia due to a longer life span [48]. Up to this day, the majority of studies on this topic involved investigation of the risk factors for the occurrence of dementia connected to aging. A longer life span of women does not fully explain their greater majority among those suffering from AD, but it does raise the total prevalence of all types of dementia in women in the group of the oldest subjects [47]. Our sample was for in the most part heterogeneous in terms of age, i.e. it included a relatively wide array of ages so it would be useful to examine the connection between dementia and gender with this sample which is stratified by our patients' years.

Women also made up the majority within the MCI diagnostic category which would, when taking this stage into consideration, explain the assumption of the greater sensitivity of this population category on cognitive changes and their readiness to seek help earlier, but also it would explain the traditionally greater pressure of different roles which continues even after women go into their retirement years in Serbia. Also, the greater eagerness to seek help in the MCI group could be explained by the patients' younger ages and their greater educational level as well, i.e. the patients were generally better informed and this difference was determined among the patients of this group in comparison to those in the other groups. During first contact, the subjects with the MCI diagnosis, on the cognitive screening level, showed average results which were within physiological limits (MMSE: 26.8 ± 3.2 and TCS: 4.1 ± 1.3). These were individuals who most often did not gravitate towards risk behavior (smoking, alcohol), and the majority of patients' reasons for coming to the Center very rarely had anything to do with them being related to individuals suffering from dementia. On the other hand, a great number of patients from the MCI group had verified reductive changes on the brain which was seen through their CT scan. More than half of the subjects had registered HTN and also a similar percentage of patients had bilateral carotid stenosis which was registered through an ultrasound, and there was a smaller number of patients that suffer from vascular lesions and CVI. Our data are in accordance with the results by Camarda et al. [48] which confirmed the presence of atrophic and vascular changes on the brain in patients with MCI and thus this gives great importance to conducting check-ups for cardiovascular risk factors in the prevention of dementia, which the Center also greatly insists on. Before coming to the Center the subjects from this group had very rarely undergone medicament therapy, and if they had, they had mainly taken antidepressants. This is in accordance with the information from the literature stating that depression is 2.6 times more present in individuals with MCI in comparison to the healthy population [49, 50].

The group of patients with AD diagnosis was the next group in line in terms of occurrence in the Center (28.2%). This category contained up to 211 (22.6%) patients with the onset of the illness before the age of 65 – YOD, but in spite of this the patients were on average older than the MCI subjects, the subject from the group with the affective disorder diagnosis and the Other heterogeneous group, as well as the FTD group [51, 52]. On the other hand, the patients suffering from AD were significantly better educated than the subjects with VaD (within the scope of three year high school education) but also had the widest array in terms of educational range – from practically illiterate subjects to members from institutions of academic education. Although, according to the opinions of caregivers who often accompanied patients before their arrival to the Center, their illness lasted for a relatively short time (three years on average), the result from the screening test in the initial visit showed very extreme cognitive deterioration (MMSE =16.2). This data suggests that, unfortunately, there is a high level of unknowingness and prejudices connected to what is conventionally considered normal aging.

Different European health care systems have different structures and referral pathways but all seem problematic for dementia care [53]. According to the recently published data, there is a robust perception that AD is underdiagnosed and undertreated throughout Europe due to mistaken, absent and delayed diagnosis [54]. This is in line with data from primary care setting and population based epidemiological studies showing that almost one half of dementia patients remain undiagnosed in the community [55, 59]. Stigma has an strong influence on delays in recognition and diagnosis in primary care and exists among all European countries, it is associated with reluctance toward an early diagnosis and pessimism about prognosis, which in turn enhances therapeutic nihilism [56].

There are three levels of access to mental health in dementia care: micro-level (the person with dementia and their family), meso-level (the professional first contacted) and macro-level (the factors shaping the responses of specialists and those providing ongoing care) [56]. At each level there may be obstacles that will make it impossible to maximize the available assistance to the patient. While at the micro level the main obstacle is the lack of awareness of patients and their families about dementia, at the median level there is limited experience of general practitioners (GPs) on dementia and their embarrassment about discussing memory loss. At macro level, these are the issues of coordination of the service within the system and the question of taking over or transferring responsibilities within certain elements of the system [57].

Nearly two thirds of patients with AD from our sample lived in their own home, but only a quarter were capable of self-care. This can primarily be explained through cultural distinctions which insist on the family being responsible for taking care of an ill family member on one side, but also the poor financial support, insufficient institutional care and insufficient aid from society for individuals suffering from illnesses and their families, all due to which caregivers are subject to great and long lasting pressure [58]. Even for the 30% of the subjects suffering from AD, from our sample the observers listed the presence of cognitive changes in relatives as well. However, this hetero-anamnesis fact does not have a high specific value considering that it is present in a similar percentage as are the other diagnostic categories of the patients. Namely, family members rarely had reliable information on the illness existing among relatives which is objectively determined. For the most part, these were merely statements based on the opinions of the caregivers/relatives, which, in the majority of cases, is a very heterogeneous group of possible disorders. Subjects suffering from AD, in our sample, more often than not, in comparison to the others, had a deficit in vitamin B12, as well as HTN which is in accordance with published data which confirm the presence of vascular risk factors in this group of patients along with the importance of conducting check-ups in order to control them [59]. Despite the advanced stage of cognitive changes heading towards dementia, only every ninth patient had an appropriate therapy assigned prior to coming to the Center. This worrying fact shows us that this group is not directed towards a sufficient number of specialized services which would over a period of time recognize the illness and treat it in an adequate way. In the EU countries the situation is not unified but there is a concordance between specialists and GPs that dementia patients are undertreated (except for specialist in Spain, 54% of whom believed patients are adequately treated) [54]. Moore and Cahill [59] showed that despite the availability of highly sophisticated pre- and post-diagnostic tools, the majority of Swedish and Irish GPs showed therapeutic nihilism and reluctance to openly speak to their patients about dementia [60]. The reasons relate to insufficient diagnosis or excessively delayed diagnosis, the limited therapeutic effect, cost of the drug to the health care system and government restrictions [61–63].

In comparison to all subcategories of dementia in our sample of patients, the second place in terms of frequency was the VaD category (7.8%), which is slightly less than what is published in epidemiological research [61–63]. In our sample women made up the majority of this group, in their early eighties, and, in comparison to the majority of the other patients, they also had the lowest level of education. This lower level of education is recognized as a very important risk factor for the development of dementia, especially of the vascular type, because it is closely connected to tendencies of risk behavior and absence of control [61, 63]. When they came to the Center for their first visit, cognitive changes are already evident at the screening test level (MMSE) and they were under the borderline score for dementia. The majority of the patients from this group suffered with long-term HTN, with lesions and cerebrovascular insult (CVI) which were on CT scans along with confirmed significant hemodynamic changes in terms of bilateral carotid stenosis. Less than one fifth were smokers and an equal fraction consumed alcohol. In this group, the highest number of patients had diabetes mellitus, mainly type 2, with rarely present thyroid dysfunction. A very small number of patients were on the therapy before visiting the Center. The most frequent therapy involved medicine from one of the groups for the medical treatment of dementia, much less frequent for the treatment of depression, which is unexpected considering that depression often follows cerebrovascular changes [64–66].

Besides MCI, AD, and VaD, a particular number of patients that were referred to the Center were those from the group with affective disorder such as depression, anxiety disorder and some forms of psychosis (3.8%). This group of patients represented a differential-diagnostic challenge in terms of the importance of differentiating treatable forms from “real” dementia where emotional changes are the prodromal signs of illness. In our sample, the youngest patients belonged to this group, and on average had roughly three years of high school education, cognitive abilities within limits of normal values (MMSE = 25.9) and, in accordance with the majority of other characteristics, were similar to the patients from the MCI group. This shows that it is highly likely that there is overlapping within these two diagnostic categories due to which these patients are further being observed in the span of one year in the Center.

Patients diagnosed with FTD, DLB, PDD and Mixed Dementia were much less frequent in our sample, in comparison to epidemiological data from other studies [66–69]. The reason for this may be due to the dispersion of the patients towards other subspecialized centers (for example there is an FTD variant with motor neuron diseases which is in the scope of other current epidemiological research in Serbia or VaD or Mixed Dementia which is included in Cerebrovascular Diseases (CVD) Clinics and other national centers for CVD, which is why they were unavailable for our records. The FTD group was made up of relatively younger patients in our sample and male patients were the majority, however, taking the heterogeneousness of this diagnostic category into consideration which we were not analyzed, and also the difference in distribution of the patients by the

gender within each subcategory, this data could not be compared with the epidemiological data from other research. The patients from our FTD group were of a somewhat higher educational level – on average completed four years of high school education, but were also significantly cognitively compromised at their first visit (MMSE 18.1 ± 7.3). Also, a very small number of subjects were independent in activities for everyday functioning (17.4%), and slightly above average on the level of our entire sample were the subjects from the FTD group who in their medical history had serious head injuries with loss of consciousness. Even though more than half of the patients had HTN and carotid stenosis, slightly over a third had vascular lesions confirmed *via* a CT scan; however, much less frequently registered were the cases with CVI. On the other hand, in this group of subjects, brain atrophy was registered in over 90% of cases. In comparison to the other patient groups, a significant number of patients prior to coming to the Center were given therapy for dementia as well as neuroleptic therapy.

In our sample, the DLB group contained the least number of patients (0.6%) which significantly differs from published data [70, 71]. One explanation could be found in the clinical features because this category of patients is for the most part referred to psychiatric establishments. The patient profile from our sample included the oldest patients, in which men were significantly more frequent, had a completed high school education, but had globally more significantly deteriorated cognitions at the moment of their first visit (MMSE 19.8 ± 5.3). Accordingly, less than a third of patients were independent in everyday life. Almost two thirds of these subjects have had hypertension for several years, bilateral carotid stenosis, and applied imaging techniques showed that a third had vascular lesions, and nearly three quarters also had brain atrophy.

In the evaluation of cognitive changes that are typical for dementia in comparison to normal aging, neuropsychological testing nowadays prevails over all other methods [72]. General diagnostic or also called screening tests such as MMSE and CDT are relatively rough tests and are isolated as less efficient in diagnostics, however when applied together have an overall greater efficiency [72]. Accordingly, MMSE and CDT had the greatest application among our sample – 96% and 78.2%, respectively, followed by the Addenbrooke's cognitive examination – final revised (ACE – R) testing which was applied on slightly less than two thirds of the patients. The less frequent application of this test was due to the fact that it requires greater effort regarding the complexity of the task at hand and its duration, which in the case of subjects that have severe cognitive deficit, becomes impossible to carry out [73]. From the oriented tests domain, the most frequently applied are fluency tests due to their simplicity in terms of application, on the one side, and the high sensitivity in the differentiating of cognitive deficit etiology, on the other one [41]. Following this test is the verbal declarative memory test – the Ray anditory verbal learning test (RAVLT) (68.2%) which turned out to be more applicable with patients who have visual or reading deficits in comparison to other tests of verbal memory – free and cued Selective Reminding test (FCSRT) (51.4%). Both tests are highly sensitive and specific to distinguish MCI from AD as well as healthy population of respondents, but exact data on this as well as their metric characteristics in our population are under preparation.

It is important to emphasize that the Center is considered to be on a tertiary level of health care system in Serbia which is why its access to individual patients is currently limited. This, in the same time, presents the main limitation of this overview, and our results can be deemed as preliminary.

## Conclusion

According to the knowledge of researchers, this paper is the first of its kind which aims to show the profile of patients from a heterogeneous group of illnesses known as dementia in the Serbian population. In that sense, we tried to give a general overview of patients through most frequent diagnostic categories in order to provide a framework for planning activities of the Center but also of the health care system in Serbia. Also, through this overview, we wanted provide an organizational model which would inspire the establishment and the networking of medical centers specialized for dementia on a national level, the standardization of epidemiological criteria and the formation of a unique registry for dementia in the Republic of Serbia. All of these represents a prerequisite for the establishment of a national strategy for the battle against this, obviously, disease of the future which will spread on a greater population level, and this paper is the first step in achieving this goal.

## REFERENCES


1. *Arie T*. The first year of the Goodmayes psychiatric service for old people. Lancet 1970; 2(7684): 1179–82.
2. *Pitt B*. Psychogeriatrics: An Introduction to the Psychiatry of Old Age. Edinburgh:Chuchill-Livingstone; 1975.
3. *Benbow SM, Jolley D*. Organisation of mental health services for older people. In: *Pathy M, Sinclair A, Morley E,* editors. Principles and Practice of Geriatric Medicine. 4th ed. Chichester: John Wiley; 2006; p. 1163–71.
4. *Fraser M*. Memory clinics and memory training. Chapter 10. In: *Arie T*, editor. Recent advances in psychogeriatrics 2. Edinburgh: Churchill Livingstone; 1992. p. 105–16.
5. *Ames D, Flicker L, Helme R*. A memory clinic at a geriatric hospital: Rationale, routine and results from the first 100 patients. Med J Aust 1992; 156(9): 618–22.
6. *Jolley D, Moniz-Cook E*. Memory clinics in context. Indian J Psychiatry 2009; 51(Suppl 1): S70–6.
7. *Hejl A, Hogh P, Waldemar G*. Potentially reversible conditions in 1000 memory clinic patients. J Neurol Neurosurg Psychiatry 2002; 73(4): 390–4.
8. *Freter S, Bergman H, Gold S, Chertkow H, Clarfield AM*. Prevalence of potentially reversible dementias and actual reversibility in a memory clinic cohort. CMAJ 1998; 159(6): 657–62.

9. *Vernooij-Dassen MJ, Moniz-Cook ED, Woods RT, De Lepeleire J, Leuschner A, Zanetti O,* et al. Factors affecting the timely recognition and diagnosis of dementia across Europe: from awareness to stigma. Int J Geriatr Psychiatry. 2005; 20(4): 377–86.
10. *van Hout HP, Vernooij-Dassen MJ, Hoefnagels WH, Grol RP.* Measuring the opinions of memory clinic users: patients, relatives and general practitioners. Int J Geriatr Psychiatry 2001; 16(9): 846–51.
11. *Streams ME, Wackerbarth SB, Maxwell A.* Diagnosis seeking at subspecialty memory clinics: trigger events. Int J Geriatr Psychiatry 2003; 18(10): 915–24.
12. *Dautzenberg PL, van Marum RJ, van Der Hammen R, Paling HA.* Patients and families desire a patient to be told the diagnosis of dementia: a survey questionnaire of a Dutch memory clinic. Int J Geriatr Psychiatry 2003; 18(9=: 777–9.
13. *LoGiudice D, Hassett A, Cook R, Flicker L, Ames D.* Equity of access to a memory clinic in Melbourne? Non-English speaking background attenders are more severely demented and have increased rates of psychiatric disorders. Int J Geriatr Psychiatry 2001; 16(3): 327–34.
14. *Logiudice D, Waltrowicz W, Brown K, Burrows C, Ames D, Flicker L.* Do memory clinics improve the quality of life for carers. A randomised pilot trial? Int J Geriatr Psychiatry 1999; 14(8): 626–32.
15. *Thomas Antérion C, Gely-Nargeot MC, Pancrazi MP.* Management of memory disorders in anxious patients consulting a memory clinic. Rev Neurol (Paris) 2000; 156(8–9): 775–9. (French)
16. *Damian M, Krumm B, Syren M, Hentschel F.* Is there a referral bias in the diagnoses of patients of a memory clinic? Z Gerontol Geriatr 2003; 36(3): 197–203. (German)
17. *Walstra GJ, Derix MM, Hijdra A, van Crevel H.* An outpatient clinic for memory disorders: initial experiences. Ned Tijdschr Geneeskd 1992; 136(7): 328–32. (Dutch)
18. *Werner P, Heinik J, Aharon J.* Process and organisational characteristics of memory clinics in Israel: a national survey. Arch Gerontol Geriatr 2001; 33: 191–201.
19. *Rösler A, Gönnenwein C, Müller N, Sterzer P, Kleinschmidt A, Frölich L.* The fuzzy frontier between memory complaints and early dementia: a survey of patient management in German memory clinics. Dement Geriatr Cogn Disord 2004; 17(3): 222–30.
20. *Diehl J, Staehelin H, Wiltfang J, Hampel H, Calabrese P, Monsch A,* et al. German-speaking memory clinics: the state of the art. Z Gerontol Geriatr 2003; 36(3): 189–96. (German)
21. *Monsch AU, Ermini-Fünfschilling D, Mulligan R, Meier D, Juillerat AC, Michel JP,* et al. Memory clinics in Switzerland. Collaborative group of Swiss memory clinics. Ann Med Interne (Paris) 1998; 149(4): 221–7.
22. *Cheung G, Strachan J.* A survey of memory clinics in New Zealand. Australas Psychiatry 2008; 16(4): 244–7.
23. *Verhey FR, Ramakers I, Jolles J, Scheltens P, Vernooij-Dassen M, Olde Rikkert M.* Development of memory clinics in the Netherland. Tijdschr Geronotol Geriatr 2007; 38(5): 237–45. (Dutch)
24. *Kopelman M, Crawford S.* Not all memory clinics are dementia clinics. Neuropsychol Rehabil 1996; 6(3): 187–202.
25. *Matthews FE, Arthur A, Barnes LE, Bond J, Jagger C, Robinson L,* et al. Medical Research Council Cognitive Function and Ageing Collaboration. A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: results of the Cognitive Function and Ageing Study I and II. Lancet 2013; 382(9902): 1405–412.
26. *Qiu C, von Strauss E, Bäckman L, Winblad B, Fratiglioni L.* Twenty-year changes in dementia occurrence suggest decreasing incidence in central Stockholm, Sweden. Neurology 2013; 80(20): 1888–94.
27. *Christensen K, Thinggaard M, Oksuzyan A, Steenstrup T, Andersen-Ranberg K, Jeune B,* McGue et al. Physical and cognitive functioning of people older than 90 years: A comparison of two Danish cohorts born 10 years apart. Lancet 2013; 382(9903): 1507–13.
28. *Lobo A, Saz P, Marcos G, Dia JL, De-la-Camara C, Ventura T*, et al. Prevalence of dementia in a southern European population in two different time periods: The ZARADEMP Project. Acta Psychiatr Scand 2007; 116(4): 299–307.
29. *Krysinska K, Sachdev P, Brodaty H.* Dementia registries around the world: a review and recommendations 2016. Available from: https://cheba.unsw.edu.au/sites/default/files/Krysinska%20et%20al%20-%20Dementia%20registries%20around%20the%20world%20report%20November%202016.pdf
30. *Centers for Disease Control and Prevention.* International Classification of Diseases, Injuries, and Causes of Death. Ninth Revision (ICD-9). Atlanta, GA: CDC/National Center for Health Statistics; 1979.
31. *McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM.* Clinical diagnosis of alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on alzheimer's disease. Neurology 1984; 34(7): 939–44.
32. *Román GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH,*et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993; 43(2): 250–60.
33. *McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA,* et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with lewy bodies (DLB): Report of the consortium on DLB international workshop. Neurology 1996; 47(5): 1113–24.
34. *McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ.* Work Group on Frontotemporal Dementia and Pick's Disease. Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick's Disease. Arch Neurol 2001; 58(11): 1803–9.
35. *American Psychiatric Association.* Diagnostic and statistical manual of mental disorders. 4th ed. (DSM-IV). Washington DC, American Psychiatric Association; 1994.
36. *Folstein MF, Folstein SE, McHugh TR.* "Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12(3): 189–98.
37. *Mioshi E, Dawson K, Mitchell J, Arnold R, Hodges JR.* The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive testbattery for dementia screening. Int J Geriatr Psychiatry 2006; 21(11): 1078–85.
38. *Mattis S.* Dementia rating scale: professional manual. Odessa, Fl: Psychological Assessment Resources; 1988.
39. *Freedman M, Leach L, Kaplan E, Winocur G, Shulman K, Delis DC.* Clock-drawing: a neuropsychological analysis. New York, NY: Oxford University Press; 1994.
40. *Lezak MD.* Neuropsychological assessment. New York, NY: Oxford University Press; 1995.
41. *Buschke H.* Cued recall in amnesia. J Clin Neuropsychol 1984; 6(4): 433–40.
42. *Grober E, Buschke H.* Genuine memory deficits in dementia. Dev Neuropsychol 1987; 3(1): 13–36.
43. *Kaplan E, Goodglass H, Weintrab S.* The Boston Naming Test. Philadelphia: Lea & Febiger; 1983.
44. *Reisberg B, Finkel S, Overall J, Schmidt-Gollas N, Kanowski S, Lehfeld H,* et al. The Alzheimer's disease activities of daily living international scale (ADL-IS). Int Psychogeriatr 2001; 13(2): 163–81.
45. *Seshadri S, Wolf PA, Beiser A, Au R, McNulty K, White R,* et al. Lifetime risk of dementia and Alzheimer's disease. The impact

of mortality on risk estimates in the Framingham Study. Neurology 1997; 49(6): 1498–504.
46. *Podcasy JL, Epperson CN.* Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin Neurosci 2016; 18(4): 437–46.
47. *Azad NA, Al Bugami M, Loy-English I.* Gender differences in dementia risk factors. Gend Med 2007; 4(2): 120–9.
48. *Camarda C, Pipia C, Azzarello D, Battaglini I, Romeo G, Chiodi M,* et al. Vascular risk factors, vascular diseases, and imaging findings in a hospital-based cohort of mild cognitive impairment types. Curr Alzheimer Res 2018; 15(7): 679–90.
49. *Bartels C, Wagner M, Wolfsgruber S, Ehrenreich H, Schneider A.* Alzheimer's disease neuroimaging initiative. Impact of SSRI therapy on risk of conversion from mild cognitive impairment to Alzheimer's dementia in individuals with previous depression. Am J Psychiatry 2018; 175(3): 232–41.
50. *Caselli RJ, Langlais BT, Dueck AC, Henslin BR, Johnson TA, Woodruff BK,* et al. Personality Changes During the Transition from Cognitive Health to Mild Cognitive Impairment. J Am Geriatr Soc 2018; 66(4): 671–8.
51. *Seltman RE, Matthews BR.* Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. CNS Drugs 2012; 26(10): 841–70.
52. *Iliffe S, De Lepeleire J, Van Hout H, Kenny G, Lewis A, Vernooij-Dassen M.* DIADEM Group. Understanding obstacles to the recognition of and response to dementia in different European countries: a modified focus group approach using multinational, multi-disciplinary expert groups. Aging Ment Health 2005; 9(1): 1–6.
53. *Martinez-Lage P, Frolich L, Knox S, Berthet K.* Assessing physician attitudes and perceptions of Alzheimer's disease across Europe. J Nutr Health Aging 2010; 14(7): 537–44.
54. *Hausner L, Frölich L, Gardette V, Reynish E, Ousset PJ, Andrieu S,* et al. On Behalf Of The Ictus-Eadc Study Group. Regional variation on the presentation of Alzheimer's disease patients in memory clinics within Europe: data from the ICTUS study. J Alzheimers Dis 2010; 21(1): 155–65.
55. *Iliffe S, Robinson L, Brayne C, Goodman C, Rait G, Manthorpe J,* et al. Primary care and dementia: 1. diagnosis, screening and disclosure. Int J Geriatr Psychiatry 2009; 24(9): 895–901.
56. *Pope C, Mays N.* Qualitative research in health care. 2nd ed. London: BMJ Books; 1999.
57. Amity. Life without memory. Available from: http://www.amity-yu.org/wp-content/uploads/2017/02/zivot_bez_secanja_istrazivanje_potrebe_za_dnevnim_centrom_za_dementne_policy_amity.pdf (Serbian)
58. *Chui HC, Ramirez-Gomez L.* Clinical and imaging features of mixed Alzheimer and vascular pathologies. Alzheimers Res Ther 2015; 7(1): 21.
59. *Moore V, Cahill S.* Diagnosis and disclosure of dementia--a comparative qualitative study of Irish and Swedish General Practitioners. Aging Ment Health. 2013; 17(1): 77–84.
60. *Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K,* et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. World Federation of Neurology Dementia Research Group. Lancet Neurol 2008; 7(9): 812–26.
61. *Forette F, Boller F.* Hypertension and the risk of dementia in the elderly. Am J Med 1991; 90(3A): 14S–19S.
62. *Rizzi L, Rosset I, Roriz-Cruz M.* Global epidemiology of dementia: Alzheimer's and vascular types. Biomed Res Int. 2014; 2014: 908915.
63. *Vita AJ, Terry RB, Hubert HB, Fries JF.* Aging, health risks, and cumulative disability. N Engl J Med 1998; 338(15): 1035–41.
64. *Van der Mast RC, Vinkers DJ, Stek ML, Bek MC, Westendorp RG, Gussekloo J,* et al.. Vascular disease and apathy in old age. The Leiden 85-Plus Study. Int J Geriatr Psychiatry 2008; 23(3): 266–71.
65. *Van Dalen JW, Van Wanrooij LL, Moll van Charante EP, Richard E, van Gool WA.* Apathy is associated with incident dementia in community-dwelling older people. Neurology 2018; 90(1): e82–e89.
66. *Aarsland D, Zaccai J, Brayne C.* A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord 2005; 20(10): 1255–63.
67. *Vasconcellos LF, Pereira JS.* Parkinson's disease dementia: Diagnostic criteria and risk factor review. J Clin Exp Neuropsychol 2015; 37(9): 988–93.
68. *Custodio N, Montesinos R, Lira D, Herrera-Pérez E, Bardales Y, Valeriano-Lorenzo L.* Mixed dementia: A review of the evidence. Dement Neuropsychol 2017; 11(4): 364–70.
69. *Vann Jones SA, O'Brien JT.* The prevalence and incidence of dementia with Lewy bodies: a systematic review of population and clinical studies. Psychol Med 2014; 44(4): 673–83.
70. *Kane JPM, Surendranathan A, Bentley A, Barker SAH, Taylor JP, Thomas AJ,* et al. Clinical prevalence of Lewy body dementia. Alzheimers Res Ther 2018; 10(1): 19.
71. *Stefanova E, Pavlović A, Semnic M, Janjić V, Petrović V, Milošević V,* et al. Vodič za Alchajmerovu bolest 2013.god. Beograd: Ministrastvo zdravlja ; 2013. (Serbian)
72. *Aprahamian I, Martinelli JE, Neri AL, Yassuda MS.* The accuracy of the Clock Drawing Test compared to that of standard screening tests for Alzheimer's disease: results from a study of Brazilian elderly with heterogeneous educational backgrounds. Int Psychogeriatr 2010; 22(1): 64–71.
73. *Henry JD, Crawford JR, Phillips LH.* Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. Neuropsychologia 2004; 42(9): 1212–22.

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# Analysis of malignancy predictors for follicular thyroid tumors

## Analiza prediktora maligniteta folikulskih tumora štitaste žlezde

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**Abstract**

**Background/Aim.** Establishing a preoperative diagnosis of thyroid follicular tumors is difficult due to the fact that the cell morphology of adenomas and carcinomas are similar and that capsular and vascular invasion cannot be determined by cytology. We analyzed predictive factors of follicular carcinoma in order to enable a surgeon to indicate operative treatment and to perform an adequate operation for each patient with a follicular neoplasm. **Methods.** In this retrospective study, we analyzed medical records of all patients with follicular thyroid tumors operated at an endocrine surgery unit of a tertiary referral academic hospital, between 2008 and 2012. A total of 263 operated patients were included and divided into follicular adenomas (n = 97) and follicular carcinomas (n = 166) based on the histopathology results. The most important demographic and clinical characteristics were analyzed by univariate and multivariate logistic regression analysis. **Results.** In adenoma group (19 males, 78 females) age range was 19–79, mean age 50. In carcinoma group (35 males, 131 females) age range was 15–78, mean age 48. Univariate analysis showed that thyroglobulin concentration ≥ 500 ng/mL, tumor diameter < 30 mm, presence of more than one thyroid nodule and an afunctional/hypofunctional nodule were significantly more frequent in follicular carcinoma than in follicular adenoma. Independent predictive factors of malignancy were: elevated preoperative thyroglobulin concentration (≥ 500 ng/mL) and presence of more than one nodule. Based on our results we formed a nomogram, a two-dimensional diagram designed to enable estimation of preoperative probability of malignancy. **Conclusion.** Elevated preoperative thyroglobulin concentration, ≥ 500 ng/mL, and the presence of more than one nodule are independent predictors of malignancy for follicular thyroid carcinomas.

**Key words:**
**thyroid neoplasms; diagnosis; diagnosis, differential; thyroidectomy; thyroglobulin; nomograms.**

**Apstrakt**

**Uvod/Cilj.** Prema raspoloživim dijagnostičkim metodama nije moguće preoperativno razlikovati benigne od malignih folikulskih tumora štitaste žlezde, a najčešće ni intraoperativno zbog veoma slične ćelijske morfologije folikulskih adenoma i folikulskih karcinoma i nemogućnosti citološkog dokaza invazije kapsule ili krvnih sudova karakteristične za folikulske karcinome. U ovoj studiji, istraživali su se mogući prediktivni faktori maligniteta kod bolesnika s folikulskim karcinomom štitaste žlezde koji bi omogućili ispravnu selekciju bolesnika za hirurško lečenje, a potom i izvođenje adekvatnog tipa operacije kod bolesnika s folikulskom tireoidnom neoplazmom. **Metode.** Ovom retrospektivnom studijom su obuhvaćeni svi bolesnici operisani zbog postojanja folikulskog tumora štitaste žlezde u tercijarnoj univerzitetskoj zdravstvenoj ustanovi endokrine hirurgije, tokom petogodišnjeg perioda (2008–2012). U istraživanje su bila uključena 263 operisana bolesnika. Na osnovu definitivnog histopatološkog nalaza ispitanici su bili podeljeni u dve grupe: folikulske adenome (n = 97) i folikulske karcinome (n = 166). Najvažnije demografske i kliničke karakteristike operisanih bolesnika analizirane su univarijantnom i multivarijantnom logističkom regresionom analizom. **Rezultati.** U grupi bolesnika operisanih zbog folikulskog adenoma (19 osoba muškog i 78 ženskog pola) starosna dob je iznosila 19–79 godina s prosečnom starošću od 50 godina. U grupi bolesnika operisanih zbog folikulskog karcinoma (35 muških, 131 ženska osoba) starosna dob je bila u rasponu 15–78 godina, a prosečna starost 48 godina. Univarijantnom analizom pokazano je da se koncentracija tireoglobulina ≥ 500 ng/mL, promer tumora < 30 mm, prisustvo više od jednog

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tireoidnog čvora i nalaz afunkcijskog/hipofunkcijskog čvora značajno češće nalaze kod folikulskog karcinoma u odnosu na folikulski adenom. Nezavisni prediktivni faktori maligniteta bili su povišena preoperativna koncentracija tireoglobulina (≥ 500 ng/mL) i prisustvo više od jednog čvora. Ovi rezultati su, u cilju primene u praksi, prikazani i nomogramom, dvodimenzionalnim dijagramom dizajniranim da omogući približno preoperativno grafičko izračunavanje verovatnoće postojanja maligniteta. **Zaključak.** Povišena preoperativna koncentracija tireoglobulina, ≥ 500 ng/mL, i prisustvo više od jednog čvora su nezavisni prediktori maligniteta folikulskih karcinoma štitaste žlezde.

**Ključne reči:**
**tireoidna žlezda, neoplazme; dijagnoza; dijagnoza, diferencijalna; tireoidektomija; tireoglobulin; nomogrami.**

## Introduction

Primary thyroid malignancies, according to their cell origin are divided into two groups: larger, from follicular cells (papillary, follicular, oxyphilic and anaplastic carcinoma – more than 90%) and smaller, which originate from C-cells (medullary carcinoma, less than 10%).

The aim of the modern medicine is to know the nature of the tumor preoperatively or at least intraoperatively. The best tests to predict malignancy and the need for surgery in patients with thyroid nodules are fine-needle aspiration biopsy (FNAB) and measurement of serum calcitonin for medullary cancer. The reported accuracy of FNAB ranges from 70–90%. It is useful in the diagnostics of goiter, some benign thyroid tumors (like colloid adenoma or cysts), papillary and anaplastic carcinoma; but it is not reliable in distinguishing benign from malignant follicular and Hurthle-cell neoplasms.

Follicular adenoma and follicular carcinoma give the same cytological diagnosis – follicular lesion that includes both, benign follicular tumors (adenomas) and malignant (follicular carcinomas and follicular variant of papillary cancer). The role of the intraoperative frozen-section examination is controversial for those two types of thyroid tumor, too.

Consequently, it is difficult to establish a correct preoperative diagnosis for follicular tumors because of a very similar benign and malignant cytological morphology and because of the fact that capsular and vascular invasion cannot be verified by cytological examination [1]. Up to 70% of these patients with a diagnosis of follicular lesion undergo surgery for benign disease with risk of surgical complications. The need for a thyroidectomy completion increases the risk of complications and the costs [2, 3].

Malignant follicular neoplasms include follicular carcinoma and the follicular variant of papillary carcinoma. Follicular carcinoma is rare, but the follicular variant of papillary cancer is more often present on histopathology findings thus it has a bigger differential diagnostic and clinical importance.

In this study, we looked for possible predictive factors of malignant follicular neoplasms in order to enable a surgeon to indicate operative treatment and to perform an adequate operation for each patient with a follicular neoplasm.

## Methods

In this retrospective study, we analyzed medical records of all patients with follicular tumors of the thyroid operated at an endocrine surgery unit of a tertiary referral academic hospital, in a five-year period (2008–2012). The study was approved by the Ethic Committee of the tertiary referral university hospital. A total of 263 patients were included and divided, on the basis of definite histopathology, into two groups: 1) follicular adenomas (97 patients) and 2) follicular carcinomas and follicular variant of papillary cancer (166 patients – 11 follicular carcinomas and 155 patients with follicular variant of papillary cancer). The most important demographic and clinical characteristics were analyzed (n = 34) including gender (male/female), age (≤ 50/> 50 years), smoking (smokers/nonsmokers), duration of disease (> 60/≤ 60 months), type of operation, tumor diameter (< 30/≥ 30 mm), type of nodule (dominant, non-dominant/solitary), multifocality of the tumor (yes/no), microcalcifications (yes/no), echostructure (iso-, hyper-, heteroechoic/hypoechoic), vascularization of nodule (irregular/regular), scintigraphy (afunctional and hypofunctional/functional and hyperfunctional), thyroid functional status (hypothyroid, euthyroid, hyperthyroid), level of serum thyroglobulin (≥ 500/< 500 ng/mL), anti-thyreoglobulin (Tg) antibodies (increased/normal), anti-thyroperoxidase (TPO) antibodies (increased/normal), coexisting benign thyroid diseases (yes/no), coexisting Hashimoto thyroiditis (yes/no), coexisting Graves' disease (yes/no), coexisting multinodular goiter (yes/no), coexisting thyroid adenoma (yes/no), coexisting malign thyroid diseases (yes/no), coexisting oxyphilic carcinoma (yes/no), coexisting papillary carcinoma (yes/no), coexisting micropapillary carcinoma (yes/no), coexisting malignant tumors of other organs (yes/no), presence of arterial hypertension (yes/no), diabetes mellitus (yes/no), ABO, Rh, presence of benign (yes/no) and malignant (yes/no) family thyroid diseases and other malignant family diseases (yes/no). Dichotomy of continuing variables was made on the base of data distribution and referral literature value.

The Cox regression model was used in statistical data processing. All the variables were tested by univariate logistic regression analysis and those with $p < 0.05$ were included in the multivariate logistic regression analysis to test for independence in the prediction of malignancy with a 95% confidence interval (CI) for the odds ratio (OR). A $p$ value < 0.05 was considered as statistically significant. Based on our results we formed a nomogram, a two-dimensional diagram designed to enable calculation of preoperative probability of malignancy. The software package SPSS 12.0 for windows was used for all statistical analyses.

## Results

Over the study period, there were 263 patients who underwent surgical treatment: 97 (36.9%) with benign histology of follicular adenoma and 166 (63.1%) with malignant

histology of follicular carcinoma (n = 11) or follicular variant of papillary carcinoma (n = 155). Results are presented in Tables 1 to 3.

**Table 1**
**Demographic and clinical characteristics of all patients**

| Characteristics | Adenoma | Carcinoma |
|---|---|---|
| | n (%) | n (%) |
| Gender | | |
| male | 19 (19.6) | 35 (21.1) |
| female | 78 (80.4) | 131 (78.9) |
| Age (years) | | |
| ≤ 30 | 12 (12.4) | 21 (12.7) |
| 31–40 | 15 (15.5) | 37 (22.3) |
| 41–50 | 18 (18.6) | 36 (21.7) |
| 51–60 | 26 (26.8) | 41 (24.7) |
| 61–70 | 21 (21.6) | 20 (12.0) |
| ≥ 71 | 5 (5.2) | 11 (6.6) |
| Smoking | | |
| smokers | 24 (32.4) | 45 (36.6) |
| former smokers | 15 (20.3) | 18 (14.6) |
| nonsmokers | 35 (47.3) | 60 (48.8) |
| Disease duration (months) | | |
| ≤ 11.9 | 13 (13.8) | 35 (22.3) |
| 12–35.9 | 19 (20.2) | 27 (17.2) |
| 36–59.9 | 15 (16.0) | 27 (17.2) |
| 60–119.9 | 21 (22.3) | 26 (16.6) |
| 120–239.9 | 16 (17.0) | 28 (17.8) |
| Thyroid functional status | | |
| hypothyroidism | 4 (4.1) | 8 (4.8) |
| euthyroidism | 85 (87.6) | 147 (89.1) |
| hyperthyroidism | 8 (8.2) | 10 (6.1) |
| Thyroglobulin (ng/mL) | | |
| ≥ 500 | 3 (5.4) | 19 (17.1) |
| < 500 | 53 (94.6) | 92 (82.9) |
| Anti-Tg antibodies | | |
| increased | 8 (18.6) | 22 (21.4) |
| normal | 35 (81.4) | 81 (78.6) |
| Anti-TPO antibodies | | |
| increased | 10 (24.4) | 23 (26.4) |
| normal | 31 (75.6) | 64 (73.6) |
| Type of operation | | |
| hemithyroidectomy | 44 (45.4) | 46 (27.7) |
| lobectomy with partial resection | 0 (0.0) | 5 (3.0) |
| near-total thyroidectomy | 5 (5.2) | 11 (6.6) |
| thyroidectomy with/without dissection. | 48 (49.5) | 104 (62.7) |

**Anti-TPO antibodies – antithyroperoxidase antibodies; Anti-Tg antibodies – anti-thyroglobulin antibodies.**

In the adenoma group (19 males, 78 females) age ranged from 19 to 79 with a mean age of 50 years. In the carcinoma group (35 males, 131 females) age ranged from 15–78 with a mean age of 48 years. The mean tumor diameter in the adenoma group was 37.5 mm (median 36, range 12–150 mm) and 33.4 mm (median 30, range 3–90 mm) in the carcinoma group. The mean preoperative level of thyroglobulin in the adenoma group was 226.6 ng/mL, and 320.3 ng/mL in the carcinoma group.

**Table 2**
**Tumor characteristics in all patients**

| Characteristics | Adenoma | Carcinoma |
|---|---|---|
| | n (%) | n (%) |
| Tumor diameter (mm) | | |
| < 30 | 43 (44.3) | 98 (59.0) |
| ≥ 30 | 54 (55.7) | 68 (41.0) |
| Type of nodule | | |
| solitary | 56 (57.7) | 71 (43.0) |
| dominant | 35 (36.1) | 65 (39.4) |
| non-dominant | 6 (6.2) | 29 (17.6) |
| Multifocal tumor | | |
| no (unifocal) | 94 (97.9) | 112 (67.9) |
| two tumors | 0 (0.0) | 34 (20.6) |
| three tumors | 2 (2.1) | 19 (11.5) |
| Microcalcifications | | |
| yes | 13 (14.4) | 32 (20.1) |
| no | 77 (85.6) | 127 (79.9) |
| Ehostructure | | |
| isoechoic | 10 (14.7) | 14 (12.7) |
| hyperechoic | 7 (10.3) | 6 (5.5) |
| hypoechoic | 19 (27.9) | 38 (34.5) |
| heteroechoic | 32 (47.1) | 52 (47.3) |
| Nodule vascularisation | | |
| regular | 39 (84.8) | 65 (75.6) |
| irregular | 7 (15.2) | 21 (24.4) |
| Scintigraphy | | |
| afunctional | 29 (50.9) | 56 (71.8) |
| hypofunctional | 15 (26.3) | 18 (23.1) |
| functional | 3 (5.3) | 0 (0.0) |
| hyperfunctional | 10 (17.5) | 4 (5.1) |

Patients in the carcinoma group significantly more often had more than one nodule ($p = 0.012$) below 30 mm in diameter ($p = 0.021$), afunctional on scintigraphy ($p < 0.01$), serum thyroglobulin level ≥ 500 ng/mL ($p = 0.045$) and coexisting thyroid adenomas ($p < 0.01$).

Coexisting malignant thyroid diseases ($p < 0.01$), thyroid micropapillary ($p = 0.027$) and papillary carcinomas ($p < 0.01$) were significantly more frequent in the group of adenomas.

There were no significant differences between these two groups regarding gender, age, smoking, disease duration, consistence of nodule, microcalcifications, echostructure, nodule vascularization, thyroid functional status, levels of anti-Tg and anti-TPO antibodies, coexisting benign thyroid diseases (Hashimoto thyroiditis, Graves' disease, nodular and multinodular goiter), coexisting oxyphilic carcinoma and malignant tumors of other organs, arterial hypertension, diabetes mellitus, ABO, Rh, benign and malignant family thyroid diseases and other malignant diseases in family.

All variables that can be preoperatively determined were included in the univariate regression analysis. Results are presented in Table 4.

Univariate analysis showed that thyroglobulin concentration greater or equal than 500 ng/mL, tumor diameter < 30 mm, presence of more than one thyroid nodule and an afunctional/hypofunctional nodule on scintigraphy were significantly more frequent in patients with follicular carcinoma compared to patients with follicular adenoma.

**Table 3**
**Coexisting thyroid and other diseases in all patients**

| Coexisting diseases | Adenoma | Carcinoma |
|---|---|---|
| | n (%) | n (%) |
| Benign thyroid diseases | | |
| yes | 56 (57.7) | 115 (69.3) |
| no | 41 (42.3) | 51 (30.7) |
| Hashimoto thyreoiditis | | |
| yes | 16 (16.5) | 18 (10.8) |
| no | 81 (83.5) | 148 (89.2) |
| Graves disease | | |
| yes | 0 (0.0) | 4 (2.4) |
| no | 97 (100.0) | 162 (97.6) |
| Nodular/multinodular goiter | | |
| yes | 38 (39.2) | 49 (29.5) |
| no | 59 (60.8) | 117 (70.5) |
| Thyroid adenoma | | |
| yes | 2 (2.1) | 44 (26.5) |
| no | 95 (97.9) | 122 (73.5) |
| Malign thyroid diseases | | |
| yes | 25 (25.8) | 10 (6.0) |
| no | 72 (74.2) | 156 (94.0) |
| Oxyphilic carcinoma | | |
| yes | 2 (2.1) | 1 (1.6) |
| no | 95 (97.9) | 165 (99.4) |
| Papillary carcinoma | | |
| yes | 12 (12.4) | 2 (1.2) |
| no | 85 (87.6) | 164 (98.8) |
| Micropapillary carcinoma | | |
| yes | 11 (11.3) | 7 (4.2) |
| no | 86 (88.7) | 159 (95.8) |
| Malignant tumors of other organs | | |
| yes | 4 (4.1) | 3 (1.8) |
| no | 93 (95.9) | 163 (98.2) |
| Arterial hypertension | | |
| yes | 42 (43.3) | 61 (36.7) |
| no | 55 (56.7) | 105 (63.3) |
| Diabetes mellitus | | |
| yes | 4 (4.1) | 14 (8.4) |
| no | 93 (95.9) | 152 (91.6) |
| ABO | | |
| A | 26 (36.6) | 59 (41.3) |
| B | 11 (15.5) | 26 (18.2) |
| AB | 2 (2.8) | 8 (5.6) |
| 0 | 32 (45.1) | 50 (35.0) |
| Rh | | |
| positive | 61 (87.1) | 117 (81.8) |
| negative | 9 (12.9) | 26 (18.2) |
| Benign thyroid diseases (family) | | |
| yes | 24 (25.0) | 30 (18.2) |
| no | 72 (75.0) | 135 (81.8) |
| Malignant thyroid diseases (family) | | |
| yes | 2 (2.1) | 7 (4.3) |
| no | 92 (97.9) | 157 (95.7) |
| Other malignant diseases (family) | | |
| yes | 9 (9.6) | 14 (8.6) |
| no | 85 (90.4) | 148 (91.4) |

All variables with a $p$ value < 0.05 were included in the multivariate logistic regression model (Table 5). Independent predictive factors were elevated preoperative thyroglobulin concentration, ≥ 500 ng/mL, and a presence of more than one nodule. Scintigraphy findings were excluded from analysis because of a small number of patients with them. The whole model with all predictors was statistically significant ($p < 0.001$). There was no significant multi-collinearity among the predictors.

According to multivariate regression analysis, statistically significant predictors for follicular thyroid cancer were: type of nodule (dominant and non-dominant/solitary) (OR = 2.71, 95% CI 1.36–5.38), which means that patients with more than one nodule have almost three times a bigger chance to have follicular cancer in relation to patients with a solitary nodule; preoperative serum thyroglobulin concentration ≥ 500 ng/mL with OR = 4.18, 95% CI 1.14–15.33. Patients with Tg ≥ 500 ng/mL had over four times a bigger chance for follicular cancer.

Based on our results we formed a nomogram, a two-dimensional diagram designed to enable calculation of preoperative probability of malignancy (Figure 1). It may help to improve clinical management of patients with follicular lesions.

## Discussion

The incidence of malignancy in patients with thyroid follicular tumors lies between 12% and 30% [4–7]. In our study it was 63.1%. The research of Paramo and Mesko [8] (71 patients with follicular neoplasm) showed that the incidence of malignancy was 13% in men and 13% in women. In the study of Petric et al. [9], the malignancy rate was 43% in males and 23% in female patients with follicular and Hurthle cell neoplasms with a diameter of 2 cm or less.

The frozen section findings and FNAB are not a reliable method for distinguishing between benign a malignant follicular nodules. The discrimination between follicular adenoma and carcinoma can only be made postoperatively. Possible predictive factors of follicular carcinoma can help a surgeon to indicate operative treatment and to perform an adequate operation for each patient with a follicular thyroid neoplasm.

Average age of our patients was similar in both groups (adenoma group 48 years, carcinoma group 50 years). Our findings extend previous reports that age and gender are not predictive factors of malignancy [4, 5, 7, 8, 10, 11], but the findings of Petric et al. [9] were the opposite, where male patients had a higher risk of carcinoma. Similar result regarding sex reported Reparia et al. [12], but they also reported that age of the patient was not a predictor of malignancy. Unlike this, according to the research of Paramo and Mesko [8] age ≤ 45 years was a predictive parameter of malignancy in follicular neoplasm of the thyroid.

In the present study, univariate analysis showed that a tumor diameter < 30 mm was significantly more frequent in patients with follicular carcinoma.

**Table 4**

**Univariate regression analysis**

| Independent variable | *p* | OR | 95% CI | |
|---|---|---|---|---|
| Gender (male/female) | 0.772 | 0.91 | 0.49 | 1.70 |
| Age | 0.195 | 0.99 | 0.97 | 1.01 |
| Age (50 years) | 0.109 | 0.66 | 0.40 | 1.10 |
| Smoking (smokers/nonsmokers) | 0.840 | 0.94 | 0.53 | 1.68 |
| Disease duration (> 60/≤ 60 months) | 0.399 | 0.80 | 0.48 | 1.34 |
| Tumor diameter (< 30 mm/≥ 30 mm) | **0.022** | 1.81 | 1.09 | 3.00 |
| Type of nodule (dominant and non-dominant/solitary) | **0.022** | 1.81 | 1.09 | 3.00 |
| Microcalcifications (yes/no) | 0.265 | 1.49 | 0.74 | 3.02 |
| Echostructure (iso, hyper, heteroechoic/hypoechoic) | 0.360 | 0.73 | 0.38 | 1.42 |
| Nodule vascularization (irregular/regular) | 0.222 | 1.80 | 0.70 | 4.62 |
| Scintigraphy (afunctional and hypofunctional/functional and hyperfunctional) | **0.005** | 5.47 | 1.68 | 17.81 |
| Thyroid functional status | 0.501 | 0.77 | 0.37 | 1.63 |
| Serum Tg | 0.157 | 1.00 | 1.00 | 1.00 |
| Serum Tg (≥ 78 ng/mL/< 78 ng/mL) | 0.672 | 1.16 | 0.59 | 2.28 |
| Serum Tg (≥ 500 ng/mL/< 500 ng/mL) | **0.045** | 3.65 | 1.03 | 12.91 |
| Anti-TPOAb (positive/negative) | 0.805 | 1.11 | 0.47 | 2.63 |
| Coexisting Hashimoto thyroiditis. (yes, no) | 0.190 | 0.62 | 0.30 | 1.27 |
| Coexisting goiter (yes, no) | 0.109 | 0.65 | 0.38 | 1.10 |
| Coexisting Graves' disease (yes, no) | 0.999 | - | - | - |
| Arterial hypertension (yes, no) | 0.294 | 0.76 | 0.46 | 1.27 |
| Diabetes mellitus (yes, no) | 0.191 | 2.14 | 0.68 | 6.70 |

**OR – odds ratio; CI – confidence interval; Tg – thyreoglobulin; Anti TPOAb – antithyreoperoxidase antibodies.**
**Note: statistically significant values are bolded.**

**Table 5**

**Multivariate regression analysis**

| Independent variable | *p* | OR | 95% CI | |
|---|---|---|---|---|
| Tumor diameter (< 30 mm / ≥ 30 mm) | 0.063 | 1.92 | 0.96 | 3.83 |
| Type of nodule (dominant and non-dominant/solitary) | 0.004 | 2.71 | 1.36 | 5.38 |
| Thyroglobulin (≥ 500 ng/mL/< 500 ng/mL) | 0.031 | 4.18 | 1.14 | 15.33 |

**OR – odds ratio; CI – confidence interval.**

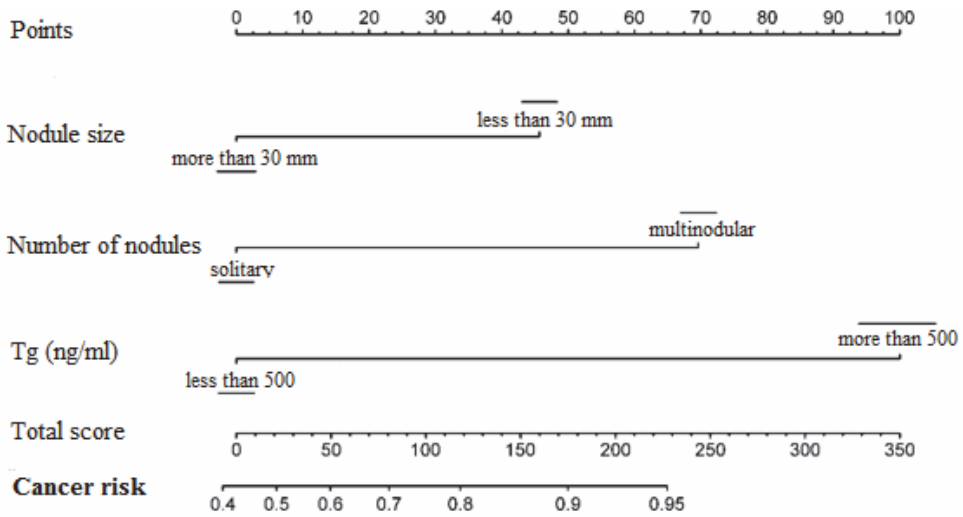


**Fig. 1 – Nomogram-preoperative probability of malignancy for follicular thyroid tumors.**

The diameter of follicular tumors as a predictive factor for carcinoma has been mentioned in literature many times [1, 9, 12, 13]. In the study of 616 patients with follicular adenoma, follicular carcinoma and follicular variant of papillary cancer, nodules ≥ 4 cm were associated with increased odds of a benign lesion which is in correlation to our research. In the same study, a family history of thyroid cancer was associated with increased odds of malignancy which was not proven in our study [10]. Paramo and Mesko [8] reported that a tumor > 4 cm is a predictive parameter of malignancy in follicular neoplasms. Gulcelik et al. [2] in their study of 98 patients with follicular neoplasm did not find a statistical significance, although the mean nodule size was slightly larger in malignant nodules. Risk of malignancy was higher in nodules measuring 2 cm or larger according to the results of Reparia et al. [12]. In the study of Petric et al. [9] the tumor diameter did not correlate with the malignancy rate. Tumor volume was found to be an independent predictor for follicular thyroid cancer (FTC) in all patients with a cytological diagnosis of follicular lesion [14].

Adenomas are usually solitary, less than 3 cm, but a significant numbers of exceptions exist [15]. In our study, univariate analysis showed that the presence of more than one thyroid nodule was significantly more frequent in patients with follicular carcinoma and it was an independent predictive factor in the multivariate logistic regression model. Gulcelik et al. [2] published similar results, in their study the presence of a solitary nodule was not predictive for malignancy. Unlike this, Najafian et al. [16] came out with data that multinodularity on physical examination was associated with an increased odds of a benign lesion.

Deviation of our results regarding incidence of malignancy, tumor size and type of nodule may be caused by a minority of follicular carcinomas in regard to the number of follicular variant of papillary carcinomas in the carcinoma group.

Serum thyroglobulin is primarily used in the postoperative cancer monitoring for differentiated thyroid carcinomas, but it could indicate differentiated cancer with controversial usefulness. In our study, the mean preoperative thyroglobulin level in the adenoma group was 226.6 ng/mL and 320.3 ng/mL in the carcinoma group. Thyroglobulin level over 500 ng/mL were significantly more frequent in patients with follicular carcinoma. Patients with thyroglobulin level over 500 ng/mL have over four times a bigger chance to have cancer. Petric at al. [9] showed that patients with a preoperative thyroglobulin values over 80 ng/mL had a malignancy rate of 35%, while in those with a lower level it was 19%. Several investigators published that there is a higher level of preoperative thyroglobulin in patients with well differentiated thyroid carcinomas compared to patients with benign goiters [17–19]. Kim et al. [14] showed that it was found to be an independent predictor for follicular cancer in all patients with a cytological diagnosis of a follicular lesion. On the other hand, Suh et al. [20] claimed that an elevated thyroglobulin level had no predictive value in follicular tumors, even in a high threshold value of 500 ng/mL.

The presence of hypoechoic solid features is generally considered to be the most reliable suspicious finding, but the value of ultrasonography is still controversial [21, 22]. Our results didn't confirm that echostructure (hypoechoic nodule) and the presence of microcalcifications had a higher frequency of malignancy, although it was reported by many authors [2, 4, 5, 21, 22]. Calò et al. [10] published, in accordance with our research, that the presence of microcalcifications was not significantly associated with malignancy. The results of Zdon et al. [11] also showed that the disease duration was not a significant predictor of malignancy.

Interestingly, coexisting primary hyperparathyroidism was not found in any of the 263 patients. Out of the 4,033 patients who underwent thyroidectomy at our institution from 2009–2014, in 114 (2.8%) a parathyroidectomy was simultaneously performed. Out of these 114 patients, 42 (37 %) had normocalcaemic primary hyperparathyroidism [23].

One of the main limitations of our study was the low rate of patients with follicular carcinoma, but it is considered a rare tumor. To overcome this limitation it would be necessary to analyze a higher number of such patients which would be possible through a multicentric study or in a longer period of time. On the other hand, the relatively short period where all operations were performed in one tertiary referral centre at a highly specialized endocrine surgery unit with thyroid expert pathologists and the uniformity of data can be considered a strength of the study.

## Conclusion

According to our results, an elevated preoperative thyroglobulin concentration level, greater than or equal to 500 ng/mL, and the presence of more than one thyroid nodule are independent predictors of malignancy for follicular thyroid carcinomas. The nomogram presented in this study could help to improve the clinical management of patients with follicular thyroid lesions.

## R E F E R E N C E S


1. *Ito Y, Miyauchi A.* Prognostic Factors and Therapeutic Strategies for Differentiated Carcinomas of the Thyroid. Endocr J 2009; 56: 177–92.
2. *Gulcelik NE, Gulcelik MA, Kuru B.* Risk of Malignancy in Patients With Follicular Neoplasm. Arch Otolaryngol Head Neck Surg 2008; 134(12): 1312–5.
3. *Hamburger JI, Husain M.* Contribution of intraoperative pathology evaluation to surgical management of thyroid nodules. Endocrinol Metab Clin North Am 1990; 19(3): 509–22.
4. *Sahin M, Gursoy A, Tutuncu NB, Guverner DN.* Prevalence and prediction of malignancy in cytologically indeterminate thyroid nodules. Clin Endocrinol (Oxford) 2006; 65(4): 5148.
5. *Miller B, Burkey S, Lindberg G, Snyder WH, Nwariaku FE.* Prevalence of malignancy withincytologically indeterminate thyroid nodules. Am J Surg 2004; 188(5): 459–62.
6. *Goldstein RE, Netterville JL, Burkey B, Johnson JE.* Implications of follicular neoplasms, atypia, and lesions suspicious for malignancy diagnosed by fine-needle aspiration of thyroid nodules. Ann Surg 2002; 235(5): 656–62.

7. *Raber W, Kaserer K, Niederle B, Vierhapper H.* Risk factors for malignancy of thyroid nodules initially identified as follicular neoplasia by fine-needle aspiration: results of a prospective study of one hundred twenty patients. Thyroid 2000; 10(8): 709–12.
8. *Paramo JC, Mesko T.* Age, tumor size, and in-office ultrasonography are predictive parameters of malignancy in follicular neoplasms of the thyroid. Endocr Pract 2008; 14(4): 447–51.
9. *Petric R, Besic H, Besic N.* Preoperative serum thyroglobulin concentration as a predictive factor of malignancy in small follicular and Hürthle cell neoplasms of the thyroid gland. World J Surg Oncol 2014; 12: 282.
10. *Calò PG, Medas F, Santa Cruz R, Podda F, Erdas E, Pisano G, Nicolosi A.* Follicular nodules (Thy3) of the thyroid: is total thyroidectomy the best option? BMC Surg 2014; 14: 12.
11. *Zdon MJ, Fredland AJ, Zaret PH.* Follicular neoplasms of the thyroid. Predictors of malignancy? Am Surg 2001; 67(9): 880–4.
12. *Reparia K, Min SK, Mody DR, Anton R, Amrikachi M.* Clinical outcomes for "suspicious" category in thyroid fine-needle biopsy: Patient's sex and nodule size are possible predictors of malignancy. Arch Pathol Lab Med 2009; 133(5): 787–90.
13. *Gulcelik NE, Gulcelik MA, Kuru B.* Risk of Malignancy in Patients With Follicular Neoplasm. Arch Otolaryngol Head Neck Surg 2008; 134(12): 1312–5.
14. *Kim HJ, Mok JO, Kim CH, Kim YJ, Kim SJ, Park HK,* et al. Preoperative serum thyroglobulin and changes in serum thyroglobulin during TSH suppression independently predict follicular thyroid carcinoma in thyroid nodules with a cythological diagnosis of follicular lesion. Endocr Res 2017; 42(2): 154–62.
15. *Davis NL, Gordon M, Germann E, Robins RE, McGregor GI.* Clinical parameters predictive of malignancy of thyroid follicular neoplasms. Am J Surg 1991; 161(5): 567–9.
16. *Najafian A, Olson MT, Schneider EB, Zeiger MA.* Clinical presentation of patients with a thyroid follicular neoplasm: are there preoperative predictors of malignancy? Ann Surg Oncol 2015; 22(9): 3007–13.
17. *Besic N, Sesek M, Peric B, Zgajnar J, Hocevar M.* Predictive factors of carcinoma in 327 patients with follicular neoplasm of the thyroid. Med Sci Monit 2008; 14(9): CR459–67.
18. *Hrafnkelsson J, Tulinius H, Kjeld M, Sigvaldason H, Jónasson JG.* Serum thyroglobulin as a risk factor for thyroid carcinoma. Acta Oncol 2000; 39(8): 973–7.
19. *Panza N, Lombardi G, De Rosa M, Pacilio G, Lapenta L, Salvatore M.* High serum thyroglobulin levels. Diagnostic indicators in patients with metastases from unknown primary sites. Cancer 1987; 60(9): 2233–6.
20. *Suh I, Vriens MR, Guerrero MA, Griffin A, Shen WT, Duh QY*, et al. Serum thyroglobulin is a poor diagnostic biomarker of malignancy in follicular and Hurthle-cel neoplasms of the thyroid. Am J Surg 2010; 200(1): 41–6.
21. *Koike E, Noguchi S, Yamashita H, Murakami T, Ohshima A, Kawamoto H,* et al. Ultrasonographic characteristics of thyroid nodules: prediction of malignancy. Arch Surg 2001; 136(3): 334–7.
22. *Leenhardt L, Hejblum G, Franc B, Fediaevsky LD, Delbot T, Le Guillouzic D,* et al. Indications and limits of ultrasound-guided cytology in the management of nonpalpable thyroid nodules. J Clin Endocrinol Metab 1999; 84(1): 24–8.
23. *Jovanovic MD, Zivaljevic VR, Diklic AD, Rovcanin BR, V Zoric G, Paunovic IR.* Surgical treatment of concomitant thyroid and parathyroid disorders: analysis of 4882 cases. Eur Arch Otorhinolaryngol 2017; 274(2): 997–1004.

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# Single stage bilateral total hip arthroplasty – 10 years of experience

## Bilateralna totalna artroplastika kukova u jednom aktu – desetogodišnje iskustvo

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**Abstract**

**Background/Aim.** Coxarthrosis is a chronic degenerative joint disorder which occurs in approximately 4% of population, and bilateral occurrence is estimated at 42%. Patients with bilateral coxarthrosis can undergo bilateral hip replacement in one or two stage procedure. Aim of this article was to present our experience in simultaneous bilateral hip arthroplasty, and to recommend it in patients with adequate indications. **Methods.** We processed data for 113 patients of both genders, operated at the Clinic for Orthopedic Surgery and Traumatology of the Military Medical Academy in Belgrade, Serbia from 2005 until 2015 where single stage bilateral hip arthroplasty was performed. Identical operative technique was applied in all patients as well as standard antibiotic and thromboprophylactic treatment. Follow-up period was 30 days postoperatively. **Results.** Mean age of patients was 56 ± 10.2 years, whereby 45 (39.8%) of them were males and 68 (60.2%) females. Primary coxarthrosis was etiologic factor for the majority of patients, 69 (61.1%). Postoperative hospitalization duration was 10.3 days on average (range from 3 to 34 days). We noticed the following complications: one luxation of the endoprosthesis, one deep infection and one thrombosis of the leg. There was no mortality. Majority of operated patients were grouped as the American Society of Anesthesiologists (ASA) scores 1-3. Mean blood transfusion was 1,275 mL (range from 300 to 2,830 mL). **Conclusion.** One stage bilateral hip arthroplasty can be performed routinely and safely in facilities with possibility for interdisciplinary approach and adequate selection of patients. Data from our study which indicate significantly increased need for blood substitution, emphasize the need to introduce contemporary perioperative blood loss management principles. The rate of complications observed in our study was within rates published in the literature concerning hip arthroplasty.

**Key words:**
**arthroplasty, replacement, hip; surgical procedures, operative; comorbidity; postoperative complications; serbia.**

**Apstrakt**

**Uvod/ Cilj**. Koksartroza je hronično degenerativno oboljenje koje se javlja kod oko 4% populacije, a procenjuje se da oko 42% bolesnika ima bilateralno oboljenje. Bolesnicima sa bilateralnom koksartrozom može se učiniti artroplastika oba kuka u jednom ili dva akta. Cilj ovog rada bio je prezentacija našeg iskustva sa izvođenjem simultane bilateralne artroplastike kukova i preporuka za izvođenje procedure kod bolesnika sa adekvatnom indikacijom. **Metode.** Obradili smo podatke za 113 bolesnika oba pola koji su operisani na Klinici za ortopedsku hirurgiju i traumatologiju Vojnomedicinske akademije u Beogradu, Srbija, u periodu od 2005. do 2015. godine, a kojima je učinjena artroplastika oba kuka u jednom aktu. Kod svih bolesnika je primenjena identična operativna tehnika uz identičnu standardnu antibiotsku i tromboprofilaktičku terapiju. Postoperativno praćenje bolesnika je iznosilo 30 dana. **Rezultati.** Prosečna starost bolesnika bila je 56 ± 10.2 godina; muškog pola je bilo 45 (39,8%), a ženskog pola 68 (60,2%) bolesnika. Primarna koksartroza je bila etiološki faktor kod većine bolesnika, njih 69 (61,1%). Prosečna dužina hospitalizacije postoperativno bila je 10,3 dana (od 3 do 34 dana). Zabeležili smo sledeće komplikacije: jedna luksacija endoproteze, jedna duboka infekcija i jedna tromboza vena noge. Nije bilo smrtnih slučajeva. Najveći broj operisanih bolesnika pripadao je grupama *American Society of Anesthesiologists* (ASA) skoring sistema 1-3. Prosečna količina transfuzionisane krvi iznosila je 1 275 mL (od 300 do 2 830 mL). **Zaključak**. Bilateralna artroplastika kukova u jednom aktu može se izvoditi rutinski i bezbedno u ustanovama koje omogućavaju multidisciplinarni pristup i adekvatnu selekciju bolesnika. Podaci iz naše studije ukazuju na povećanu potrebu za supstitucijom krvi što naglašava potrebu za uvođenjem savremenih principa pe-

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rioperativne kontrole gubitka krvi. Učestalost komplikacija zabeleženih u ovoj studiji je u okviru stepena učestalosti saopštenih u literaturi koja se bavi artroplastikama kukova.

**Ključne reči:**
**artroplastika kuka; hirurgija, operativne procedre; komorbiditet; postoperativne komplikacije; srbija.**

## Introduction

Total hip arthroplasty represents replacement of damaged articular surfaces of the femoral head and acetabulum with the artificial one. The most common indication for hip replacement is arthrosis. Coxarthrosis is a chronic, degenerative disease with prevalence of 4% in general population, while 42% of patients have bilateral disease [1]. Initial stadiums are treated conservatively by physiotherapy procedures, and with the advance of the disease total hip arthroplasty becomes the treatment of choice.

Patients with bilateral coxarthrosis can be operated in a single stage procedure with implantation of hip endoprosthesis bilaterally, or in two stage procedure, when second hip is operated after some time. Published literature shows perplexity considering safety of the single stage procedure. The procedure supporters claim that there is no significant difference regarding perioperative complications after simultaneous bilateral procedure comparing to the two stage procedure [2–5]. Opponents of this method report higher rate of systemic complications, thromboembolic complications, cardiopulmonary complications, infections, more frequent revision surgery, higher need for blood transfusion and higher mortality rate [6–11]. Likewise, there is no consensus regarding selection of patients neither an absolute indication for performing simultaneous bilateral total hip arthroplasty. Some authors performed simultaneous bilateral procedure on patients without significant comorbidity [2, 12].

Trying to establish objective criteria for selection, using score systems have started. Nowadays, most frequently used is the American Society of Anesthesiologists (ASA) score. Recommendations vary a lot: some authors advise performing procedure in patients classified as ASA score 1 or 2 [5, 13, 14], while others performed surgery in patients belonging from ASA scores 1–4 [4]. There is a consensus that simultaneous bilateral procedure leads to the reduction of cost and hospital stay [11, 13, 15]. Also, there is agreement that performing single stage bilateral procedure contributes to significantly better outcome of patients with bilateral coxarthrosis. In one study authors report that complete functional recovery occurs only after bilateral hip replacement, and the functional scores on operated hip in unilateral surgery are lower than after replacing second hip, and conclude that optimal result is obtained after second hip replacement [16]. Some authors consider that simultaneous bilateral total hip arthroplasty has an advantage, compared to the two stage procedure, because both operated sides are equally painful, and thus evenly used during rehabilitation, which results in avoiding the undesirable spare use of the operated hip [7].

Our study aimed at presenting our experience with bilateral hip arthroplasty and making recommendations regarding the choice of patients.

## Methods

At the Clinic for Orthopedic Surgery and Traumatology of the Military Medical Academy (MMA) in Belgrade, Serbia 113 simultaneous bilateral hip arthroplasties were performed between 2005 and 2015. The selection criteria were: the patients' wish to operate both hips in one act, as well as the estimation of a surgeon and the approval of internist and anesthesiologist consultants for performing such procedure. Data are extracted from protocols of the Clinic for Orthopedic Surgery and Traumatology of the MMA and protocols of the Department for Hospital Infection Control. Identical antibiotic and thromboprophylactic protocols were administered to all patients. The type of anesthesia was indicated by anesthesiologists, therefore 110 (97.3%) patients received general anesthesia and 3 (2.7%) spinal anesthesia. Patients were positioned in lateral decubitus, posterolateral approach was used with excision of the posterior capsule, while rotator muscle reconstruction was not performed. There were no wound drains. All implanted endoprostheses were cementless, press fit technique was applied, additional screw fixation of acetabulum was performed according to the surgeons' estimation. After finishing implantation of the first hip, a patient was rotated and the second hip was replaced. The same set of sterile instruments was used for both sides. Members of the operative team changed their sterile dressings and gloves for the second operation. On the first postoperative day patient was verticalized with crutches and full weight bearing.

## Results

Mean age of the patients was 56 ± 10.2 years (28–79 years). Forty five (39.8%) patients were males and 68 (60.2%) females. Noticed comorbidities are shown in Table 1.

**Table 1**

**Recorded comorbidities**

| Comorbidities | Patients, n (%) |
|---|---|
| Arterial hypertension | 55 (48.7) |
| Cardiomyopathy | 8 (7.1) |
| Angina pectoris | 3 (2.7) |
| Diabetes mellitus | 8 (7.1) |
| Hypothyreosis | 1 (0.9) |
| Hyperthyreosis | 2 ( 1.8) |
| Varices cruris | 5 (4.4) |
| Brain ischemic disease | 1 (0.9) |
| Rheumatoid arthritis | 6 (5.3) |
| Morbus. Bechterew | 4 (3.5) |

On the basis of comorbidity patients were classified in the ASA scoring system groups. The mean ASA score was 2.1. The distribution of patients in the ASA scoring groups is shown in Table 2.

**Table 2**

**Distribution of patients in the ASA scoring system groups**

| ASA score | Patients, n (%) |
|---|---|
| 1 | 16 (14.2) |
| 2 | 70 (61.9) |
| 3 | 26 (23) |
| 4 | 1 (0.9) |
| Total | 113 (100) |

**ASA - American Society of Anesthesiologists**

Mean body mass index (BMI) was 26.3 ± 3.2 kg/m$^2$ (17–37 kg/m$^2$). Primary coxarthrosis was etiologic factor in the majority of patients – 69 (61.1%), (Figure 1).

Mean postoperative hospitalization stay was 10.2 ± 4.8 days (3–34 days). By following hematologic status and clinical parameters of anemia, blood substitution was indicated. Mean amount of transfusion was 1,275.64 ± 567.626 mL and 97.3% of patients were transfused.

There was one deep wound infection, *Pseudomonas spp*. were isolated in the culture. This complication was managed by surgical debridement and antibiotic therapy according to antibiogram. One patient acquired luxation of one hip postoperatively. After attempting manual reposition, radiography was performed and migration of acetabular part of endoprosthesis was detected. This complication was managed by acetabular part revision surgery. Postoperatively, one patient had clinical signs of leg thrombosis, consulted vascular surgeon indicated Doppler ultrasound and laboratory examination. As the thrombosis was confirmed, therapy was successfully continued with low molecular weight heparin. One patient suffered dyspnea postoperatively, pulmonologist was consulted, and bilateral pneumonia was established as diagnosis. Symptoms resigned after applying antibiotics and supportive therapy as indicated by the consultant.

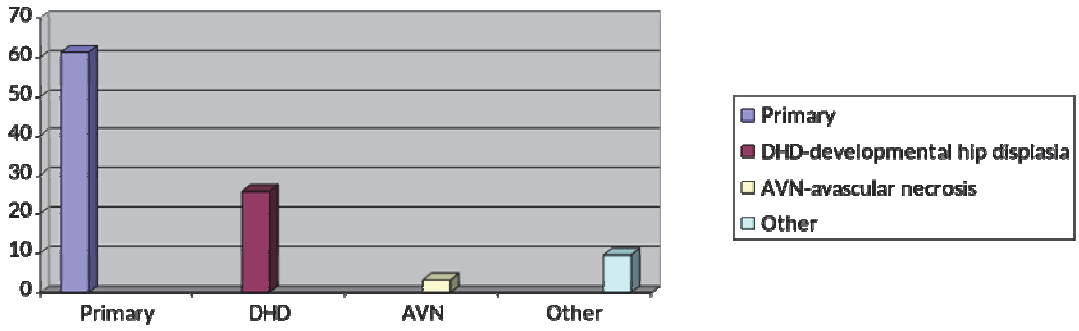


**Fig. 1 – Etiology of coxarthrosis.**

## Discussion

The first bilateral hip arthroplasty in a single procedure was described in 1967 [17], and Jaffe and Charnley [18] (1971) have published an article analyzing their experience with this procedure. Authors concluded that there was an increased risk for complications performing simultaneous procedure with the advantages of shorter hospital stay, single anesthesia and single rehabilitation period. Subsequently, several authors reported their results of bilateral hip arthroplasty in a single procedure without consensus for patients selection criteria and perioperative safety compared to the unilateral procedure.

Comorbidity is the most important factor considering a possibility for the simultaneous bilateral procedure. Some authors use general terms such as: patients should be without significant comorbidities [2, 12], patients should be in good overall health [8, 19], or they endeavor to perform the procedure on healthy and younger patients [20].

In available literature, authors who used scoring systems for standardizing selection of patients uniformly use the ASA score, but the viewpoint of groups of ASA scores that should be candidates for safely performing these procedure are controversial: some recommend performing procedure in the ASA 1 and 2 groups [5, 13, 14], others operated on patients with the ASA scores 1-4 [4], while some of authors claim that the procedure is safe for performing in patients graded as the ASA scores 1 and 2, and probably the ASA scores 3 and 4 [21]. In our study the majority of patients were with the ASA score 2, but operation was also successfully performed on patients with the ASA score 3 [26 (23%) patients], and even one patient (0.97%) with the ASA score 4.

Analysis of our patients confirmed findings of authors that claimed not to find increased percentage of complications while performing bilateral simultaneous procedure comparing to the unilateral one. Our complication rates were within rates cited in the literature [22]. Symptomatic forms of thromboembolic complications in total hip arthroplasty with thromboprophylactic therapy have a rate around 1.3% [22]. We detected 1 (0.88%) complication of this type that was managed successfully without consequences. Despite the trend towards raising comorbidity in hip arthroplasties during years, reported mortality rates are gradually decreasing. Reported mortality rates vary from 0.3% to 0.6% [23]; our series showed no mortality during 30-day postoperative follow-up.

Infection is one of the most devastating complications of total joint arthroplasty. Advances in surgical technique, implants improvements, managing of comorbidity known to contribute to infections lead to significant reduction of repor-

ted infection rates in total hip arthroplasty. Currently reported rates of infection in joint arthroplasties range 0.7–2.3% [24], with significantly higher surgical site infection rate in knee arthroplasties compared to hip arthroplasties [25]. We recorded one infection in 113 operated patients (0.88%), having rate within that reported in the literature.

Endoprosthesis dislocation is the third most frequently reported complication. Majority of dislocations (70%) happen within the first 6 weeks after the surgery. It is considered that early dislocations have better outcome compared to the late ones (more than 3 months after surgery). Inadequate endoprosthesis components orientation is considered as a major cause for an early dislocation, while in late dislocations there are multiple etiologic factors [26, 27]. We recorded one (0.88%) dislocation caused by inadequate acetabular component fixation and consequent migration with unfavorable orientation.

Elective orthopedic surgery, especially arthroplastic procedures are related to blood loss and the need for transfusion. Allogenic transfusions carry risks: trend towards infection [28], immunomodulation, and pathogen transmission [29]. Various techniques were developed to reduce the possibility of these complications and the need for transfusion: autologous blood pre-donation, intraoperative blood cell salvage, erythropoietin, tranexamic acid, and normovolemic hemodilution [30]. Reported rates for allogenic transfusion of red blood cells are 21–70% [31, 32]. In our series of 113 patients, 110 of them received transfusion, mean volume 1,275 mL. Average number of transfusions was 3. It is considerably higher value compared to the contemporary trends in blood substitution in elective orthopedic surgery. Explanation for this could be in the absence of routine use of tranexamic acid and clear protocols for blood substitution, so it is necessary to raise work quality in this area in the future.

## Conclusion

Based on our ten-year experience we consider simultaneous bilateral total hip arthroplasty in selected patients a safe procedure that does not carry higher risks of perioperative complications compared to the two stage procedure. Multidisciplinary approach is advisable in patient selection, and procedure should be conducted in facilities which enable this approach. Analysis of our results supports the conclusion that the ASA scores 1, 2 and 3 patients are good candidates for safe procedure, while we have no sufficient data for the ASA 4. One of the most important findings of our study is the need to introduce contemporary perioperative blood management protocol as a routine.

## REFERENCES


1. *Allen K, Golightly Y.* State of the evidence. Curr Opin Rheumatol 2015; 27(3): 276-283.
2. *Aghayev E, Beck A, Staub L, Dietrich D, Melloh M, Orljanski W*, at al. Simultaneous bilateral hip replacement reveals superior outcome and fewer complications than two- stage procedures: a prospective study including 1819 patients and 5801 follow-ups from a total joint replacement registry. BMC Musculoskelet Disord 2010; 11: 245.
3. *Stavrakis AI, SooHoo NF, Lieberman JR.* Bilateral Total Hip Arthroplasty has Similar Complication Rates to Unilateral Total Hip Arthroplasty. J Arthroplasty 2015; 30(7): 1211–4.
4. *Garland A, Rolfson O, Garellick G, Kärrholm J, Hailer N.* Early postoperative mortality after simultaneous or staged bilateral primary total hip arthroplasty: an observational register study from the swedish Hip arthroplasty register. BMC Musculoskelet Disord 2015; 16: 77.
5. *Trojani C, d'Ollone T, Saragaglia D, Vielpeau C, Carles M, Prudhon JL.* One stage bilateral total hip arthroplasty: Functional outcome and complications in 112 patients Orthop Traumatol Surg Res 2012; 98(6): S120–3.
6. *Namba RS, Inacio MC, Paxton EW.* Risk factors associated with surgical site infection in 30 491 primary total hip replacements. J Bone Joint Surg Br 2012; 94(10): 1330–8.
7. *Berend ME, Ritter MA, Harty LD, Davis KE, Keating EM, Meding JB*, at al. Simultaneous bilateral versus unilateral total hip arthroplasty. J Arthroplasty 2005; 20(4): 421–6.
8. *Berend KR, Lombardi AV Jr, Adams JB.* Simultaneous vs staged cementless bilateral total hip arthroplasty: perioperative risk comparison. J Arthroplasty 2007; 22(6 Suppl 2): 111–5.
9. *Yeager AM, Ruel AV, Westrich GH.* Are bilateral total joint arthroplasty patients at a higher risk of developing pulmonary embolism following total hip and knee surgery? J Arthroplasty 2014; 29(5): 900–2.
10. *Rasouli MR, Maltenfort MG, Ross D, Hozack WJ, Memtsoudis SG, Parvizi J.* Perioperative morbidity and mortality following bilateral total hip arthroplasty. J Arthroplasty 2014; 29(1): 142–8.
11. *Glait SA, Khatib ON, Bansal A, Hochfelder JP, Slover JD.* Comparing the incidence and clinical data for simultaneous bilateral versus unilateral total hip arthroplasty in NewYork State between 1990 and 2010. J Arthroplasty 2015; 30(11): 1887–91.
12. *Popović Z, Rajović J, Radunović A.* Total bilateral hip arthroplasty in one surgical procedure. Vojnosanit Pregl 2007; 64(10): 697–700. (Serbian)
13. *Lamo-Espinosa J, Troncoso S, Valentí-Azcárate A, Díaz de Rada P, Valentí-Nín JR.* Clinical and radiological short-term complications after single stage bilateral uncemented total hip arthroplasty. Musculoskelet Surg 2015; 99(1): 67–73.
14. *Romagnoli S, Zacchetti S, Perazzo P, Verde F, Banfi G, Viganò M.* Simultaneous bilateral total hip arthroplasties do not lead to higher complication or allogeneic transfusion rates compared to unilateral procedures. Int Orthop 2013; 37(11): 2125–30.
15. *Reuben JD, Meyers SJ, Cox DD, Elliott M, Watson M, Shim SD.* Cost comparison between bilateral simultaneous, staged, and unilateral total joint arthroplasty. J Arthroplasty 1998; 13(2): 172–9.
16. *Wykman A., Olsson E.* Walking ability after total hip arthroplasty: a comparison of gait analysis in unilateral and bilateral cases. J Bone Joint Surg Br 1992; 74(1): 53–6.
17. *Lazansky M.* A Study of Bilateral LowFriction Arthroplasty. Internal Publication No. 3. Appley Bridge, UK: Centre for Hip Surgery, Wrightington Hospital; 1967.
18. *Jaffe WL, Charnley J.* Bilateral Charnley low-friction arthroplasty as a single operative procedure. A report of fifty cases. Bull Hosp Joint Dis 1971; 32(2): 198–214.
19. *Macaulay W, Salvati EA, Sculco TP, Pellicci PM.* Single-stage bilateral total hip arthroplasty. J Am Acad Orthop Surg 2002; 10(3): 217–21.

20. *Parvizi J, Tarity TD, Sheikh E, Sharkey PF, Hozack WJ, Rothman RH.* Bilateral total hip arthroplasty: one-stage versus two-stage procedures. Clin Orthop Relat Res 2006; 453: 137–41.
21. *Bhan S, Pankaj A, Malhotra R.* One- or two-stage bilateral total hip arthroplasty: a prospective, randomised, controlled study in an Asian population. J Bone Joint Surg Br 2006; 88(3): 298–303.
22. *Samama CM, Ravaud P, Parent F, Barre J, Mertl P, Mismetti P.* Epidemiology of venous thromboembolism after lower limb arthroplasty: the FOTO study. J Thromb Haemost 2007; 5(12): 2360–7.
23. *Liu SS, Della Valle AG, Besculides MC, Gaber LK, Memtsoudis SG.* Trends in mortality, complications, and demographics for primary hip arthroplasty in the United States. Int Orthop 2009; 33(3): 643–51.
24. *Jaekel DJ, Ong KL, Lau EC, Watson HN, Kurtz SM.* Epidemiology of total hip and knee arthroplasty infection. In*: Springer BD, Parvizi J,* editors. Periprosthetic joint infection of the hip and knee. New York: Springer; 2014. pp. 1–14. Chapter 1.
25. *Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J.* Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty 2008; 23(7): 984–91.
26. *Skutek M, Bourne RB, MacDonald SJ.* (i) International epidemiology of revision THR. Curr Orthop 2006; 20(3): 157–61.
27. *Sanchez-Sotelo J, Berry DJ.* Epidemiology of instability after total hip replacement. Orthop Clin North Am 2001; 32(4): 543–52, vii.
28. *Patel PD, Potts A, Froimson MI.* The dislocating hip arthroplasty: prevention and treatment. J Arthroplasty 2007; 22(4 Suppl 1): 86–90.
29. *Innerhofer P*, *Walleczek C*, *Luz G*, *Hobisch-Hagen P*, *Benzer A*, *Stöckl B*, et al. Transfusion of buffy coat-depleted blood components and risk of postoperative infection in orthopedic patients. Transfusion 1999; 39(6): 625–32.
30. *Rawn J.* The silent risks of blood transfusion. Curr Opin Anaesthesiol 2008; 21(5): 664–8.
31. *Aderinto J, Brenkel IJ.* Pre-operative predictors of the requirement for blood transfusion following total hip replacement. J Bone Joint Surg Br 2004; 86(7): 970–3.
32. *Rosencher N, Kerkkamp HE, Macheras G, Munuera LM, Menichella G, Barton DM,* et al. Orthopedic Surgery Transfusion Hemoglobin European Overview (OSTHEO) study: blood management in elective knee and hip arthroplasty in Europe. Transfusion. 2003; 43(4): 459–69.

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# Validation of Serbian version of chronic obstructive pulmonary disease assessment test

## Validacija sprske verzije upitnika za procenu hronične opstruktivne bolesti pluća

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**Abstract**

**Background/Aim.** The Chronic obstructive pulmonary disease (COPD) Assessment Test (CAT) is a simple and reliable tool designed to measure overall COPD related health status and complement physician assessment in routine clinical practice. Objective of this study was to evaluate the validity of the Serbian version of CAT. **Methods.** Study included 140 outpatients in the stable COPD, recruited from two centres: Clinic for Pulmonology, Clinical Center of Serbia, Belgrade, and Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica. All patients completed pulmonary function testing – spirometry, the CAT and the modified Medical Research Council (mMRC) dyspnea scale at baseline visit. The CAT test-retest reliability was tested in 20 patients by the same investigator (physician). **Results.** We demonstrated that Serbian version of CAT had high internal consistency with Cronbach's alpha 0.88. Test-retest analysis showed good correlation between CAT scores in two time points (Spearman's $\varrho = 0.681$, $p < 0.01$). In our study the CAT correlated moderately to mMRC scale ($\varrho = +0.57$), weakly to $FEV_1$ ($\varrho$ -0.214), was positively related to number of exacerbations, but did not showed exact regularity with change in the Global Initiative for Chronic Obstructive lung disease (GOLD) stage. **Conclusion.** The Serbian version of CAT is a reliable, simple and easy-to-use tool that can be used in everyday clinical practice to assess the health status of COPD patients in Serbia.

**Key words:**
**pulmonary disease, chronic obstructive; surveys and questionnaires; serbia; comorbidity; forced expiratory volume.**

**Apstrakt**

**Uvod/Cilj.** Upitnik za procenu hronične opstruktivne bolesti pluća (HOBP) (engl. *COPD Assessment Test* - CAT) je jednostavan i pouzdan test namenjen za merenje ukupnog zdravstvenog stanja bolesnika sa HOBP i koristan je za upotrebu u svakodnevnoj kliničkoj praksi. Cilj ovog istraživanja bio je da se proceni validnost i opravdanost primene srpske verzije CAT. **Metode.** U studiji je učestvovalo 140 bolesnika u stabilnom stanju HOBP, ispitivanih u ambulantnim uslovima na Klinici za pulmologiju, Kliničkog centra Srbije u Beogradu i Institutu za plućne bolesti Vojvodine u Sremskoj Kamenici. Tokom prvog pregleda bolesnicima je učinjeno ispitivanje plućne funkcije (spirometrija), popunili

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su CAT upitnik i mMMR (*modified Medical Research Council*) skalu za procenu stepena dispneje. Pouzdanost CAT test-retesta je ispitivana kod 20 bolesnika od strane istog istraživača. **Rezultati.** Pokazali smo da srpska verzija CAT ima visoku internu konzistentnost sa Cronbach-ovim alfa 0.88. Test-retest analiza pokazala je dobru korelaciju između CAT rezultata u dve vremenske tačke (Spearmanov r = 0,681; $p < 0,01$). CAT je umereno korelirao sa mMRC skalom (r = + 0,57), blago sa forsiranim ekspiratornim volumenom u prvoj sekundi ($FEV_1$), (r -0,214), uz pozitivnu korelaciju sa ukupnim brojem pogoršanja HOBP, ali bez jasne regularnosti sa promenom GOLD (*Global Initiative for Chronic Obstructive Lung Disease*) stadijuma. **Zaključak.** Srpska verzija CAT je pokazala visoku internu konzistentnost i test-retest pouzdanost. Ona predstavlja pouzdano, jednostavno i lako sredstvo za upotrebu koje se može koristiti u svakodnevnoj kliničkoj praksi za procenu zdravstvenog stanja kod bolesnika sa HOBP u Srbiji.

**Ključne reči:**
**pluća, opstruktivne bolesti; hronične; ankete i upitnici; srbija; komorbiditet; ekspiratorni volumen, forsirani.**

## Introduction

The Global initiative for Chronic Obstructive Lung Disease (GOLD) strategy document defined chronic obstructive pulmonary disease (COPD) as a preventable and treatable disease, with persistent respiratory symptoms and airflow limitation [1]. Smoking is the main risk factor for disease development along with environmental exposures to biomass fuels and air pollution. Also, individual predisposing factors such as genetic abnormalities, abnormal lung development, and accelerated aging contribute to COPD developing. A course of the disease is often progressive and associated with significant comorbidities which increase its morbidity and mortality.

COPD is one of the most common diseases with a global prevalence of 11.7% [2]. Prevalence of chronic bronchitis symptoms in Belgrade, Serbia was 21.6% [3]. Also, in the other study COPD diagnosis, confirmed by the pulmonologist, was in 21.9% of the patients and newly diagnosed COPD in 10.9% of the patients [4].

COPD is one of the fastest growing causes of death and it is expected to be the 3rd cause of mortality by 2020 worldwide [1]. The disease may cause disability and the quality of life is one of the treatment goals. Health status assessment in COPD patients is a routine in clinical research studies with comprehensive but time-consuming
tools such as the St. George's Respiratory Questionnaire (SGRQ), the Chronic Respiratory Disease Questionnaire (CRQ) and the COPD Clinical Questionnaire (CCQ) [5–7].

The COPD Assessment Test (CAT) is a simple and reliable tool designed to measure overall COPD-related health status and complement physician assessment in routine clinical practice [8]. It is a short, self-administered, eight-item questionnaire that includes symptoms, limitation of daily activity, sleep quality and energy, providing a single score. It is easy to complete by the patient and interpret by the clinician. The CAT was developed and validated internationally, in the Europe and United States, and has been translated into many languages worldwide [9].

The GOLD recognized the importance of the CAT in the multidimensional system of assessment of the disease severity and selection of pharmacological treatment, as well as monitoring of the disease.

The aim of this cross-sectional study was to evaluate the validity of the Serbian version of the CAT.

## Methods

The study was conducted in accordance with the Good Clinical Practice as outlined in the Declaration of Helsinki 2000. All necessary approvals for the trial were obtained from respective institutional review and ethical boards.

### *Study population and design*

The study was independently conducted, and 140 patients were recruited from two Serbian centres: the Clinic for Pulmonology, Clinical Center of Serbia, Belgrade (98 subjects) and the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica (42 subjects) from May 2017 to January 2018.

They were outpatients in the stable stage of COPD, older than 40 years of age, smokers or ex-smokers. COPD was diagnosed according to the GOLD criteria [1], not earlier than 6 months prior to the study. Inclusion criteria were COPD stable stage and written informed consent. Exclusion criteria were: active respiratory disorder other than COPD, immunosuppression, or subjects unable to complete the questionnaires. Stable COPD is defined as no change in respiratory state in duration of 4 weeks that requires no change in therapy, or systemic steroids and/or antibiotics use. Patient characteristics included demographic information, smoking, COPD and exacerbations history, therapy and comorbidities (cardiovascular diseases – heart failure, arterial hypertension, ischemic heart disease, arrhythmia, peripheral artery diseases, osteoporosis, depression, diabetes, and gastroesophageal reflux). At the baseline visit, the patient's breathlessness was assessed using the Modified Medical Research Council (mMRC) dyspnea scale, and spirometry test was performed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) spirometry guidelines [10]. Patients are further classified according to the airflow limitation severity based on post-bronchodilator forced expiratory volume in the first second ($FEV_1$) in patients with $FEV_1$/forced volume vital capacity (FVC) < 0.70 to the GOLD stages: mild – GOLD 1 ($FEV_1 \geq 80\%$), moderate

– GOLD 2 ($FEV_1$ 50–79%), severe –GOLD 3 ($FEV_1$ 30–49%), and very severe –GOLD 4 (FEV1 < 30%). The CAT was administered to all the patients at the baseline visit by the same investigator (physician). The CAT was again administered to twenty patients by the same investigator, at the second visit, 14 days after the first one.

*Questionnaire*

The CAT is a disease-specific questionnaire assessing health status in individuals with COPD. The CAT and CAT logo is a trade mark of GlaxoSmithKline group of companies. The CAT can be freely used. For this study we used already available Serbian translation of CAT written in consistent and understandable language, so there was no need for back-translation analysis. The CAT consists of the following eight items, each formatted as a minimum and maximum score of 0 to 5, respectively: cough, phlegm, chest tightness, and breathlessness going up hills/stairs, activity limitations at home, confidence leaving home, sleep, and energy. Individual item scores are summarized to provide a total CAT score that can range from 0 (floor) to 40 (ceiling).

*Statistical analysis*

Internal consistency of the CAT questionnaire was tested by Cronbach's α coefficient analysis. Correlation analysis between the CAT score and mMRC dyspnea scale was performed, so as correlation between CAT score and pulmonary function measures [$FEV_1$ (L, %), FVC (L, %), $FEV_1$/FVC]. Test-retest analysis obtained as correlation analysis between CAT scores in two time points, was performed by the same investigators (physicians). Differences between continuous variables were tested by using Student's *t* test, ANOVA for variables with normal distribution, or Mann-Whitney *U* test or Kruskal-Wallis nonparametric ANOVA for parameters which distribution deviated from normal Gaussian distribution pattern. Differences in frequency of categorical variables were tested with $\chi^2$ test. Correlation analysis performed by using Spearman's nonparametric correlation methods. All differences were set at 0.05 alpha.

## Results

From May 2017 to January 2018, 140 patients with COPD completed the CAT questionnaire and mMRC dyspnea scale, and 20 patients completed the CAT test in two time points, performed by the same investigator (physician). General characteristic of subjects (mean age 64.4 ± 9.3 years; 84 men and 56 women) are summarized in Table 1. The majority of subject were ex-smokers (n = 82; 60%), and the rest were active smokers (n = 58; 41%). There were no subjects who never smoked. The mean body mass index (BMI) was 26.6 ± 5.4 $kg/m^2$. Average $FEV_1$ was 47.6 ± 19.1% which indicated moderate to severe airflow limitation. Average mMRC score was 1.93 ± 1.11 and average CAT was 19.5 ± 8.9, which implied that patients had more symptoms and more pronounced breathlessness (Table 1).

The Cronbach's α was 0.887 for the CAT test. Neither Cronbach's α item deleted value was not larger than basic value of 0.887, so we concluded that all questions are consistent with the questionnaire topic. We found significant positive correlation between the CAT score and mMRC score ($\rho = +0.570$; $p < 0.001$). The CAT score showed weak but significant negative correlation with FVC (L): $\rho = -0.274$; $p < 0.01$) and $FEV_1$ (L): (-0.214; $p < 0.05$), but did not correlate with any other pulmonary function measure. Test-retest analysis showed good correlation between CAT scores in two time points (Spearman's $\rho = 0.681$; $p < 0.01$).

**Table 1**

**General anthropometric, demographic, pulmonary function data and average modified Medical Research Council Dyspnea Scale (mMRC) and Chronic Obstructive Pulmonary Disease Assessment Test (CAT) score in the study population**

| Parameter | Values |
|---|---|
| Age (years), mean ± SD | 64.4 ± 9.3 |
| Gender (male/female), n (%) | 84/56 (60/40) |
| Nonsmoker/ex-smoker/current smoker, n (%) | 0/82/58 (0/59/41) |
| BMI ($kg/m^2$), mean ± SD | 26.6 ± 5.4 |
| Post-BD FEV1 (L), mean ± SD | 1.27 ± 0.59 |
| Post-BD FEV1 (%), mean ± SD | 47.6 ± 19.1 |
| Post-BD FVC (L), mean ± SD | 2.73 ± 0.92 |
| Post-BD FVC (%), mean ± SD | 81.9 ± 21.0 |
| Post-BD FEV1/FVC, mean ± SD | 46.2 ± 12.3 |
| mMRC score, mean ± SD | 1.93 ± 1.11 |
| Total CAT score, mean ± SD | 19.5 ± 8.9 |

**BMI – body mass index; BD-$FEV_1$ – bronchodilator-forced expiratory volume in the first second; BD-FVC – bronchodilator-forced volume vital capacity; SD – standard deviation.**

Next, patients were classified according to the GOLD stage (I–IV). In the GOLD stage groups we compared different general, anthropometric, clinical and pulmonary function parameters, exacerbation status and maintenance therapy (Table 2). There was no difference in distribution of ex-smokers and smokers, and cumulative smoking status expressed in pack/years among GOLD I–IV groups. Patients with longer disease duration tended to be in higher GOLD stage groups, but there was no significant difference. Average COPD duration was from 4 years in the GOLD I group to 8.5 years in the GOLD IV group. Patients with higher GOLD stage had significantly more acute exacerbation episodes (GOLD III and IV groups compared to II and I), and also higher number of exacerbations requiring hospitalization (especially the GOLD IV group compared to other three groups). Regarding maintenance therapy higher percentage of patients used long acting muscarinic antagonists (LAMA) and inhaled glucocorticoid (ICS)/long acting bronchidilators (LABA) in higher GOLD stage groups. The mMRC breathlessness score was highest in the GOLD IV group compared to other three GOLD groups. On the contrary, the CAT total score did not show exact regularity with the GOLD stage change (Table 2).

**Table 2**

**Demographic, clinical and functional characteristics, exacerbations and therapy of the study population related to the GOLD stadium classification**

| Parameter | GOLD I (n = 10) | GOLD II (n = 48) | GOLD III (n = 53) | GOLD IV (n = 30) | *p* |
|---|---|---|---|---|---|
| Age, years | 63.4 ± 6.4 | 65.2 ± 9.8 | 65.7 ± 8.4 | 58.8 ± 9.3[c] | 0.041 |
| Smoking status, (smoker/ex smoker), n (%) | 5/5 (8.6/6.0) | 19/29 (32.8/34.9) | 22/31 (37.9/37.3) | 12/18 (20.7/21.7) | 0.945 |
| BMI (kg/m$^2$), mean ± SD | 30.0 ± 6.7 | 27.7 ± 5.5 | 26.8 ± 4.7 | 23.2 ± 4.9[a] | 0.038 |
| Pack years, mean ± SD | 42.1 ± 20.0 | 33.8 ± 17.4 | 34.6 ± 17.3 | 33.7 ± 20.5 | 0.725 |
| COPD duration, years* | 4.0 (3.00–9.50) | 6.0 (4.0–9.0) | 8.0 (4.0–12.0) | 8.5 (5.50–13.50) | 0.137 |
| Acute exacerbation, n* | 1.00 (0.0–1.0) | 1.00 (0.0–2.0) | 2.00 (1.0–3.0)[aa] | 3.00 (1.5–3.0)[aaa,bb] | < 0.001 |
| Exacerbation requiring hospitalization, n* | 0 (0–0) | 0 (0–0) | 0 (0–1) | 1 (0–2)[a,b] | 0.006 |
| FEV1 (%), mean ± SD | 86.6 ± 9.9 | 60.8 ± 8.2[aaa] | 39.7 ± 5.8[aaa,bbb] | 24.4 ± 3.4[aaa,bbb,ccc] | < 0.001 |
| FEV1 (L), mean ± SD | 2.38 ± 0.63 | 1.60 ± 0.47[aaa] | 1.08 ± 0.29[aaa,bbb] | 0.69 ± 0.17[aaa,bbb,ccc] | < 0.001 |
| FVC (%), mean ± SD | 107.6 ± 11.1 | 94.3 ± 13.5 | 78.6 ± 17.3[aaa,bbb] | 59.4 ± 15.4[aaa,bbb,ccc] | < 0.001 |
| FVC (L), mean ± SD | 3.64 ± 0.66 | 3.05 ± 0.91 | 2.63 ± 0.84[aa] | 2.10 ± 0.674[aaa,bbb,ccc] | < 0.001 |
| FEV1/FVC, mean ± SD | 65.1 ± 8.5 | 53.2 ± 8.9[aaa] | 42.9 ± 8.6[aaa,bbb] | 34.4 ± 9.4[aaa,bbb,ccc] | < 0.001 |
| LAMA therapy, n (%) | 5 (71) | 31 (93.9) | 37 (97.4) | 20 (100) | 0.024 |
| LABA therapy, n (%) | 1 (14.3) | 6 (18.2) | 7 (18.4) | 4 (20.0) | 0.852 |
| ISC/LABA therapy, n (%) | 3 (42.9) | 17 (51.5) | 30 (78.9) | 16 (80.0) | 0.025 |
| mMRC dyspnea score, mean ± SD | 1.00 ± 0.82 | 1.70 ± 1.10 | 2.11 ± 1.08 | 2.50 ± 0.95[aa,b] | 0.009 |
| CAT total score* | 15.0 (9.0–21.0) | 18.5 (10.5–24.5) | 21.0 (16.0–25.0) | 20.0 (15.0–28.0) | 0.314 |

**GOLD – Global initiative for Chronic Obstructive Lung Disease**; **BMI – body mass index; COPD – chronic obstructive pulmonary disease; $FEV_1$ – forced expiratory volume in the first second; FVC – forced volume vital capacity; LAMA – long acting muscarinic antagonist; LABA – long acting bronchodilators; ICS – inhaled glucocorticoid; mMRC – modified Medical research council; CAT – COPD assessment test.**

***parameters with non-normal distribution presented as median (25th–75th percentile) values; *p* from parametric (ANOVA) or from non-parametric (Kruskal-Wallis) tests; a, b, c letters indicate significant difference compared to the GOLD I, the GOLD II and the GOLD III group, respectively (one letter – $p < 0.05$, two letters – $p < 0.01$, three letters – $p < 0.001$).**

Relation between the CAT score and presence of comorbidities, all and cardiovascular, and number of exacerbation/year are summarized in Table 3.

**Table 3**

**CAT score values, comorbidities and exacerbation rates**

| Impairment | Patients n (%) | mean ± SD | *p* |
|---|---|---|---|
| Comorbidities | | | |
| none | 70 (50) | 17.2 ± 8.5 | 0.184 |
| At least one | 70 (50) | 20.1 ± 10.2 | |
| CVB comorbidity | | | |
| none | 59 (42) | 18.2 ± 9.0 | 0.539 |
| At least one | 81 (58) | 16.9 ± 8.7 | |
| Exacerbations | | | |
| none | 26 (18) | 12.0 ± 6.4 | |
| 1 | 33 (24) | 17.3 ± 10.2 | 0.003 |
| ≥ 2 | 81 (58) | 20.0 ± 8.3[aa] | |

**CAT – Chronic Obstructive Pulmonary Disease Assessment Test; CVB – cerebrovascular burden.**

***p* from ANOVA ([aa] $p < 0.001$ vs. group without exacerbation).**

There was no significant difference in the CAT score between subjects with or without investigated comorbidities. On the contrary, an increase in exacerbation number was related consistently with higher CAT score ($p < 0.01$), so patients with 2 or more exacerbations during one year had significantly higher CAT score (Table 3). We also compared CAT scores between body mass index (BMI subgroups, but neither any regularity nor statistical significance were found.

## Discussion

The study demonstrated good internal consistency and reliability of the CAT score in a population of COPD patients in Serbia. The CAT correlated moderately with the mMRC scale, did not differ significantly across spirometric GOLD stages and was higher in patients who experienced frequent exacerbations.

The CAT is a short (8-item), self-administered questionnaire developed by Jones et al. [5, 8] for the purpose of measuring health status of patients with COPD. It was derived from the data from three international observational

prospective studies including 1,503 COPD patients from Belgium, France, Germany, the Netherlands, Spain and the USA following rigorous methodological approach and was subsequently validated in the subgroup of patients from the USA. This study showed excellent consistency of the questionnaire with the Cronbach's alpha of 0.88 and a good reliability [8]. Soon after it was developed, the CAT was incorporated into the GOLD guidelines as a part of a multidimensional assessment of COPD patients. Currently, the CAT is the preferred method for symptom assessment over traditionally used the unidimensional mMRC scale that measures only breathlessness, as it is more comprehensive (GOLD). Also, the CAT demonstrated a very good correlation with the SGRQ that is commonly used to access the impact of COPD on health status in clinical trials but is too complex for use in a busy every day practice [8].

Since the CAT questionnaire was developed and validated in English language it is possible that cultural, social, and linguistic differences may affect its performance in other populations. Hence, after its publication in 2009, the CAT has been translated and validated in various countries including Japan [11], Indonesia, Korea, Vietnam [12], Thailand [13], Brazil [14], Turkey [15], Iran [16] and Arabic speaking countries [17]. To our knowledge our study was the first that validated the use of the CAT in Serbian language.

We demonstrated that the Serbian version of CAT has high internal consistency with Cronbach's alpha 0.88 that is identical to original version of CAT [8] and comparable to other validation studies in which Cronbach's alpha ranged from 0.73 to 0.98 thus exhibiting high item correlation [16, 18]. The demographic characteristics of our study population were similar to derivation cohort [8] with 60% men, mean age 64 years, but our patients had more severe airway obstruction (mean FEV1 47.6 ± 19.1% predicted compared to 52.3 ± 18.9% predicted in the US and 57.8 ± 19.9% predicted in the EU cohort). The mean values of the CAT score was 19.5 ± 8.9 indicating that patients had high symptom burden and pronounced breathlessness which is comparable to CAT scores in studies from Belgium, France, Germany, US, Portugal and Asian population [12, 14, 19].

Test-retest reproducibility measured at two time points in our study was good (Spearman's $\rho = 0.681$) and consistent with other validation studies. When compared to other important functional and physiological variables, the CAT correlated moderately to the mMRC scale ($\rho = +0.57$) and weakly to FEV1 ($\rho$ -0.214). This is in line with previous studies in which the correlation between the CAT and mMRC scale ($\rho = 0.29$–$0.61$) and FEV1 ($\rho = -0.56$–$-0.23$) was found to be moderate at best [18]. Although in our study more severe COPD patients (the groups 3 and 4) had higher CAT scores the difference between the COPD groups was not significant. By contrast, a cross-sectional European study [19] showed a constant increase of the CAT score across COPD stages with 3 points difference between the classes.

In our study, the CAT was positively related to number of exacerbations. Frequent exacerbators (≥ 2 exacerbations) had higher CAT scores compared to non exacerbators (20.0 ± 8.3 vs. 12.0 ± 6.4, respectively). Previous studies have also demonstrated that infrequent exacerbators have lower values of the CAT score compared to patients with ≥ 2 exacerbations [20, 21]. In addition, CAT values are shown to be higher in patients experiencing exacerbation compared to stable patients with a mean difference of 4.7 units between the groups [8, 19, 22].

We found no difference in CAT scores in patients with and without comorbidities. This is consistent with study by Kwon et al. [12] in Asian population but differs from large European study where presence of 3 or more comorbidities has been associated with higher CAT scores [19]. This may be due to a difference in sample size, as study by Jones et al. [12] included significantly higher number of patients that allowed subgroup analysis. Similarly to aforementioned studies we found no difference in CAT scores between patients with and without cardiovascular disease. Also, in contrast to previous data that showed that patients with lower BMI have higher CAT scores [12] in our study the CAT score did not differ across BMI groups.

Our study has several limitations. First, the study included COPD patients from two academic pulmonary hospitals that are not necessarily generalizable to all COPD patients in Serbia. Second, although number of patients is comparable to other questionnaire validation studies, our study was likely underpowered to detect between group differences which may explain the observed dissimilarities (such as non-significant differences in CAT values across GOLD stages and BMI groups) when compared to larger studies. Nevertheless, this is the first study to validate the CAT in Serbian language that confirmed its reliability and consistency.

## Conclusion

The Serbian version of the CAT is an easy to administer and reliable tool that could be used in everyday clinical practice for assessment of health status in Serbian COPD patients.

## REFERENCES


1. *Global Initiative for Chronic Obstructive Lung Disease.* GOLD 2017 Global Strategy for the Diagnosis, Management and Prevention of COPD. Available from: http://goldcopd.org.
2. *Adeloye D, Chua S, Lee Ch, Basquill C, Papana A, Theodoraton E*, et al. Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. J Glob Health 2015; 5(2): 020415.
3. *Milenkovic B, Mitic-Milikic M, Rebic P, Vukcevic M, Dudvarski-Ilic A, Nagorni-Obradovic L*, et al. Asthma and chronic bronchitis symptoms among adult population of Belgrade. Srp Arh Celok Lek 2011; 139 (3–4): 149–54.
4. Vukoja M, Rebic P, Lazic Y, Mitic-Milikic M, Milenkovic B, Zvezdin B, et al. Early detection of asthma and chronic obstructive pulmonary disease in primare care patients. Med Pregl 2013; 66(1–2): 46–52.

5. *Jones PW, Quirk FH, Baveystock CM*. The St George s Respiratory Questionnaire. Respir Med 1991; 85 Suppl B: 25–31 discussion 33–7.
6. *Larson JL, Covey MK, Berry JK, Wirtz S, Kim MJ*. Reliability and validity of the Chronic Respiratory Disease Questionnaire. Am Rev Respir Dis 1993; 147: A 530.
7. *van der Molen T, Willemse BW, Schokker S, ten Hacken NH, Postma DS, Juniper EF*. Development, validity and responsiveness of the Clinical COPD Questionnaire. Health Qual Life Outcomes 2003; 1: 13.
8. *Jones PW, Harding G, Berry P, Wiklung I, Chen WH, Kline Leidy N*. Development and first validation of the COPD Assessment Test. Eur Respir J 2009; 34(3): 648–54.
9. COPD Assessment Test. Available from: http://www.catestonline.org/.
10. *Miller MR, Hankinson J, Brusasco V, Burgas V, Burgas F, Casaburi R*, et al. Standardisation of spirometry. Eur Respir J 2005; 26(2): 319–38.
11. *Tsuda T, Suematsu R, Kamohara K, Kurose M, Arakawa I, Tomioka R*, et al. Development of the Japanese version of the COPD Assessment Test. Respir Investig 2012; 50(2): 34–9.
12. *Kwon N, Amin M, Hui DS, Jung KS, Lim SY, Ta HD*, et al. Validaty of the COPD assessment test translatedi Into local languages for Asian patients. Chest 2013; 143(3): 703–10.
13. *Pothirat C, Kiatboonsri S, Chuchattaworn C*. Validation of the new COPD assessment test translated into Thai in patients with chronic obstructive pulmonary disease. BMC Pulm Med 2014; 14: 193.
14. *Silva GP, Morano MT, Viana CM, Magalhães CB, Pereira ED*. Portuguese-language version of the COPD Assessment Test: validation for use in Brazil. J Bras Pneumol 2013; 39(4): 402–8. (English, Portuguese)
15. Yorgancıoğlu A, Şakar Coşkun A. Is the diagnosis of asthma different in elderly? Tuberk Toraks 2012; 60(1): 81–5.
16. *Fakharian A, Masouleh SK, Karimzadeh S, Farhadi T*. Validation of the COPD assessment test in patients with COPD in Iran. Chron Obstruct Pulmon Dis 2017; 2: 22.
17. *Al-Moamary MS, Al-Hajjaj MS, Tamim HM, Al-Ghobain MO, Al-Qahtam HA, Al-Kassimi FA*. The reliability of an Arabic translation of the chronic obstructive pulmonary disease assessment test. Saudi Med J 2011; 32(10): 1028–33.
18. *Gupta N, Pinto LM, Morogan A, Bourbeau J*. The COPD assessment test: a systematic review. Eur Resp J 2014; 44(4): 873–84.
19. *Jones PW, Brusselle G, Dal Negro RW, Ferrer M, Kardos P, Levy ML*, et al. Properties of the COPD assessment test in a cross-sectional European study. Eur Respir J 2011; 38(1): 29–35.
20. *Gao Y, Hou Q, Wang H*. Assessment of Health Status in Patients with Newly Diagnosed Chronic Obstructive Pulmonary Disease. PloS One 2013; 8(12): e82782.
21. *Varol Y, Ozacar R, Balci G, Usta L, Taymaz Z*. Assessing the effectiveness of the COPD Assessment Test (CAT) to evaluate COPD severity and exacerbation rates. COPD. 2014; 11(2): 221–5.
22. *Agustí A, Soler JJ, Molina J, Muñoz MJ, García-Losa M, Roset M*, et al. Is the CAT questionnaire sensitive to changes in health status in patients with severe COPD exacerbations? COPD 2012; 9(5): 492–8.

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# Dental aspects of purging bulimia

## Dentalni aspekti bulimije praćene povraćanjem

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**Abstract**

**Background/Aim.** Bulimia is in many cases followed by frequent vomiting, which in long term can result in irreversible loss of dental tissue, most commonly manifested as dental erosion. Frequent purging, xerostomia, lack of oral hygiene and acidic environment are also suitable for caries development. The aim of the research was to determine the presence, localization and degree of dental erosion using Basic Erosive Wear Examination (BEWE) index system, as well as to determine the Decayed, Missing and Filled Teeth (DMFT) index in purging bulimic patients. **Methods.** The study involved 30 purging bulimic patients and 30 healthy subjects. Used methods were survey (questionnaire) and clinical examination. The clinical examination included intraoral inspection and assessment of dental status using BEWE and DMFT index. **Results.** On the bases of conducted research, it has been found that dental erosion are significantly more often present in purging bulimics compared to the controls ($\chi^2 = 5.963$, $p < 0.05$), that eroded lesions are more severe in the bulimic group ($t = 3.925$, $p < 0.05$) and predominantly located on oral surfaces of the teeth ($\chi^2 = 10.561$, $p < 0.05$). DMFT index values showed no significant difference between bulimic patients and controls ($t = 0.741$, $p = 0.461$). **Conclusion.** Dental erosion are often encountered in patients suffering purging bulimia, especially on oral surfaces of anterior teeth that come into direct contact with gastric acid, so many bulimics exhibit high values of erosive tooth wear on mentioned surfaces. DMFT index score did not show significant differences compared to healthy participants, but due to complexity of carious process further investigation is necessary.

**Key words:**
**bulimia; dental caries; oral health; risk assessment; surveys and questionnaires; tooth erosion; vomiting.**

**Apstrakt**

**Uvod/Cilj.** Bulimija je često praćena učestalim povraćanjem, koje u dužem vremenskom periodu može rezultirati ireverzibilnim gubitkom zubnog tkiva, koje se manifestuje dentalnim erozijama. Učestalo povraćanje, kserostomija, loša oralna higijena i kisela sredina pogoduju i razvoju karijesa. Cilj rada bio je utvrđivanje prisustva, lokalizacije i stepena dentalnih erozija upotrebom *Basic Erosive Wear Examination* (BEWE) indeksa, kao i utvrđivanje vrednosti indeksa karijesnih, ekstrahovanih i plombiranih zuba (KEP) kod obolelih od bulimije praćene povraćanjem. **Metode.** Istraživanjem je obuhvaćeno 30 ispitanika obolelih od bulimije praćene povraćanjem i 30 zdravih ispitanika. Korišćene su metode anketnog ispitivanja (upitnik) i kliničkog pregleda. Klinički pregled podrazumevao je intraoralnu inspekciju uz beleženje statusa prisutnih zuba upotrebom BEWE i KEP indeksa. **Rezultati.** Utvrđeno je da su dentalne erozije bile značajno češće kod obolelih od bulimije praćene povraćanjem u odnosu na zdrave ispitanike ($\chi^2 = 5.963$, $p < 0.05$), da su bile težeg stepena kod obolelih od bulimije ($t = 3.925$, $p < 0.05$), kao i da su bile češće na oralnim površinama zuba ($\chi^2 = 10.561$, $p < 0.05$). Između ispitivanih grupa nisu utvrđene značajne razlike u vrednostima KEP indeksa ($t = 0.741$, $p = 0.461$). **Zaključak.** Dentalne erozije se često sreću kod obolelih od bulimije praćene povraćanjem, posebno na oralnim površinama frontalnih zuba koje su u direktnom kontaktu sa želudačnom kiselinom, zbog čega na njima postoji značajan gubitak zubne supstance. Vrednosti KEP indeksa nisu se značajno razlikovale između ispitivanih grupa, ali su zbog složenosti karijesnog procesa neophodna dalja istraživanja.

**Ključne reči:**
**bulimija; zub, karijes; usta, zdravlje; rizik, procena; ankete i upitnici; zub, erozija; povraćanje.**

### Introduction

Eating disorders (ED) are serious mental, psychiatric and behavioral disorders with multifactorial etiology involving sociocultural, psychological and biological factors [1]. As one of the most common ED, bulimia is characterized by repeated episodes of binge eating and food restriction, strong fear of gaining weight, loss of control over food intake and

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inappropriate behavior in order to eliminate food (self-induced vomiting, abuse of purgatives and diuretics) [2, 3]. Since the incidence and prevalence of these disorders has increased in the past 20 years, numerious studies regarding etiology, prevention and medical complications have been conducted [4].

Regarding dental aspects of bulimia, frequent purging and impact of gastric juice are the most dominant factors causing hard tissue loss due to high acidity (mean pH 2.9), which is below critical pH for enamel dissolution [5, 6]. Dental erosion (DE) are early and most common oral finding among bulimics, usually encountered on the palatal surfaces of teeth as a consequence of intense and repeated gastric juice influence, without bacterial involvement or trauma [7–9]. Vomiting at least once a week is considered to be a risk factor for progression of dental erosion that can become clinically apparent after 6 months up to 2 years [5, 10, 11]. The number and severity of erosion increase with the duration of the ED, but many modifying factors that influence erosive process must be taken into consideration [12, 13]. Unfortunately, the process is irreversible, so early diagnosis of bulimia is crucial for prevention of oral complications [13]. Accompanying characteristics of bulimia, such as lack of oral hygiene, rich carbohydrate nutrition during binge, xerostomia and dominance of aciduric microbes, could increase risk of caries development [5, 6, 11, 12, 14]. However, studies concerning relationship between caries and bulimia are equivocal, and require questioning of many factors that influence and modify carious process [12–15]. In order to diagnose dental manifestations of bulimia and perceive all the factors that play a role in their evolvent, adequate knowledge about clinical features of mentioned lesions is necessary, as well as usage of detailed questionnaire.

The aim of the research was to determine the presence, localization and degree of dental erosion using Basic Erosive Wear Examination (BEWE) index system, as well as to determine the Decayed, Missing and Filled Teeth (DMFT) index in purging bulimic patients and healthy controls.

## Methods

A prospective, observational clinical study was conducted in the period 2015–2017 at Dentistry Clinic of Vojvodina (Department of Dental diseases and Endodontics), University of Novi Sad, Serbia. The study involved 30 patients diagnosed with purging bulimia and treated at the Psychiatric Clinic of Clinical Center Vojvodina, Serbia. Study included patients aged 18–35 years that suffered from bulimia followed by frequent vomiting (minimum 2–3 times a week in acute phase) for at least 3 years. Control group involved 30 healthy subjects and was matched with bulimics in age and gender.

The exclusion criteria included: patients under 18 and older than 35 years; patients diagnosed with bulimia less than 3 years ago; patients suffering non-vomiting type of bulimia, other eating disorders followed by vomiting; gastrointestinal disorders accompanied by vomiting; neurological, psychosomatic, metabolic and endocrine disorders followed by vomiting; chronic alcoholism and pregnancy.

After being properly informed about research, all participants signed the informed consent form.

Data collection was done using a standardized questionnaire administered to each patient. Questionnaire contained: general and sociodemographic data (name, age, gender and occupation), medical history (duration of bulimia and frequency of purging), oral hygiene habits (technique, duration, intensity and frequency of brushing), parafunctional habits (grinding and chlenching teeth, nibbling on foreign objects) and dietary habits (frequency of sweet and acid diet intake). Questionnaire was designed to give comprehensive insight into etiological and modifying factors for appearance and development of dental erosion and caries.

The clinical examination included intraoral inspection and assessment of dental status using Basic Erosive Wear Examination (BEWE) and Decayed, Missing and Filled Teeth (DMFT) index. Examination was conducted under artificial light source using a dental probe and a mirror, with precleaning and drying of the tooth surfaces. Inspection included examination of all present teeth, recording healthy, decayed, missing teeth and fillings.

The presence of dental erosion was determined on the basis of objective findings and clinical signs of erosive tooth wear. Presence of erosion was noted in the upper and lower jaw, on the vestibular, oral and occlusal surfaces of the teeth, as well as in certain groups of teeth (incisors, canines, premolars, molars). BEWE index was used to determine degree of erosive wear and severity was graded according to the following criteria [15]: 0 – no erosive tooth wear; 1 – initial loss of surface texture; 2* – distinct defect, hard tissue loss $< 50\%$ of the surfaced area; 3* – hard tissue loss $\geq 50\%$ of the surface area; *scores 2 and 3 often involve dentin.

All teeth surfaces were examined and the highest score recorded. The examination was repeated for all teeth in a sextant but only the surface with the highest score was recorded for each sextant. The cumulative score of all sextants was calculated providing relevant information on the extent of erosive changes [15].

DMFT index is well established as the key measure of caries experience in dental epidemiology. The DMFT index is applied to the permanent dentition and is expressed as the total number of teeth that are decayed (D), missing (M), or filled (F) [16]. When a carious lesion(s) or both carious lesion(s) and a restoration were present, the tooth was recorded as a D. When a tooth has been extracted due to caries, it was recorded as an M. When a permanent or temporary filling was present, or when a filling was defective but not decayed, this was counted as an F. Teeth restored for reasons other than caries were not counted as an F [17].

Statistical analysis was processed using the standard statistical program licensed at the University of Novi Sad, IBM SPSS Statistics r20.0, Minitab v 16. The *t*-test and $\chi^2$ test were used for group comparison. To evaluate relationship between continuous or ordinal variables Pearson and Spearman correlation were used. The threshold of significance retained was $p < 0.05$.

## Results

Bulimic group involved 30 participants, 28 female and 2 male with the average age of 24.6 ± 4.42 years and control group involved 30 participants with same female predominance (14 : 1) and similar average age (Table 1). The analysis of age ($t$ = -0.1, $p$ = 0.921) and gender ($\chi^2$ = 0, $p$ = 1) did not find any significant difference between the two groups. Results regarding patient occupation did not show significant difference ($\chi^2$ = 0.667, $p$ = 0.955) (Table 1).

Average duration of bulimia was 4.75 ± 2.58 years, and in most patients (76.7%) it lasts for 3–5 years (Table 2). Data regarding frequency of vomiting show that 43% of bulimic patients purge several times a day, 27% at least once a day and 30% vomit several times a week (Table 2).

Oral hygiene habits of participants are shown in (Table 3). All results regarding oral hygiene habits, technique ($\chi^2$ = 2.31, $p$ = 0.315), duration ($\chi^2$ = 0.202, $p$ = 0.904), intensity ($\chi^2$ = 0.664, $p$ = 0.717) and frequency ($t$ = 2.54, $p$ = 0.8), did not show significant difference between groups.

Parafunctional habits of participants are also shown in Table 3. Statistical differences were not found between groups concerning grinding/chlenching ($\chi^2$ = 0.077, $p$ = 0.781) or nibbling habit ($\chi^2$ = 0, $p$ = 1).

**Table 2**

**Duration of bulimia and vomiting frequency in the bulimic group**

| Parameter | Values |
|---|---|
| Bulimia duration (years), n (%) | |
| 3–5 | 23 (76.7) |
| 6–8 | 4 (13.3) |
| 9–11 | 2 (6.7) |
| > 12 | 1 (3.3) |
| Average bulimia duration (years), mean ± SD | 4.75 ± 2.58 |
| Vomiting frequency, n (%) | |
| several times a day | 13 (43) |
| once a day | 8 (27) |
| several times a week | 9 (30) |

**SD – standard deviation.**

**Table 1**

**Age, gender and occupation in the bulimic and the control group**

| Parameters | Bulimic group | Control group | $p$ |
|---|---|---|---|
| Average age (years), mean ± SD | 24.6 ± 4.42 | 24.73 ± 5.81 | 0.921 |
| Gender, n (%) | | | |
| female | 28 (93.3) | 28 (93.3) | 1 |
| male | 2 (6.7) | 2 (6.7) | |
| Occupation, n (%) | | | |
| students | 25 (83.4) | 25 (83.4) | |
| healthcare employees | 1 (3.3) | 2 (6.7) | |
| education employees | 1 (3.3) | 1 (3.3) | 0.955 |
| lawyers and economists | 2 (6.7) | 1 (3.3) | |
| engineers | 1 (3.3) | 1 (3.3) | |

**SD – standard deviation.**

**Table 3**

**Oral hygiene and parafunctional habits in the bulimic and the control group**

| Parameters | Bulimic group n (%) | Control group n (%) | $p$ |
|---|---|---|---|
| Brushing technique | | | |
| vertical | 8 (26.6) | 7 (23.3) | |
| horizontal | 2 (6.7) | 6 (20) | 0.315 |
| rotary | 20 (66.7) | 17 (56.7) | |
| Brushing duration (min) | | | |
| < 3 | 20 (66.6) | 20 (66.6) | |
| > 3 | 5 (16.7) | 4 (13.4) | 0.904 |
| not sure | 5 (16.7) | 6 (20) | |
| Brushing intensity | | | |
| mild | 2 (6.7) | 2 (6.7) | |
| moderate | 18 (60) | 15 (50) | 0.717 |
| vigorous | 10 (33.3) | 13 (43.3) | |
| Brushing frequency (daily) | | | |
| > 3 times | 4 (13.4) | 3 (10) | |
| 2–3 times | 24 (80) | 24 (80) | 0.8 |
| 1–2 times | 1 (3.3) | 3 (10) | |
| < 1 | 1 (3.3) | - | |
| Parafunctional habits | | | |
| grinding and chlenching | 21 (70) | 20 (66.7) | 0.781 |
| nibbling of foreign objects | 17 (56.7) | 17 (56.7) | 1 |

**Table 4**

**Dietary habits (frequency of consumption) in the bulimic and the control group**

| Parameters | Bulimic group n (%) | Control group n (%) | *p* |
|---|---|---|---|
| Sweets | | | |
| every day | 21 (70) | 25 (83.4) | 0.617 |
| 1–2 times a week | 6 (20) | 3 (10) | |
| 1–2 times a month | 1 (3.3) | 1 (3.3) | |
| rarely/never | 2 (6.7) | 1 (3.3) | |
| Yogurt | | | |
| every day | 19 (63.3) | 11 (36.7) | 0.208 |
| 1–2 times a week | 6 (20) | 12 (40) | |
| 1–2 times a month | 5 (16.7) | 1 (3.3) | |
| rarely/never | - | 6 (20) | |
| Herbal tea | | | |
| every day | 15 (50) | 11 (36.7) | 0.539 |
| 1–2 times a week | 6 (20) | 5 (16.7) | |
| 1–2 times a month | - | 8 (26.6) | |
| rarely/never | 9 (30) | 6 (20) | |
| Carbonated beverages | | | |
| every day | 5 (16.7) | 11 (36.7) | < 0.05 |
| 1–2 times a week | 2 (6.7) | 8 (26.7) | |
| 1–2 times a month | - | 4 (13.3) | |
| rarely/never | 23 (76.6) | 7 (23.3) | |
| Non-carbonated beverages | | | |
| every day | 5 (16.7) | 6 (20) | < 0.05 |
| 1–2 times a week | 3 (10) | 11 (36.7) | |
| 1–2 times a month | 1 (3.3) | 4 (13.3) | |
| rarely/never | 21 (70) | 9 (30) | |
| Vinegar | | | |
| every day | 10 (33.3) | 12 (40) | 0.06 |
| 1–2 times a week | 8 (26.7) | 12 (40) | |
| 1–2 times a month | 1 (3.3) | 4 (13.3) | |
| rarely/never | 11 (36.7) | 2 (6.7) | |
| Energy drinks | | | |
| every day | 1 (3.3) | - | 0.589 |
| 1–2 times a week | 2 (6.7) | 3 (10) | |
| 1–2 times a month | 2 (6.7) | 6 (20) | |
| rarely/never | 25 (83.3) | 21 (70) | |
| Citrus fruit | | | |
| every day | 11 (36.7) | 8 (26.6) | 0.817 |
| 1–2 times a week | - | 11 (36.7) | |
| 1–2 times a month | 11 (36.7) | 7 (23.4) | |
| rarely/never | 8 (26.6) | 4 (13.3) | |

Analysis of diatary habits noted that on daily bases many bulimic patients consumed sweets, yogurt and herbal teas, while majority of them rarely or never consumed energy drinks, carbonated and noncarbonated juices. Sweets and vinegar were daily consumed by great number of participants in the control group, while most of them rarely or never consumed energy drinks (Table 4). Significant differences between groups were found regarding intake of carbonated ($t$ = -3.684, $p < 0.05$) and non-carbonated juices ($t$ = -2.428, $p < 0.05$), which were both more frequently consumed by participants in the control group (Table 4).

Dental erosion were significantly more often present in purging bulimics compared to the controls ($\chi^2 = 5.963$, $p < 0.05$), with significantly higher total ($\chi^2 = 5.765$, $p < 0.05$) and average number ($t = 2.243$, $p < 0.05$) of erosion per patient (Table 5).

In bulimic group most of erosive lesions were found on incisors (41.5%), while in control group erosion were dominantly present on molars (38.9%). The analysis of erosion localization in different teeth groups did not find any significant difference between groups ($\chi^2 = 1.044$, $p = 0.791$) (Table 5). Analyzing tooth surfaces, significant difference

between groups was noted ($\chi^2 = 10.561$, $p < 0.05$), referring that majority of lesions in bulimic group (43.9%) were located on oral surfaces, and in control group predominantly on vestibular surfaces (44%) (Table 5). Regarding jaw location, there were no significant difference between examined groups ($\chi^2 = 0.717$, $p = 0.397$) (Table 5).

Severity of eroded lesions assessed by BEWE index showed that bulimic patients had significantly higher average values of BEWE score ($t = 3.925$, $p < 0.05$) (Table 5). Significant differences between groups were found in second ($t = 3.089$, $p < 0.05$) and fourth ($t = 2.565$, $p < 0.05$) sextant, showing higher average BEWE score in bulimic group (Table 5).

Average values of DMFT index showed no significant difference between groups ($t = 0.741$, $p = 0.461$) (Table 6). The analysis of average number of decayed ($t = -0.917$, $p = 0.363$), missing ($t = 1.969$, $p = 0.054$) and filled teeth ($t = 0.787$, $p = 0.434$) did not find significant differences between two groups (Table 6).

**Table 5**

**Dental erosion (DE) presence, localization and severity (BEWE index) in the bulimic and the control group**

| Parameters | Bulimic group | Control group | *p* |
|---|---|---|---|
| Patients diagnosed with DE, n (%) | 27 (90) | 19 (63.3) | < 0.05 |
| Total number of DE | 82 | 54 | < 0.05 |
| Average number of DE, mean ± SD | 2.73 ± 1.53 | 1.8 ± 1.69 | < 0.05 |
| Localization of DE in tooth groups,*n (%) | | | |
| incisors | 34 (41.5) | 18 (33.3) | 0.791 |
| canines | 6 (7.3) | 5 (9.3) | |
| premolars | 15 (18.3) | 10 (18.5) | |
| molars | 27 (32.9) | 21 (38.9) | |
| Localization of DE on tooth surfaces, *n (%) | | | |
| vestibular | 18 (22) | 24 (44.4) | < 0.05 |
| oral | 36 (43.9) | 11 (20.4) | |
| occlusal | 28 (34.1) | 19 (35.2) | |
| Localization of DE in jaws, *n (%) | | | |
| upper jaw | 41 (50) | 31 (57.4) | 0.397 |
| lower jaw | 41 (50) | 23 (42.6) | |
| BEWE index score, mean ± SD | 2.67 ± 1.6 | 1.23 ± 1.19 | < 0.05 |
| BEWE index score in sextants, mean ± SD | | | |
| I | 0.17 ± 0.38 | 0.13 ± 0.35 | 0.723 |
| II | 0.9 ± 0.96 | 0.27 ± 0.58 | < 0.05 |
| III | 0.27 ± 0.58 | 0.23 ± 0.5 | 0.814 |
| IV | 0.57 ± 0.82 | 0.13 ± 0.43 | < 0.05 |
| V | 0.5 ± 0.78 | 0.27 ± 0.58 | 0.193 |
| VI | 0.23 ± 0.57 | 0.23 ± 0.64 | 0.832 |

**BEWE – Basic Erosive Wear Examination;** n **– number of patients; *n – number of teeth; SD – standard deviation.**

**Table 6**

**Average decayed, missing, and filled teeth (DMFT) index scores in the bulimic and the control group**

| Parameters | Bulimic group (mean ± SD) | Control group (mean ± SD) | *p* |
|---|---|---|---|
| DMFT index score | 8.87 ± 3.43 | 8.1 ± 4.51 | 0.461 |
| decayed teeth score | 1.93 ± 2.35 | 1.47 ± 1.5 | 0.363 |
| missing teeth score | 1.23 ± 1.14 | 0.67 ± 1.09 | 0.054 |
| filled teeth score | 6.23 ± 2.46 | 5.5 ± 4.47 | 0.434 |

## Discussion

The aim of the research was designed after analysis of numerous scientific papers regarding bulimia and its oral manifestations, considering claims of significantly greater possibility of developing erosive and caries lesions in these patients, as well as eventual changes of lifestyle habits [6,12,13]. While fast modernization of society raises the prevalence of diseases such as bulimia, oral manifestations of ED are not sufficiently recorded and monitored by dentists in Southea-

stern Europe, so dental protocols for prevention and treatment of bulimic patients are still missing.

In most studies involving bulimic patients, it is very difficult to reach a representative sample because large number of patients are hiding their disorder, they are young and not interested in cooperation [12, 18]. This is the reason why most of the modifying factors affecting DE and caries can not fully be investigated and confirmed in bulimic patients [12].

Questionnaire was designed as set of questions that resembles the ones that were used in other original scientific papers with similar subject matter, considering all data that seemed to be anamnesticly significant for analysis of presence, localization and degree of DE, tooth decay and life habits in bulimic patients [18, 19]. The clinical examination included intraoral inspection. DMFT index is considered to be the standard for continuous and cumulative annual monitoring of decayed, missed and filled teeth [14], and BEWE index was used as simple scoring system that easily indicates severity of tooth wear in everyday practice [15].

The age of participants was selected according to the scientific findings referring to the age when bulimia usually occurs (age 18–35), and control group was gender and age matched. The average age of bulimic patients is 24.6 ± 4.42 years and 83.3% of them were students, which corresponds to the fact that bulimia is most commonly present in university population [13]. Paszynska et al. [20] examined a group of 33 patients suffering bulimia and found that the average age of the diseased was 21.2 ± 3.2 years, and three years ago same authors repeated the study with 25 patients of the same age [21], while another study included 62 bulimic patients of the average age 27.7 [19].

Gender affiliation of the participants was in favor of the female sex, with a ratio of 14 : 1, which corresponds to the claims that bulimia more commonly affects women (10 : 1–20 : 1) [13].

Patients's vocation is considered to be a significant data because certain professions have an increased risk of additional exposure to acidic agents, such as chemical and metal industry, professional swimmers, sommeliers, etc. [10], but in this study none of the participants belonged to high risk groups.

The average duration of bulimia was 4.75 ± 2.58 years, and this period can be considered as a sufficient for occurrence of irreversible oral changes [10]. Paszynska et al. [20] had similar findings, examining 33 bulimic patients with average duration of 3.5 ± 2.4 years and the frequency of vomiting on average twice a day. Uhlen et al. [19] examined 62 bulimics with an average disease duration of 10.6 years, and Schlueter et al. [22] found that 7 years lasting bulimia accompanied by vomiting 2.5–5 times a day, can lead to clinically manifested erosion. Duration of disease and frequency of vomiting are significant factors affecting erosion development, but meta-analysis from 2015 found that the correlation between these factors and erosive process is complex and there is no linear association of these parameters [14]. In this study duration of bulimia significantly correlated with DE appearance ($r = -0.214$, $p < 0.05$) and localization on the oral surfaces ($r = -0.385$, $p < 0.05$), but did not significantly correlate with severity of erosion ($r = -0.340$, $p = 0.06$). Higher frequency of vomiting significantly correlates with more frequent occurrence of erosion on the oral teeth surfaces ($r = -0.118$, $p < 0.05$), while correlations with DE appearance ($r = -0.411$, $p = 0.059$) and severity ($r = -0.008$, $p = 0.996$) were not found. This finding supports the fact that appearance and progression of erosion depends on chronicity of disease and that repeated vomiting causes endogenous erosion in typical localization [5, 12].

Oral hygiene habits are especially important and considered as one of the main factors when it comes to caries appearance and progression of DE [23]. The quality of oral hygiene is assessed according to technique, duration, intensity and frequency of tooth brushing. All these parameters are also important because abrasive forces can lead to impairment of present erosive lesions. Acid demineralised enamel is non-resistant to abrasion, so softened enamel is very easy to remove by vigorous or frequent brushing, especially immediately after vomiting [7, 13, 24, 25]. Study done by Al-Zarea [26] founded that the frequency and brushing technique positively correlated with degree of enamel loss, but in this study none of the mentioned parameters correlated positively with the degree of erosive lesions. However, certain tendencies have been observed, so the participants in both groups experienced more severe erosion if brushing was intensive and more frequent.

Parafunctional habits can be significant because eroded lesions are sensitive to mechanical influence and attrition forces that can impair degree of erosive lesions. Bruxism can be a clinical sign of emotional and psychological stress, that are common symptoms of ED, so in bulimic patients attrition could cause erosive tooth wear to increase and progress faster [27]. Distinguishing abrasion and attrition apart from erosion is very important in differential diagnosis, and implies excellent knowledge about clinical symptoms of noncarious lesions combined with detailed anamnestic data. In this study, parafunctional habits did not positively correlate to severity of DE, although some studies confirm positive correlation between parafunctional habits and increased loss of hard dental tissue on eroded surfaces [26].

In bulimic patients dietary habits are mentioned as the most indicative factor in determination of dental erosion origin and localization [5, 28]. Palatinal lesions are most commonly found in patients who chronically vomit, while vestibular lesions are usually exogenous and originate from acidic food and beverages, so diet rich in citrus fruits, herbal teas and carbonated juices can increase the prevalence of exogenous erosion [28]. Regarding dietary habits significant differences between group were found for non-carbonated juices ($t = -2.482$, $p < 0.05$) and carbonated juices ($t = -3.684$, $p < 0.05$) that are consumed more often by healthy participants. The fact that control group participants intake more acidic beverages, explains predominant location of lesions on vestibular teeth surfaces in this group, confirming exogenous type of erosion, while bulimics have significantly more DE on oral surfaces which can be explained by frequent vomiting. Research by Dynesen et al. [29] found no positive correlation between consumption of acidic beverages and the presence of DE in bulimic or control group. In this study,

presence, location and degree of DE did not correlate with dietary habits in bulimic group, but in control group frequent consumption of carbonated juices correlated positively with degree of tissue loss ($\rho = 0.434$, $p < 0.05$) and localization of lesions on vestibular surfaces ($r = -2.814$, $p = < 0.05$).

DE in bulimic patients were subject of numerous studies and meta-analyzes and great majority of them pointed the fact that DE are significantly more often present in patients suffering ED compared to healthy subjects [3, 21, 29, 30]. Purging as repeated behavioral pattern increases risk of dental erosion appearance up to 5–8.5 times, and their presence 2.6–5.5 times, so DE can be detected in 90% of purging bulimics, while these lesions are not typical for non-purging types of eating disorders [14, 18, 30]. These findings are confirmed by studies that prove DE presence in 63%–86% of purging bulimics, and none of non-purging patients [31, 32].

In accordance with previous findings, our study proves presence of DE in 90% of bulimics, confirming that erosion more frequently occurs in purging bulimics ($\chi^2 = 5.963$, $p < 0.05$) who experience significantly higher number of erosive lesions compared to controls ($\chi^2 = 5.765$, $p < 0.05$) and higher average number of erosive lesions per person ($\chi^2 = 2.243$, $p < 0.05$). In 10% of bulimics who chronically vomit, lesions were absent. Study by Uhlen et al. [19] found that as many as 30.3% of purging ED patients do not have to experience erosive changes, probably because of low vomiting frequency, good salivary composition and adequate lifestyle habits.

Palatinal surfaces of upper anterior incisors and occlusal surfaces of the lower molars are considered to be typical sites of DE occurrence in purging bulimics, and it is explained by intense and frequent contact with gastric juice, fluid gravitating to the rear mouth floor, as well as poor salivation in the upper anterior teeth [19, 28]. Typical localization of DE was confirmed in this study, finding 41.5% of DE on incisors, 43.9% on oral and 34.1% of lesions on occlusal teeth surfaces in bulimic group. Regarding DE localization significant difference between groups was found ($\chi^2 = 10.561$, $p < 0.05$). Presence of DE on oral surfaces positively correlates to bulimia duration ($r = -0.385$, $p < 0.05$), as well as to frequency of vomiting ($r = -0.118$, $p < 0.05$), marking repeated purging as crucial etiological factor for DE occurrence in bulimics.

The erosive process is multifactorial, so severity of erosive lesions depends on presence and individual or cumulative effects of many modifying factors. Using BEWE index, the study has found that more severe erosive lesions were found in bulimic group compared to controls ($t = -3.925$, $p < 0.05$), which was already proven by other studies [18,19–21, 29]. Explanation for severe degree of DE lies in nature of hydrochloric acid that is considered to be an extremely erosive chemical agent due to low pH and low calcium and phosphate levels [15, 33, 34].

In bulimic group average BEWE index score was the highest in the second sextant, and these patients had significantly higher average BEWE score in second ($t = -3.089$, $p < 0.05$) and fourth ($t = 2.565$, $p < 0.05$) sextant compared to controls. These sextants match upper anterior teeth and lower lateral teeth, where most of the erosion were located in bulimic group, so this finding implicates that their severe degree could be result of aggressive etiological factor – gastric juice. In both groups, BEWE index score did not correlate with any of oral hygiene parameters of parafunctional habits.

Results regarding average DMFT index and separate analysis of decayed, missing and filled teeth showed absence of significant differences between two groups, and this result fits the fact that there were no differences in oral hygiene habits as well. Correlation between DMFT index and vomiting, oral hygiene or dietary habits was not found. Numerous studies have confirmed similar findings [12, 30, 35], but others proved lower DMFT index in bulimic patients due to obsessive-compulsive hygiene habits, especially after vomiting [19, 36]. However, many studies claim that bulimic patients have higher caries prevalence as a result of poor oral hygiene, xerostomia, high sugar intake and rich aciduric bacterial flora [5, 11, 14]. The issue of dental caries in patients with ED is multifactorial and requires detailed and simultaneous analysis of vomiting habits, salivary factors, oral hygiene and dietary habits.

## Conclusion

Self-induced vomiting can be considered as main cause of dental erosion in bulimic patients. Erosive lesions are usually found on oral surfaces of anterior teeth which come into direct contact with vomitus and gastric acid, so many bulimics exhibit severe lesions on mentioned surfaces. DMFT index did not show significant differences compared to healthy participants, but due to complexity of carious process further investigation is necessary. Dental practitioners are usually one of the first healthcare professionals to whom a previously undiagnosed ED may present, so adequate knowledge about oral manifestations of bulimia is necessary. Creating a dental protocol for these patients should be a priority, reducing complications and providing appropriate preventive and treatment measures.

## REFERENCES


1. *Culbert KM, Racine SE, Klump KL.* Research Review: What we have learned about the causes of eating disorders - a synthesis of sociocultural, psychological, and biological research. J Child Psychol Psychiatry 2015; 56(11): 1141–64.
2. *Walsh BT, Attia E, Glasofer DR, Sysko R.* Handbook of assessment and treatment of eating disorders. 1st ed. Arlington: American Psychiatric Association Publishing; 2016.
3. *Lo Russo L, Campisi G, Di Fede O, Di Liberto C, Panzarella V, Lo Muzio L.* Oral manifestations of eating disorders: a critical review. Oral Dis. 2008; 14(6): 479–84.
4. *Dynesen AW, Bardow A, Pedersen AML, Nauntofte B.* Oral findings in anorexia nervosa and bulimia nervosa with special reference to salivary changes. Oral Biosci Med. 2004; 1(3): 151–69.

5. *Frydrych AM, Davies GR, McDermott BM.* Eating disorders and oral health: A Review of literature. Aust Dent J 2005; 50(1): 6–15; quiz 56.
6. *Rosten A, Newton T.* The impact of bulimia nervosa on oral health: a review of the literature. Br Dent J 2017; 223(7): 533–9.
7. *West N, Joiner A.* Enamel mineral loss. J Dent 2014; 42(Suppl 1): S2–11.
8. *Li X, Wang J, Joiner A, Chang J.* The remineralisation of enamel: a review of the literature. J Dent 2014; 42(Suppl 1): S12–20.
9. *Kitasako Y, Sasaki Y, Takagaki T, Sadr A, Tagami J.* Age-specific prevalence of erosive tooth wear by acidic diet and gastroesophageal reflux in Japan. J Dent 2015; 43(4): 418–23.
10. *Kargul B, Bakkal M.* Prevalence, Etiology, Risk Factors, Diagnosis, and Preventive Strategies of Dental Erosion: Literature review (Part I & Part II). Acta Stomatol Croat 2009, 43(3): 165–87.
11. *Clark DB.* Patients with eating disorders: Challenges for the oral health professional. Can J Dent Hygiene 2010; 44(4): 163–70.
12. *Romanos GE, Javed F, Romanos EB, Williams RC.* Oro-facial manifestations in patients with eating disorders. Appetite 2012; 59(2): 499–504.
13. *Mehler PS, Rylander M.* Bulimia nervosa - medical complications. J Eat Disord 2015; 3: 12.
14. *Kisely S, Baghaie H, Lalloo R, Johnson NW.* Association between poor oral health and eating disorders: systematic review and meta-analysis. Br J Psychiatry 2015; 207(4): 299–305.
15. *Bartlett D, Ganss C, Lussi A.* Basic Erosive Wear Examination (BEWE): a new scoring system for scientific and clinical needs. Clin Oral Invest 2008; 12(Suppl 1): S65–8.
16. *Larmas M.* Has dental caries prevalence some connection with caries index values in adults? Caries Res 2010; 44(1): 81–4.
17. *Cappelli DP, Mobley CC.* Prevention in Clinical Oral Health Care. Philadelphia, Pa: Mosby Elsevier; 2007.
18. *Sales-Peres SHC, Araujo JJ, Marsicano JA, Santos JE, Bastos JRM.* Prevalence, sevirity and etiology of dental wear in patients with eating disorders. Eur J Dent 2014; 8(1): 68–73.
19. *Uhlen MM, Tveit AB, Stenhagen KR, Mulic A.* Self-induced vomiting and dental erosion - a clinical study. BMC Oral Health 2014; 14: 92.
20. *Paszynska E, Jurga-Krokowicz J, Shaw H.* The use of parotid gland activity analysis in patients with gastro-esophageal reflux disease (GERD) and bulimia nervosa. Adv Med Sci 2006; 51: 208–13.
21. *Paszynska E, Slopien A, Weglarz M, Linden RWA.* Parotid salivary parameters in bulimic patients – a controlled clinical trial. Psychiatr Pol 2015; 49(4): 709–20.
22. *Schlueter N, Ganss C, Pötschke S, Klimek J, Hannig C.* Enzyme activities in the oral fluids of patients suffering from bulimia: a controlled clinical trial. Caries Res 2012; 46(2): 130–9.
23. *Damle SG, Patil A, Jain S, Damle D, Chopal N.* Effectiveness of supervised toothbrushing and oral health education in improving oral hygiene status and practices of urban and rural school children: a comparative study. J Int Soc Prev Community Dent 2014; 4(3): 175–81.
24. *Otsu M, Hamura A, Ishikawa Y, Karibe H, Ichijyo T, Yoshinaga Y.* Factors affecting the dental erosion severity of patients with eating disorders.Biopsychosoc Med. 2014 Nov 19;8:25.
25. *Voronets J, Lussi A.* Thickness of softened human enamel removed by toothbrush abrasion: an in vitro study. Clin Oral Investig 2010; 14(3): 251–6.
26. *Al-Zarea BK.* Tooth surface loss and associated risk factors in northern Saudi Arabia ISRN Dent. 2012; 2012: 161565.
27. *Yoshizawa S, Suganuma T, Takaba M, Ono Y, Saki T, Yoshizawa A et al.* Phasic jaw motor episodes in healthy subjects with or without clinical signs and symptoms of sleep bruxism: a pilot study. Sleep Breath 2014; 18(1): 187–93.
28. *Hara AT, Lussi A, Zero A.* Biological factors. In: *Lussi A,* editor. Dental Erosion: From Diagnosis to Therapy. Monogr Oral Sci. Basel: Karger, 2006. p. 88–99. Chapter 7.
29. *Dynesen AW, Bardow A, Petersson B, Nielsen LR, Nauntofte B.* Salivary changes and dental erosion in bulimia nervosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106(5): 696–707.
30. *Johansson AK, Norring C, Unell L, Johansson A.* Eating disorders and oral health: a matched case - control study. Eur J Oral Sci 2012; 120(1): 61–8.
31. *Conviser JH, Fisher SD, Mitchell KB.* Oral care behavior after purging in a sample of women with bulimia nervosa. J Am Dent Assoc 2014; 145(4): 352–4.
32. *Eisenburger M, Addy M.* Evaluation of pH and erosion time on demineralization. Clin Oral Invest 2001; 5(2): 108–11.
33. *Berar AM, Lasserre JF, d'Incau E, des Varannes SB, Picos A, Chira A* et al. Factors associated with localization of dental erosion in patients from two French medical centres. HVM Bioflux 2015; 7(2): 55–9.
34. *Ximenes R, Couto G, Sougey E.* Eating disorders in adolescents and their repercussions in oral health. Int J Eat Disord 2010; 43: 59–64.
35. *Milosevic A.* Eating disorders and the dentist. Br Dent J 1999; 186(3): 109–13.
36. *Mazurek M, Szyszkowska A, Mazurek A, Szymanska J.* Selected issues concerning oral health in female patients with eating disorders: a survey study. Curr Issues Pharm Med Sci 2016; 29(3): 139–41.

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# Antioxidant and cytotoxic activities of curly dock (*Rumex crispus* L., Polygonaceae) fruit extract

## Antioksidantna i citotoksična aktivnost ekstrakta ploda štavelja (*Rumex crispus* L., Polygonaceae)

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**Abstract**

**Background/Aim.** *Rumex crispus* (curly dock) is a wild perennial herbaceous plant, which products are considered as a rich source of biologically active molecules with antioxidant and cytotoxic activities. The aim of this study was to estimate of antioxidant and cytotoxic activities of aqueous extract of curly dock fruits. **Methods.** The aqueous extract of curly dock fruits was evaluated for its antioxidant activity by *in vitro* assays for ferric-reducing antioxidant power (FRAP), NO•, OH• and 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging activities and the influence on lipid peroxidation in liposomes. The cytotoxicity of tested extract was examined *in vitro* in human cervix carcinoma (HeLa), colon adenocarcinoma (HT-29) and breast adenocarcinoma (MCF7) cells. **Results.** The tested extract showed a potential antioxidant activity manifested in scavenging of free radicals as well as an ability to decrease lipid peroxidation in liposomes. The results indicated tissue-selective cytotoxicity of *R. crispus* fruit extract *in vitro*. The most prominent antitumor activity was observed towards HeLa and MCF7 cell lines. **Conclusion.** The investigated aqueous fruit extract of *R. crispus* had potential antioxidant and cytotoxic activities, with necrosis as a main mechanism of induced cell-death. Different methods of extraction of *R. crispus* fruits, apart from aqueous, are recommended for further investigations.

**Key words:**
**antioxidants; lipid peroxidation; phytotherapy; plants, medicinal; polygonaceae.**

**Apstrakt**

**Uvod/Cilj:** *Rumex crispus* (štavelj) je višegodišnja zeljasta biljka, koja se smatra bogatim izvorom biološki aktivnih molekula sa antioksidantnom i citotoksičnom aktivnošću. Cilj rada bio je procena antioksidantnog kapaciteta i ispitivanje antitumorske aktivnosti vodenog ekstrakta ploda štavelja. **Metode.** Antioksidantna aktivnost vodenog ekstrakta ploda štavelja procenjena je na osnovu *in vitro* testova: *ferric-reducing antioxidant power* (FRAP), sposobnosti ekstrakta da neutrališe slobodne radikale NO•, OH• i 2,2-difentil-1-pikril hidrazil (DPPH) i utiče na lipidnu peroksidaciju u lipozomima. Citotoksičnost ispitivanog ekstrakta je određena *in vitro* na tumorskim ćelijskim linijama: humani karcinom cerviksa (HeLa), adenokarcinom (HT-29) i adenokarcinom dojke (MCF7). **Rezultati.** Testirani ekstrakt pokazao je potencijalnu antioksidantnu aktivnost manifestovanu velikom moći u neutralizaciji slobodnih radikala, kao i sposobnost da smanji lipidnu peroksidaciju u lipozomima. Ustanovljena je tkivno-selektivnu citotoksičnost ekstrakta ploda štavelja *in vitro*. Najizraženija antitumorska aktivnost primećena je prema HeLa i MCF7 ćelijskim linijama. **Zaključak.** Vodeni ekstrakt ploda štavelja ima potencijalnu antioksidantnu i citotoksičnu aktivnost, sa nekrozom kao glavnim mehanizmom indukovane ćelijske smrti. Za dalja istraživanja, preporučuju se i druge metode ekstrakcije ploda štavelja.

**Ključne reči:**
**antioksidansi; lipidi, peroksidacija; fitoterapija; biljke, lekovite; polygonaceae.**

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## Introduction

*Rumex crispus* (curly dock) is a wild perennial herbaceous plant in the family Polygonaceae. Its basal leaves are narrowly lanceolate, curly in the edges. The young leaves of curly dock appear in spring, and its flowering season starts from May to August. The seeds are harvested in the summer [1–4]. On the stalk, flowers and seeds are produced in clusters on branched stems. Getting caught in wool and animal fur, or carried by the wind, the seeds have been spreading to new locations. Growing almost everywhere, the curly dock can be found especially in grassy areas, marshes, waste places, near roads, mostly in acid soils, and represents a real threat for crops as noxious weed species worldwide [1, 4, 5]. Apart from being considered a seriously invasive weed, young leaves of curly dock are edible and often used as vegetables or salad [3, 6]. Furthermore, the use of its fruits has been described in Serbian and Turkish traditional medicine in treatment of stomach complaints such as dysentery for their astringent activity [6–8]. Regarding its biological effects, the roots, leaves and fruits of *R. crispus* have been widely consumed in traditional medicine for many centuries as a tonic, laxative, spasmolytic and cholagogue agent in bilious complaints, as well as an astringent for hemorrhoids and bleeding. The curly dock has also been used in folk medicine in order to control fungal and peptic disorders [2, 7, 9]. Its dried roots are a gentle and safe laxative, particularly useful for treatment of mild constipation, due to anthraquinone content which gives the roots yellow colored pigment [1, 3, 6, 10]. Therefore, its roots are a well-known antidote for stomach disorders [3]. Curly dock is also useful for treating a wide range of skin problems such as fungal disorders, spring eruption and scrofula [6, 10].

The plant products are considered as a rich source of biologically active molecules, so the extracts of *R. crispus* have been declared to possess an antioxidant, antimicrobial and antifungal activities, offering remarkable protection against any damage [2, 3, 6, 11]. The thin layer chromatography (TLC) survey confirmed the presence of polyphenols such as flavonoids, phenolic acids and procyanidins in the investigated methanol extract of curly dock fruits [6]. Previous studies have shown a high antioxidant activity of leaf and seed of this plant, but there is a lack of reports about potency of its fruits. Only its methanol fruits extract has shown a specific antioxidant potential, suggesting that it can be used as hepatoprotective agent and an active agent in the treatment of other diseases caused by oxidative stress [6]. Furthermore, a recent study revealed that curly dock seed extracts may play an important role in reactive oxygen species (ROS) scavenging against oxidative stress [2]. Moreover, methanolic extract of *R. crispus* root has shown a high level of inhibition of the HT-29 cell growth, and also has inhibited α-glucosidase and amylase effectively. Conclusively, above mentioned extract can be considered as a potent carbohydrase inhibitor, anticancerous and antioxidant [12]. In addition, the extracts of curly dock root have demonstrated the high xanthine oxidase inhibitory activity [13]. The ethanol extracts of roots, leaves and fruits of curly dock have been screened for its cytotoxic activity *in vitro* and it has been found remarkable cytotoxic activities on leukemic 1301 – human T cell leukemia lymphoblast, and EOL-1 cell lines – human eosinophilic leukemia. The analysis of morphological changes showed that the mechanism of cell-death was apoptosis [9]. It has been proven that the extract of entire plant of *R. crispus* possess high antimalarial activity *in vitro* and *in vivo* against *Plasmodium falciparum* chloroquine-sensitive and *Plasmodium falciparum* chloroquine-resistant. The antimalarial activity of curly dock originates from isolated compound nepodin (a naphthalene derivative), which appears in high concentration in roots of the plant [7]. In addition, it has been discovered that ether extracts of the leaf and the seed and an ethanol extract of the leaf of curly dock have antimicrobial activities on *Staphylococcus aureus* and *Bacillus subtilis*, which are Gram-positive bacteria [11]. Recently, it has been reported that an extract of *R. crispus* roots exhibits antifungal activity due to chrysophanol and parietin, both anthraquinones, and nepodin, that were isolated from roots [14]. These compounds were screened for *in vivo* antifungal activity against several various plant pathogenic fungi and were effective in controlling of the disease development comparable to that of synthetic fungicide fenarimol, at similar concentrations [3, 14]. The overall activities of extracts of curly dock support the traditional use of extracts from all parts of the mentioned plant in the treatment of various disorders.

The aim of this study was to measure amount of flavonoids, which are present in aqueous extract of *R. crispus* fruits, and known to possess a potential antioxidant activity [15]. Furthermore, this study was based on estimation of antioxidant capacity and potentiality of investigated extract. The aim of this study also was to evaluate *in vitro* antitumor activity of the aqueous extract of curly dock fruits and to determinate *in vitro* mechanism of cell-death induced by this extract in human tumor cell lines. The aim also was a determination of the non-tumor/tumor $IC_{50}$ effects obtained after treatment with mentioned extract.

## Methods

### *Estraction*

Ripe fruits of *R. crispus* (voucher specimen number 3874) were collected during July 2012 from a meadow at Kumodraž, the suburbs of Belgrade. The identification was confirmed by Prof. Dr Radiša Jančić, Faculty of Pharmacy, University of Belgrade, Serbia. The voucher specimen was deposited in the herbarium of Faculty of Pharmacy, University of Belgrade, Serbia. Harvested fruits were dried in room temperature in airy place avoiding sunlight. Dried plant sample was reduced to a fine powder and defatted in a Soxhlet-type apparatus with n-hexane and chloroform until exhausted. Residual plant material was extracted for 3 h at 50°C with 10-fold quantity of water and filtered. Resulting aqueous extract was evaporated under reduced pressure in order to produce a deep brownish-red powder.

### *Quantification of flavonoids*

The plant material (600 mg of dry extract of curly dock fruits) was extracted under reflux conditions (80°C) with 20 mL acetone (LaChema, Neratovice, Czech Republic), 1 mL solution

of urotropine (5 g/L; Merck, Darmstadt, Germany) and 2 mL hydrochloric acid (25%; Zorka Pharma, Šabac, Serbia) during 30 min. The extract was cooled to room temperature and filtered. The residue was reextracted under the same conditions. Both extracts were combined and the volume was completed to 100 mL of acetone solution, resulting in the stock solution. An aliquot of 20 mL of the stock solution was transferred to a separatory funnel, mixed with 20 mL of water and shaken once with 15 mL and three times more with 10 mL ethyl acetate (Zorka Pharma, Šabac, Serbia). All ethyl acetate solutions were merged and shaken twice with 50 mL of water in a separatory funnel, then transferred to flask and completed to 50 mL of ethyl acetate – the working solution. Preparing the test solution was performed as follows: an aliquot of 10 mL working solution was transferred to 25 mL volumetric flask, a volume of 1 mL 2% aluminium chloride ($AlCl_3$; Merck, Darmstadt, Germany) solution was added and made to volume with acetic acid (5%; Zorka Pharma, Šabac, Serbia) in methanol (Zorka Pharma, Šabac, Serbia). Preparing the blank solution was as follows: an aliquot of 10 mL working solution was transferred to 25 mL volumetric flask and completed to volume with acetic acid (5%) in methanol. After 30 min the absorbance of the test solution was measured (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA) at 425 nm against the blank solution. The result was expressed as the percentage of flavonoids (%) [16].

*In vitro antioxidant tests*

The ferric-reducing antioxidant power (FRAP) assay

Appropriately diluted aqueous extract of *R. crispus* fruits (100 μL with final concentrations 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL and 0.10 mg/mL) and 3.0 mL of freshly prepared FRAP-reagent [25 mL of the acetate buffer (Sigma Aldrich GmbH, Steinheim, Germany), 300 mmol/L, pH 3.6, + 2.5 mL of 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma Aldrich GmbH, Steinheim, Germany) in 40 mmol/L HCl (Sigma Aldrich GmbH, Steinheim, Germany), + 2.5 mL of 20 mmol/L $FeCl_3 \bullet 6H_2O$ (LaChema, Neratovice, Czech Republic)] were combined. After incubation at 37°C for 30 min, the intensive blue complex $Fe^{2+}$-TPTZ was formed and the absorbance was recorded at 593 nm (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA) against the blank, containing 100 μL of resembling solvent. The absorbance was measured to test the amount of iron reduced and correlated with the amount of antioxidants present in the extracts. The absorbance of the samples was compared to the calibration curve of $FeSO_4 \bullet 7H_2O$ (Kemika, Zagreb, Croatia) standard solutions, covering the concentration range 100–1000 μmol/L; the FRAP values were expressed as FRAP units, where one FRAP unit was 100 μmol/L $Fe^{2+}$ [6–18].

2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging activity (DPPH assay)

Samples of the extract, and a standard substance Trolox® (with final concentrations: 1.000 mg/mL, 0.500 mg/mL, 0.100 mg/mL, 0.050 mg/mL, 0.025 mg/mL and 0.010 mg/mL) were put into the set of test tubes and made up to 4.0 mL by the addition of methanol (Merck, Darmstadt, Germany). Finally, 1 mL of 0.5 mmol/L methanol DPPH (Sigma Aldrich GmbH, Steinheim, Germany) solution was put into each test tube. Decolorisation percentage was obtained spectrophotometrically at 517 nm (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA) after 60 min incubation at room temperature in the dark, against methanol (Merck, Darmstadt, Germany) as the blank. The percent of scavenging was calculated against the control, containing only methanol instead of the extract, and Trolox® [6, 18–20].

Inhibition of lipid peroxidation in liposomes by thiobarbituric acid (TBA assay)

In brief, 10 μL of appropriately diluted extract (final concentrations 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL and 0.10 mg/mL) was put into screw-capped glass test tubes and incubated for 60 min at 25 °C with 60 μL of liposomes [commercial product of proliposomes "Pro-Lipo S" (Lucas Meyer GmbH & Co., Hamburg, Germany)] with 30% of phosphatidylcholine of soya, pH 5–7, 20 μL of $FeSO_4 \bullet 7H_2O$ (0.1 mol/L, Kemika, Zagreb, Croatia) and 20 μL of ascorbic acid (0.1 mol/L, Sigma Aldrich GmbH, Steinheim, Germany). The reaction was ended by adding 2.3 mL of TBA-reagent [3 g TBA acid (TBA, Reanal, Budapest, Hungary) + 120 g of trichloroacetic acid (TCA) (TCA, LaChema, Neratovice, Czech Republic) + 10.4 mL of perchloric acid (Sigma Aldrich GmbH, Steinheim, Germany) in 800 mL of distilled water (obtained from a Simplicity 185 purification system, Millipore S.A., Molsheim, France)) and 0.2 mL ethylenediaminetetracetic acid (EDTA) (0.1 mol/L, Merck, Darmstadt, Germany)]. After heating at 100°C for 15 min, cooling and centrifugation, the absorbance of red colored adduct was measured at 532 nm (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA). The intensity of lipid peroxidation was expressed in nmol MDA/mL of liposomes [6, 21, 22].

Inhibition of lipid peroxidation in liposomes (TBA assay) combined with carbon tetrachloride $CCl_4$

The experiment was repeated with $CCl_4$ ($CCl_4$, in 50% ethanol, Sigma Aldrich GmbH, Steinheim, Germany) introduced to the system just before adding the diluted extracts (final concentrations 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL and 0.10 mg/mL) in order to evaluate possible antioxidant activity of the examined extract. The intensity of lipid peroxidation was expressed in nmol MDA/mL of liposomes [23, 24].

Hydroxyl radical (OH•) scavenging activity

In brief, 60 μL of liposomes [commercial product of proliposomes "Pro-Lipo S" (Lucas Meyer GmbH & Co., Hamburg, Germany) with 30% of phosphatidylcholine of soya, pH 5–7, was put into screw-capped glass test tubes with 20 μL of $FeSO_4 \bullet 7H_2O$ (0.1 mol/L, Kemika, Zagreb, Croa-

tia), 10 μL of hydrogen peroxide (0.009 mol/L, Kemika, Zagreb, Croatia) and 20 μL of deoxyribose (0.05 mol/L, Sigma Aldrich GmbH, Steinheim, Germany) and finally 10 μL of appropriately diluted extract (final concentrations 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL and 0.10 mg/mL) was added in the system and incubated for 60 min at 25°C. The reaction was ended by adding 2.3 mL of TBA-reagent [3 g TBA (TBA, Reanal, Budapest, Hungary) + 120 g of TCA acid (TCA, LaChema, Neratovice, Czech Republic) + 10.4 mL of perchloric acid (Sigma Aldrich GmbH, Steinheim, Germany) in 800 mL of distilled water (obtained from a Simplicity 185 purification system, Millipore S.A., Molsheim, France)] and 0.2 mL EDTA (0.1 mol/L, Merck, Darmstadt, Germany). After heating at 100°C for 15 min, cooling and centrifugation, the absorbance of red colored adduct was measured at 532 nm (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA). The production of OH• was expressed in nmol MDA/mg of deoxyribose [6, 21–24].

Hydroxyl radical (OH•) scavenging activity combined with $CCl_4$

In order to evaluate possible antioxidant activity of the examined extract, the experiment was repeated with the same diluted extracts added after introducing the $CCl_4$ ($CCl_4$, in 50% ethanol, Sigma Aldrich GmbH, Steinheim, Germany) to the system. The production of OH• was expressed in nmoL MDA/mg of deoxyribose [23, 24].

Nitric oxide radical (NO•) scavenging assay

NO• scavenging potential of the aqueous extract of curly dock fruits was evaluated using the method described by Garrat. A volume of 2 mL of 10 mM sodium nitroprusside (Sigma Aldrich GmbH, Steinheim, Germany) in phosphate buffer saline (Sigma Aldrich GmbH, Steinheim, Germany), pH 7.4, was combined with 0.5 mL of investigated extract (with final concentrations: 1.000 mg/mL, 0.500 mg/mL, 0.100 mg/mL, 0.050 mg/mL, 0.025 mg/mL and 0.010 mg/mL). After the incubation at 25°C during 150 min, 0.5 mL of incubation solution was withdrawn and mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33%) prepared in 20% glacial acetic acid (Sigma Aldrich GmbH, Steinheim, Germany)] at room temperature for 5 min with 1 mL of naphthylethylene diamine dihydrochloride (0.1% w/v, Sigma Aldrich GmbH, Steinheim, Germany). The tested mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm (Evolution 300 UV-VIS spectrophotometer, Thermo Scientific, Madison, WI, USA). The percent of inhibition was calculated against the control solution, containing only methanol (Merck, Darmstadt, Germany) instead of test solutions [20, 25].

*Tests on cell lines*

Cell lines

For the estimation of cell growth effects, human tumor cell lines HeLa (cervix epitheloid carcinoma, ECACC No. 93021013), MCF7 (breast adenocarcinoma, ECACC No. 86012803), HT-29 (colon adenocarcinoma, ECACC No. 91072201), and MRC-5 (human fetal lung, ECACC 84101801) were used and prepared according to previously described procedures [26–28]. Cell lines were cultivated in DMEM medium combined with 4.5% glucose, supplemented with 10% heat-inactivated fetal calf serum (FCS; NIVNS, Novi Sad, Serbia), 100 IU/mL of penicillin and 100 μg/mL of streptomycin (Galenika, Belgrade, Serbia). All tested cell lines grew attached to the surface, cultivated in 25 mL flasks (Corning, New York, USA) at 37°C, provided with 5% $CO_2$ and high humidity, and sub-cultured twice a week. A single cell suspension was obtained using 0.1% trypsin (Serva, UK) with 0.04% EDTA [26–29].

Cell growth activity

According to previous studies, the cell lines were harvested and plated into a volume of 199 μl in 96-well microtitre plates (Sarstedt, Newton, USA) at a seeding density of 3–5 × $10^3$ cells in each well, and preincubated in complete medium provided with 5% FCS, at 37°C for 24 h [26–29].

In order to achieve required final concentrations, serial twofold dilutions of the aqueous extract of *R. crispus* fruits in DMSO (1 μL) were combined with 199 μL of medium. Equal volume of solvent was added in control wells. After further incubation of microplates at 37°C for 48 h, the cell growth was evaluated by measuring the total protein content by colorimetric sulforhodamine B assay (SRB) according to Skehan et al. [30]. Colour development was measured using a Multiscan Ascent (Labsystems; Helsinki, Finland) photometer at 540 nm against 620 nm as background [26–29].

The effect on cell growth was calculated as 100 x (AT/AC) (%), where AT and AC are the absorbance of the test sample and the control, respectively [26, 29]. The concentration-cell growth (dose effect) curves were made for each treatment and $IC_{50}$ values (concentration of extract that inhibits cell growth by 50%) were calculated, using OriginPro 8 SRO (Origin-Lab Corporation, Northampton, USA). The non-tumor/tumor $IC_{50}$ ratios were calculated for HeLa, MCF-7, HT-29 and MRC-5 cell lines [26, 29].

Cell-death detection

The mechanism of cell-death in human cervix carcinoma (HeLa) and breast adenocarcinoma (MCF7) cell lines was determined by detection of apoptosis and necrosis using Cell Death Detection ELISA$^{PLUS}$ kit (Roche, Version 11). The enzyme-immunoassay, based on the sandwich principle, qualitatively and quantitatively determines cytoplasmic histone associated DNA fragments. Mouse monoclonal antibodies directed against DNA and histones, allowing specific determination of mono- and oligonucleosomes in the cytoplasmatic fraction of the cell, were used. Cell death detection experiments were performed when $IC_{50} < 100$ μg/mL criterion was met in cell growth experiments (in HeLa and MCF7 cell lines) [26].

According to study of Četojević-Simin et al. [26], the cell lines ($1 \times 10^4$ cells) were seeded in a 96-well microplate and preincubated for 24 h. After adding the extract and solvent (negative control) and incubation (about 2 h, precise timing was determined under the microscope) the plate was centrifuged, cell culture supernatants pooled for each treatment (n = 4) and used for the examination of necrosis. Cells were then lysed, centrifuged, lysates pooled (n = 4) and evaluated for apoptosis [26]. The supernatant of cell lines (for the evaluation of necrosis) and cell lysis fraction (for the evaluation of apoptosis) (n = 2), both containing cytoplasmic histone-associated DNA fragments, were treated with the anti-histone antibodies and anti-DNA antibodies coupled to peroxidase and incubated for 2 h [26]. The microplate was then washed, substrate of the peroxidase was added and colour development measured, using a Multiscan Ascent (Labsystems; Helsinki, Finland) photometer at 405 nm against 492 nm as background [26].

Background value was subtracted from the averages for each treatment. Enrichment factors (EF), both for apoptosis and necrosis, were calculated as EF = AT/AC, where AT was absorbance of the treatment and AC of the negative control (solvent). EF apoptosis/necrosis ratios were calculated as EF A/N = EF A/ EF N [26].

*Statistical analysis*

All results are expressed as mean ± standard deviation (SD). Student's *t*-test (presented as *t* values in results) was used for comparing means of two groups. One-way analysis of variance (ANOVA, presented as F values in results) was used to compare means of more than two groups. *Post-hoc* analysis was performed by using the Tukey's test. All values $p < 0.05$ were considered to be statistically significant. Statistical analysis was performed using IBM SPSS Statistics 21.

## Results

*Quantification of flavonoids*

The concentration of flavonoids in aqueous extract of *R. crispus* fruits, determined using spectrophotometric method with aluminium-chloride, was 0.67%.

*In vitro* antioxidant test

The results of the FRAP assay are shown in Table 1. The concentration of 0.05 mg/mL of the extract had the highest antioxidant capacity, but among the different concentrations of investigated extract there was no significant difference in antioxidant power (by using ANOVA, F = 1.511, $p = 0.224$). There were clear differences between the control group and the all concentrations of the extract except the sample that contained 0.03 mg/mL of the extract.

**Table 1**
**Ferric reducing antioxidant power (FRAP) of the aqueous extract of *Rumex crispus* fruits**

| Group | FRAP units, mean ± SD |
|---|---|
| Control | 1 |
| Extract, mg/mL | |
| 0.01 | 1.14 ± 0.04* |
| 0.02 | 1.13 ± 0.03* |
| 0.03 | 1.11 ± 0.07 |
| 0.04 | 1.14 ± 0.06* |
| 0.05 | 1.20 ± 0.10* |
| 0.10 | 1.19 ± 0.04* |

**Values (FRAP units) represent means ± standard deviation (SD) of six measurements (n = 6).**
***Statistically significant difference between particular concentrations of the extract and control group, $p < 0.001$.**

The results of the DPPH assay (Table 2) were reported as the percentage of scavenged DPPH free radical and as the $IC_{50}$. The investigated extract was capable of neutralizing DPPH free radicals via hydrogen donating activity [20] by 84.66%, 83.12%, 64.26%, 52.32%, 34.16% and 14.04% at concentrations 1.000 mg/mL, 0.500 mg/mL, 0.100 mg/mL, 0.050 mg/mL, 0.025 mg/mL and 0.010 mg/mL, respectively. The $IC_{50}$ value for the investigated extract was 0.046 mg/mL, while the standard substance Trolox® was able to reduce the stable DPPH free radical, reaching 50% reduction with an $IC_{50}$ 0.216 mg/mL. Analyzing intensity of inhibition of the DPPH free radical under the influence of the aqueous extract of *R. crispus* fruits, it was determined significant difference in reduction among concentrations used (F = 169.504, $p < 0.001$). Furthermore, using post-hoc analysis (Tykey's test), it was found the presence of statistically significant difference in scavenging power between consecutive concentrations used: 0.500 mg/mL and 0.100 mg/mL ($p < 0.01$), 0.100 mg/mL and 0.050 mg/mL ($p < 0.05$), 0.050 mg/mL and 0.025 mg/mL ($p < 0.01$), 0.025 mg/mL and 0.010 mg/mL ($p < 0.01$). The statistically significant difference in inhibition of the DPPH free radical was not established only between concentrations 1.000 mg/mL and 0.500 mg/mL ($p = 1.00$).

**Table 2**
**2,2-diphenyl-1-picrylhydrazyl(DPPH)-free radical scavenging activity of the aqueous extract of *Rumex crispus* fruits**

| Group | Concentrations of the extract (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 1.000 | 0.500 | 0.100 | 0.050 | 0.025 | 0.010 |
| Trolox® | 98.44 ± 1.62 | 56.72 ± 29.71 | 48.64 ± 5.34 | 31.20 ± 5.32 | 14.68 ± 1.06 | 11.38 ± 0.83 |
| Extract | 84.66 ± 5.31** | 83.12 ± 7.56** | 64.26 ± 3.12** | 52.32 ± 5.39** | 34.16 ± 3.09** | 14.04 ± 1.88* |

**Note: Results are given as % of DPPH• scavenging and represent as means ± standard deviation (SD) of six measurements (n=6).**
***$p < 0.05$, ** $p < 0.01$ - statistically significant difference between particular concentrations of the extract and Trolox®**

The results of TBA assay are shown in Table 3. A statistically significant difference of intensity of lipid peroxidation between the control group and the group with 0.01 mg/mL of the investigated extract, in absence of $CCl_4$ (by using Student's test, $t = 3.127$, $p = 0.014$) was determined, while there was no significant difference between the control and the extract group (0.01 mg/mL) in the presence of $CCl_4$. The comparisons between concentrations of the examined extract and both control groups (without and with $CCl_4$) are given in Table 3. Analyzing intensity of lipid peroxidation influenced by the examined extract, it was concluded that there was a statistically significant difference in means of the investigated parameter related to the extract concentrations, in both conditions: in absence and in presence of $CCl_4$ ($F = 429.220$, $p < 0.001$; $F = 83.174$, $p < 0.001$, respectively).

**Table 3**
**Influence on lipid peroxidation (LPx) in liposomes of the aqueous extract of Rumex crispus fruits with or without CCl4 addition**

| Group | MDA (nmol/mL of liposomes), mean ± SD | |
|---|---|---|
| | without $CCl_4$ | with $CCl_4$ |
| Control | 76.62 ± 1.61** | 220.66 ± 18.93* |
| Extract, mg/mL | | |
| 0.01 | 73.64 ± 1.39*,** | 198.46 ± 15.75* |
| 0.02 | 60.60 ± 2.79*,** | 193.38 ± 12.60*,** |
| 0.03 | 55.16 ± 1.69*,** | 157.12 ± 14.00*,** |
| 0.04 | 41.52 ± 2.17*,** | 107.92 ± 9.42*,** |
| 0.05 | 21.98 ± 2.56*,** | 91.76 ± 6.89*,** |
| 0.10 | 19.44 ± 3.02*,** | 98.86 ± 9.84*,** |

**Note: Values (nmoL MDA/mL of liposomes) represent means ± standard deviation (SD) of six measurements (n = 6).**
***Statistically significant difference between particular concentration of the extract and control, $p < 0.001$.**
****Statistically significant difference between particular concentration and control combined in the presence of $CCl_4$, $p < 0.001$.**

The results of OH• scavenging activity are shown in Table 4. There was no statistically significant difference in intensity of the production of OH• between the control group and the group with 0.01 mg/mL of the aqueous extract of curly dock fruits, in both experiments: without and in the presence of $CCl_4$ ($p = 0.224$, $p = 0.719$, respectively). The comparisons between other concentrations of the investigated extract and both control groups (control and control + $CCl_4$) are given in Table 4. Analyzing intensity of forming OH• influenced by mentioned extract, it was concluded that there was a statistically significant difference in means of the investigated parameter related to concentration, in both conditions: in absence and in presence of $CCl_4$ ($F = 18.576$, $p < 0.001$; $F = 84.554$, $p < 0.001$, respectively).

The results of NO• scavenging assay are shown in Table 5. The investigated extract significantly inhibited generation of NO• and it was related to different concentrations of the extract used ($F = 220.891$, $p < 0.001$).

**Table 4**
**Influence of the aqueous extract of *Rumex crispus* fruits on production of hydroxyl radical**

| Group | MDA (nmol/mg of deoxyribose), mean ± SD | |
|---|---|---|
| | without $CCl_4$ | with $CCl_4$ |
| Control | 2.17 ± 0.13** | 5.44 ± 0.29* |
| Extract, mg/mL | | |
| 0.01 | 2.08 ± 0.06** | 5.37 ± 0.28* |
| 0.02 | 2.05 ± 0.09** | 5.09 ± 0.07* |
| 0.03 | 1.95 ± 0.07** | 4.75 ± 0.25*,** |
| 0.04 | 1.82 ± 0.12*,** | 4.16 ± 0.12*,** |
| 0.05 | 1.52 ± 0.17*,** | 3.49 ± 0.21*,** |
| 0.10 | 1.59 ± 0.18*,** | 3.43 ± 0.18*,** |

**Note: The production of OH• was expressed in nmol MDA/mg of deoxyribose; values (nmoL MDA/mg of deoxyribose) represent means ± standard deviation (SD) of six measurements (n = 6).**
***Statistically significant difference between particular concentration of the extract and control, $p < 0.001$.**
****Statistically significant difference between particular concentration of the extract and control in the presence of $CCl_4$, $p < 0.001$.**

**Table 5**
**Influence of the aqueous extract of *Rumex crispus* fruits on production of nitric oxide radical**

| Concentration of the extract, mg/mL | NO• (% of inhibition), mean ± SD |
|---|---|
| 1.000 | -15.54 ± 0.77 |
| 0.500 | -7.26 ± 1.92 |
| 0.100 | -4.36 ± 0.34 |
| 0.050 | -0.36 ± 0.72 |
| 0.025 | 0.81 ± 0.45 |
| 0.010 | -0.03 ± 0.14 |

**Note: Values (% of NO•inhibition) represent means ± standard deviation (SD) of six measurements (n = 6).**

*Tests on cell lines*

The results of an influence of the extract tested on tumor cell growth are given in Table 6. The most pronounced antitumor activity was observed towards cervix carcinoma cell line (HeLa) with $IC_{50}$ 16.9 μg/mL and breast adenocarcinoma cell line (MCF7) with $IC_{50}$ 19.3 μg/mL. The non-tumor/tumor $IC_{50}$ ratios were calculated for HeLa, MCF-7 and HT-29 cell lines and they were NT/T = 1.54, NT/T = 1.35, NT/T < 0.42, respectively.

**Table 6**
**Influence of the aqueous extract of *Rumex crispus* fruits on cell growth in selected human cell lines**

| Group | $IC_{50}$ (μg/mL) | | | |
|---|---|---|---|---|
| | HeLa | MCF7 | MRC-5 | HT-29 |
| Extract | 16.88 ± 3.08 | 19.26 ± 3.45 | 25.98 ± 5.38 | n.a. ($IC_{32}$ = 62.5) |
| Control (doxorubicin) | 0.25 ± 0.09* | 0.26 ± 0.02* | 0.40 ± 0.03* | 0.38 ± 0.04* |

**Note: Values represent means ± standard deviation (SD) of eight measurements (n = 8) obtained in 3.91 to 1,000 μg/mL concentration range.**
***Rewriten from Četojević-Simin et al. [26]**

Apoptosis and necrosis were expressed as enrichment factor (EF) and apoptosis/necrosis ratios (A/N) obtained in HeLa and MCF7 cell lines after treatment with the investigated extract. *R. crispus* fruit extract slightly decreased apoptosis and significantly increased necrosis in both MCF7 (EFA = 0.77; EFN = 2) and HeLa (EFA = 0.97; EFN = 4) cell lines giving high overall decrease in apoptosis/necrosis ratios compared to the control (EF A/N = 0.24–0.39) (Table 7).

**Table 7**

**Apoptosis and necrosis expressed as enrichment factor (EF) and apoptosis/necrosis ratios (A/N) obtained in HeLa and MCF7 cell lines after treatment with the aqueous extract of *Rumex crispus* fruits**

| Cell line | EF A | EF N | EF A/N |
|---|---|---|---|
| HeLa | 0.97 | 4.00 | 0.24 |
| MCF7 | 0.77 | 2.00 | 0.39 |

**EF A – enhancement factor for apoptosis (EF for the control is 1); EF N – enhancement factor for necrosis (EF for the control is 1); EF A/N – EF A/ EF N.**

## Discussion

### *Quantification of flavonoids*

The interest in possible health benefits of flavonoids has increased owing to their potent antioxidant and free radical scavenging activities observed *in vitro* [30]. According to numerous previous studies, flavonoids might be responsible for many biological activities of curly dock, such as antiviral, antibacterial, anticancer, antioxidant activities and they might be capable to activate antioxidant enzymes [2, 3, 6, 7, 9, 11–14]. However, most interest of this study was devoted to the antioxidant activity of flavonoids and investigated extract, due to their ability to reduce free radical formation and to scavenge free radicals [31].

### *In vitro* antioxidant tests

Several *in vitro* methods are known to measure the total antioxidant capacity of biological samples. One of them, the FRAP method, is based on the reduction of a ferroin analog, the $Fe^{3+}$ complex with TPTZ ($Fe^{3+}$-TPTZ), to the $Fe^{2+}$ complex ($Fe^{2+}$-TPTZ) by antioxidants in acidic medium [6, 17, 18]. In the present study, there was no significant difference in antioxidant power among all the different concentrations of investigated extract. The results suggested that the mentioned extract might have an ability to reduce $Fe^{3+}$, and thus, evident ability to donate electrons due to clear differences between the control and the samples containing 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.05 mg/mL and 0.10 mg/mL of the extract. The FRAP value of investigated extract at concentration of 0.05 mg/mL was 1.20 FRAP units [120 μmol/L Fe(II)], which could be comparable to ascorbic acid and vitamin E, known as antioxidants, with FRAP values 14.61 μg/mL Fe(II) (about 2.62 FRAP units) and 12.39 μg/mL Fe(II) (about 2.22 FRAP units), respectively [32].

The free radical scavenging activity of the investigated extract was measured using DPPH assay. The unpaired electron of the stable free radical DPPH• determines the appearance of a purple color, with an absorption at maximum 517 nm. Generation of the reduced (molecular) form (DPPH) is accompanied by the disappearance of the violet color and the vanishing of absorption [6, 18–20]. Taking into account that $IC_{50}$ was the concentration of the extract or Trolox® necessary to decrease the initial DPPH concentration by 50%, it was concluded that the mentioned extract was more potent than standard substance Trolox® in scavenging the stable DPPH free radical, due to the fact that $IC_{50}$ of the extract was lower than that of the standard substance, Trolox®. The scavenging effect of the examined extract on the DPPH radical was comparable to previous study of extracts of *R. crispus* seeds, but the ascorbic acid with $IC_{50}$ = 9.51 μg/mL was more potent than the examined extract ($IC_{50}$ = 46 μg/mL) [2, 32, 33].

The measurement of the end product of lipid peroxidation, malondialdehyde (MDA), is one of the most widely accepted assays for oxidative damage and has been used as an indicator of lipid peroxidation. The effect of the aqueous extract of curly dock fruits on lipid peroxidation in liposomes was evaluated by the TBA test after lipid peroxidation induced by the $Fe^{2+}$/ascorbate system. This assay is based on the reactivity of MDA with TBA to generate a red chromogen complex MDA-TBA with an absorption maximum at 532 nm. Lipid peroxidation caused by reactive oxygen species (ROS) plays an important role in the damaging mechanism of many disorders and it is used as an indicator of oxidative stress in cells and tissues [6, 21, 22]. The investigated extract showed a potential ability to decrease lipid peroxidation in absence of $CCl_4$, especially at concentration of 0.10 mg/mL, because there was a statistically significant difference between values of 0.10 mg/mL of the extract (19.44 nmoL MDA/mL of liposomes) and the control group without $CCl_4$ (76.62 nmoL MDA/mL of liposomes). The concentration of 0.05 mg/mL of the extract combined with $CCl_4$ was the most prominent. Taking into account that there was a statistically significant difference between the control group without $CCl_4$ (76.62 nmoL MDA/mL of liposomes) and 0.05 mg/mL of the extract combined with $CCl_4$ (91.76 nmol MDA/mL of liposomes), the examined extract had a low ability to decrease lipid peroxidation in the presence of $CCl_4$ and it was not able to completely neutralize damaging effects of $CCl_4$ [23, 24]. $CCl_4$, probably showing prooxidative activity, caused the oxidative stress (generation of ROS) and lipid peroxidation in liposomes as an *in vitro* model of cell membrane. The examined extract combined with $CCl_4$ might inhibit lipid peroxidation, probably as antioxidant agent, scavenging ROS [23, 24].

The intensity of the production of OH• radicals was determined following reaction of degradation of deoxyribose, with main product MDA. Decreased absorbance of the reaction mixture indicated increased OH• radicals scavenging activity of the tested extract [22]. Analyzing the results, in this assay, the investigated extract might exhibit a hydroxyl radical scavenging activity. The OH• radical scavenging activity of the extract may be due to the presence of phenolic com-

pounds. There is lack of reports in the literature of the OH• scavenging activity of any standard substances.

Taking into account the results, the aqueous extract of curly dock fruits might have a potential activity to scavenge NO•. The concentration of 1 mg/mL od the extract showed the highest potential to neutralize the NO• radical. In addition, there is no report available in the literature on NO• scavenging activity of any standard substances.

*Tests on cell lines*

In this study, the cytotoxic effect of the aqueous fruit extract of *R. crispus* in cancer cell lines and its potential to inhibit their growth were evaluated. Dose-dependent activity was confirmed in all tested cell lines. The effect of the aqueous extract of *R. crispus* fruits on non-cancerous cells MRC-5 was lower ($IC_{50}$ = 25.98) (Table 6), suggesting its lower toxicity towards healthy cells. The $IC_{50}$ value in colon adenocarcinoma HT-29 cells was not reached, but $IC_{32}$ value that was obtained at still low concentration ($IC_{32}$ = 62.5 μg/mL) suggests its activity towards this cell type, as well. The results showed high and favourable tissue-selective antitumor activity of the curly dock fruit extract *in vitro*.

Favorable non-tumor/tumor ratios were obtained in cervix carcinoma (NT/T = 1.35) and breast adenocarcinoma (NT/T = 1.54) cell lines. The highest non-tumor/tumor $IC_{50}$ ratio was obtained in HeLa cell line, suggesting its high effectiveness towards this cancer type compared to non-tumor cells (MRC-5) (NT/T value was above 1). In colon adenocarcinoma HT-29 cell line, NT/T was < 0.42, which was below 1, suggesting higher activity of the extract towards healthy tissue (MRC-5) compared to colon cancer cells.

Taking into account that the contemporary cytotoxic therapy is based on the higher activity of antitumor drugs towards a tumor than to healthy tissue, this quality might favor the use of the curly dock extract in potential antitumor therapy.

Further tests were performed to evaluate the mechanism of cell-death for the examined extract. Enhancement factor ratios (EF A/N), when above 1, indicate favorable apoptosis/necrosis ratio, i.e. that dominant mode of cell death is apoptosis. Values of this ratio, when below 1, indicate that necrosis is dominant mode of cell death. Analysis of cytotoxicity mechanism showed that the necrosis was main mechanism of induced cell death in cervix (HeLa) and breast (MCF7) tumor cell lines after the *R. crispus* fruits water extract treatment.

## Conclusion

The results of this study suggest that the aqueous extract of *R. crispus* fruits, containing flavonoids, might have a potential antioxidant activity and free radicals scavenging power. It was also concluded that the examined extract could have the cytotoxic activity, but with necrosis as a main mechanism of induced cell death. Different method of extraction of *R. crispus* fruits, apart from aqueous, is recommended in order to pinpoint possible active principles with lower necrotic and higher apoptotic potential that will also retain high antitumor potential.

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## REFERENCES

1. *Mertoglu- Elmas G, Gunaydin K, Ozden O*. Environmental friendly alkaline sulfite anthraquinone-methanol (ASAM) pulping with Rumex crispus plant extract of woody materials. J Environ Biol 2012; 33(5): 941–4.
2. *Suh HJ, Lee KS, Kim SR, Shin MH, Park S, Park S*. Determination of singlet oxygen quenching and protection of biological systems by various extracts from seed of Rumex crispus L. J Photochem Photobiol B 2011; 102(2): 102–7.
3. *Kim JC, Choi GJ, Lee SW, Kim JS, Chung KY, Cho KY*. Screening extracts of Achyranthes japonica and Rumex crispus for activity against various plant pathogenic fungi and control of powdery mildew. Pest Manag Sci 2004; 60(8): 803–8.
4. *Zaller JG*. Ecology and non-chemical control of Rumex crispus and R. obtusifolius (Polygonaceae): a review. Weed Res 2004; 44: 414–32.
5. *Krištalova V, Hejcman M, Červena K, Pavlu V*. Effect of nitrogen and phosphorus availability on the emergence, growth and over-wintering of Rumex crispus and Rumex obtusifolius. Grass Forage Sci 2011; 66(3): 361-9.
6. *Maksimović Z, Kovačević N, Lakušić B, Ćebović T*. Antioxidant Activity of Yellow Dock (Rumex crispus L., Polygonaceae) Fruit Extract. Phytother Res 2011; 25(1): 101–5.
7. *Lee KH, Rhee KH*. Antimalarial activity of nepodin isolated from Rumex crispus. Arch Pharm Res 2013; 36: 430–5.
8. *Jančić R, Janjatović V, Obradović M, Pavlović S, Lukić P, Krstić- Pavlović N*. Rumex. In: *Sarić M,* editor. Medicinal Plants of SR Serbia. Belgrade: Serbian Academy of Sciences and Arts; 1989. p. 494–7. (Serbian)
9. *Węgiera M, Smolarz H, Bogucka-Kocka A*. Rumex L. species induce apoptosis in 1301, EOL-1 and H-9 cell lines. Acta Pol Pharm 2012; 69(3): 487–99.
10. *Baskan S, Daut-Ozdemir A, Gunaydin K, Erim FB*. Analysis of anthraquinones in Rumex crispus by micellar electrokinetic chromatography. Talanta 2007; 71(2): 747–50.
11. *Yildirim A, Mavi A, Kara AA*. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J Agric Food Chem 2001; 49(8): 4083–9.
12. *Shiwani S, Singh NK, Wang MH*. Carbohydrase inhibition and anti-cancerous and free radical scavenging properties along with DNA and protein protection ability of methanolic root exctracts of Rumex crispus. Nutr Res Pract 2012; 6(5): 389–95.
13. *Orban-Gyapai O, Lajter I, Hohmann J, Jakab G, Vasas A*. Xanthine oxidase inhibitory activity of extracts prepared from Polygonaceae species. Phytother Res 2015; 29(3): 459–65.
14. *Choi GJ, Lee SW, Jang KS, Kim JS, Cho KY, Kim JC*. Effects of chrysophanol, parietin, and nepodin of Rumex crispus on barley and cucumber powdery mildews. Crop Protection 2004; 23: 1215–21.

15. *Djeradi H, Rahmouni A, Cheriti A.* Antioxidant activity of flavonoids: a QSAR modeling using Fukui indices descriptors. J Mol Model 2014; 20(10): 2476.
16. *Petrović S, Maksimović Z, Kundaković T.* Chemical analysis of herbal drugs and ingredients. 2nd ed. Belgrade: University of Belgrade, Faculty of Pharmacy; 2013.
17. *Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M,* et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J Nutr 2003; 133(9): 2812–9.
18. *Antolovich M, Prenzler DP, Patsalides E, McDonald S, Robards K.* Methods for testing antioxidant activity. Analyst 2002; 127(1): 183–98.
19. *Cuendet M, Hostettmann K, Potterat O, Dyatmiko W.* Iridoid glucosides with free radical scavenging properties from Fagraea blumei. Helv Chim Acta 1997; 80(4): 1144–52.
20. *Deepa B, Prema G, Sai KB, Cherian KM.* Antioxidant and free radical scavenging activity of triphala determined by using different in vitro models. J Med Plants Res 2013; 7(39): 2898–905.
21. *Mimica-Dukić N, Božin B, Soković M, Simin N.* Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. J Agric Food Chem 2004; 52(9): 2485–9.
22. *Buege AL, Aust DS.* Microsomal lipid peroxidation. In: Fleisher S, Parker L, editors. Methods in Enzymology. New York: Academic Press; 1978. p. 306–10.
23. *Ćebović T In vitro* and *in vivo* antioxidant properties testing of *Viscum album* L. and *Allium cepa* L [thesis]. Novi Sad: Faculty of Sciences; 2003. (Serbian)
24. *Ćebović T.* The influence of the extracts (*Viscum album* L.) from different hosts on the formation of free radicals of oxygen and the activity of antioxidant enzymes [dissertation]. Belgrade: University of Belgrade, Faculty of Pharmacy; 2008. (Serbian)
25. *Garrat DC.* The quantitative analysis of drugs. 3rd ed. Japan, Tokyo: Chapman and Hall International Edition 1964.
26. *Četojević-Simin D, Velićanski A, Cvetković D, Markov S, Ćetković G, Tumbas Šaponjac V,* et al. Bioactivity of Meeker and Willamette raspberry (Rubus idaeus L.) pomace extracts. Food Chem 2015; 166: 407–13.
27. *Beara I, Lesjak M, Četojević-Simin D, Marjanović Ž, Ristić J, Mrkonjić Z,* et al. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of black (Tuber aestivum Vittad.) and white (Tuber magnatum Pico) truffles. Food Chem 2014; 165: 460–6.
28. *Četojević-Simin D, Velićanski A, Cvetković D, Markov S, Mrđanović J, Bogdanović V,* et al. Bioactivity of Lemon Balm Kombucha. Food Bioprocess Technol 2012; 5(5): 1756-65.
29. *Četojević-Simin D, Čanadanović-Brunet J, Bogdanović G, Đilas S, Ćetković G, Tumbas V,* et al. Antioxidative and Antiproliferative Activities of Different Horsetail (Equisetum arvense L.) Extracts. J Med Food 2010; 13(2): 452–9.
30. *Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D,* et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82(13): 1107–12.
31. *Procházková D, Boušová I, Wilhelmová N.* Antioxidant and prooxidant properties of flavonoids. Fitoterapia 2011; 82(4): 513–23.
32. *Pietta PG.* Flavonoids as antioxidants. J Nat Prod 2000; 63(7): 1035–42.
33. *Awotunde OS, Dhanabal SP, Rajeshkumar R, Chasitainya MV.* Antioxidant activities of Hydro-ethanol and Saponin extracts of *Terminalia schimeperiana* root. Der Pharmacia Lettre 2017; 9(11): 47–53.

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# Congenital anomalies: occurrence and potential risk factors

## Urođene anomalije: pojava i potencijalni faktori rizika

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**Abstract**

**Background/Aim.** Congenital malformations still represent one of the most important causes of prenatal and infant death. The study aim was to analyze occurrence, outcomes and risk factors of different types of congenital anomalies. **Methods.** The study included all pregnant women directed to Clinic of Obstetrics and Gynecology, Clinical Center of Serbia, Belgrade due to prenatally diagnosed congenital fetal anomalies during past ten years (January 1, 2008–December 31, 2017). Upon admission to our Clinic a detailed general medical and obstetrical history were taken from every patient. All women underwent genetic testing. Ultrasonography and magnetic resonance were diagnostic methods for fetal malformations confirmation. **Results.** The study included 773 pregnant women aged from 18 to 46 years. Out of registered nine different groups of fetal anomalies/malformations, the most common were malformations of the central nervous system, while majority of fetuses had combined multiple anomalies. Genetic cause for congenital anomalies was present in 25.2% of pregnancies. Medical pregnancy abortion was performed in 71.8% of cases. Only 10.2% of pregnancies ended in term. The best outcome for children was obtained in case of gastrointestinal anomalies (52% live born). Contrary, only one child with neck and thorax malformations could be saved. According to logistic regression the most important predictor of having a child with combined multiple anomalies was mother's age, while predictor of central nervous system anomalies was gestational diabetes. The significant predictor of genetic anomalies was mother's age. **Conclusion.** In our sample neurological congenital anomalies were the most common, although abnormalities of all organ systems were registered. Majority of pregnancies had to be discontinued due to combined multiple anomalies caused by genetic disorders. Older mother's age and diabetes can imply on the increased risk for fetal malformations.

**Key words:**
**age factors; congenital abnormalities; diagnosis; incidence; pregnancy; prognosis; risk factors; ultrasonography.**

**Apstrakt**

**Uvod/Cilj.** Urođene malformacije i dalje predstavljaju jedan od najvažnijih uzroka prenatalne i neonatalne smrti. Cilj rada bio je analiza pojave ishoda različitih tipova kongenitalnih anomalija. **Metode.** Studijom su bile obuhvaćene sve trudnice koje su u periodu 1. januar 2008–31. decembar 2017. godine bile upućene na Kliniku za ginekologiju i akušerstvo Kliničkog centra Srbije zbog prenatalno dijagnostikovanih kongenitalnih fetalnih anomalija. Po prijemu na našu Kliniku uzimani su detaljni opšti medicinski i akušerski podaci od svake pacijentkinje. Sve trudnice su podvrgnute genetskom testiranju. Ultrazvuk i magnetna rezonanca su bili dijagnostičke metode za potvrdu malformacija fetusa. **Rezultati.** Studijom su obuhvaćene 773 trudnice koje su imale od 18 do 46 godina života. Od registrovanih devet različitih grupa fetalnih anomalija/malformacija, najčešće su bile malformacije centralnog nervnog sistema, dok je većina fetusa imala više kombinovanih anomalija. Genetički uzrok kongenitalnih anomalija bio je prisutan u 25,2% trudnoća. Medicinski prekid trudnoće obavljen je u 71,8% slučajeva. Samo 10,2% trudnoća je završeno u terminu. Najbolji ishod za decu dobijen je u slučajevima gastrointestinalnih anomalija (52% živorođenih). Nasuprot tome, samo jedno dete sa malformacijama vrata i grudnog koša se moglo spasiti. Prema logističkoj regresiji najvažniji prediktor da dete ima kombinovane višestruke anomalije je bila starost majke, dok je prediktor anomalija centralnog nervnog sistema bio gestacijski dijabetes. Značajan prediktor genetskih anomalija bila je starost majke. **Zaključak.** U našem uzorku najčešće su bile neurološke kongenitalne anomalije, iako su registrovane abnormalnosti svih organskih sistema. Većina trudnoća se morala prekinuti zbog kombinovanih višestrukih anomalija uzrokovanih genetskim poremećajima. Starije životno doba majke i dijabetes mogu ukazivati na povišen rizik od fetalnih malformacija.

**Ključne reči:**
**životno doba, faktori; anomalije; dijagnoza; incidenca; trudnoća; prognoza; faktori rizika; ultrasonografija.**

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## Introduction

Congenital anomalies or malformations of the fetus are disorders of the structure, behavior, function and metabolism that arose before birth [1]. They result from disturbed development and growth during the embryonic and fetal intrauterine period. Etiology can be chromosomal and genetic, infectious, medicamentous, multifactorial, etc [2].

According to the literature data the incidence of fetal anomalies is 1.5% per year. The incidence of anomalies in pregnancies ending in spontaneous abortion is 3.3%, with intrauterine fetal death is 13%, while 0.4% of fetal anomalies are diagnosed after birth [3, 4]. Infant mortality rate from congenital malformations in a period of 15 years prior to our study (1993 to 2007) in Serbia was 1.8 (confidence interval 1.5–2.1) out of which 45.1% was caused by genetic disorders [5].

Whether the developmental anomalies occurred during organogenesis (malformation) or after their initial normal organ formation (disruption and deformation) the timely diagnosis of fetal anomalies allows medical practitioners to make an appropriate decision on the further pregnancy management [2, 6]. It is necessary to detect and discontinue pregnancy with fetal anomalies incompatible with life in time, or to begin the appropriate treatment of diagnosed malformation as soon as possible to enable not only survival, but also good quality of life for the child [7].

Although recently much has been done to improve malformation early diagnosis and treatment, there is still a debate about all risk factors causing congenital anomalies. Therefore, the aim of this study was to analyze occurrence and outcomes of different types of congenital anomalies from the tertiary referral center during a ten-year period. Moreover, study aimed at examining potential predictors of congenital anomalies based on patients' characteristics and medical history data.

## Methods

Study included all consecutive pregnant women directed to the Clinic of Obstetrics and Gynecology, Clinical Center of Serbia in Belgrade due to prenatally suspected congenital fetal anomalies. Our Clinic is one of the three tertiary referral centers for medically indicated late pregnancy terminations in Serbia in charge of patients from central Serbia (Šumadija) as well as the most complex cases from the whole country. The study was approved by the Institutional Review Board. Women were prospectively included in the study during a period of ten years (from January 1, 2008 to December 31, 2017). All investigated patients signed informed consent for the study. The main inclusion criterion was prenatally verified (clinical and/or laboratory) congenital anomaly/malformation of the fetus.

Upon admission to our Clinic a detailed general medical and obstetrical history were taken from every patient regarding age, hereditary and chronic illnesses, parity, gestational complications and outcomes of previous pregnancies (pregnancy losses, previous congenital anomalies). During the examined pregnancy we registered all gestational illnesses and complications (diabetes, rhesus D – RhD immunization) as well as the infections that could potentially cause fetal malformations [toxoplasmosis, rubella, varicella, herpes simplex virus (HSV), hepatitis B virus (HBV), cytomegalovirus (CMV), intestinal bacteria and others].

All women underwent screening for genetic abnormalities – in the first trimester (11 to 14 gestational weeks) Double test and in the second trimester (16 to 19 gestational weeks) Triple test were performed. For these tests we used Brahms Kryptor analyzer and fluorocytometric immunoassay with SsdwLab 5 software. Moreover, fetal karyotypisation was done in order to make the final diagnosis of potential genetic disorder. In case of suspected specific chromosomal numerical or structural disorders and rearrangements polymerase chain reaction (PCR) with appropriate primers was also done. Cell samples for genetic analyses were obtained by chorionic willi biopsy, amniocentesis or cordocentesis (depending on the gestational week).

Ultrasound (US) biometry and pregnancy monitoring were performed through ACCUVIX device (Samsung Medison, Seoul, Sought Korea), with 3.75 MHz abdominal and vaginal probe. Pregnancies were dated by last menstrual period and US biometric parameters. Biometrical parameters were also used for diagnosing fetal intrauterine growth restriction (IUGR). Moreover, US was used to assess amniotic fluid volume based on the deepest fluid pocket measurements (oligoamnion < 2 cm; normal fluid 2–8 cm; polyhydramnion > 8 cm). These two patological findings were specially registered as they can somethimes indicate other pregnancy complications including that fetuses have congenital anomalies.

US was the main diagnostic method for assessing fetal malformations. All examinations were performed by three obstetrics and gynecology specialists and perinatology US experts (study authors). Moreover, in some cases magnetic resonance (MR) imaging of the fetus was also done using the Siemens 1.5 Tesla Symphony apparatus. The final diagnosis as well as the decision for anomaly treatment or pregnancy termination were done in accordance with neonatologists and pediatric surgeons, members of the Congenital Anomalies/Malformations Consilium of our Clinic.

In case of minor fetal anomalies, which could be surgically corrected after birth, it was decided to continue the pregnancy and these patients were regularly checked-up throughout the second and third pregnancy trimester according to the high-risk pregnancy guidelines. The main positive pregnancy outcome assessed in the study was having liveborn children.

Contrary, if the anomaly was of genetic origin, surgically uncorrectable or incompatible with life, pregnancy was terminated after the parents signed the informed consent. We noted the method of pregnancy termination for every patient (curettage in the first or early second trimester, instillation or feticide) as well as the way of abortion/delivery (Caesarean section or vaginal delivery with or without prostaglandins PGM15 or PGE2, Foley catheter or oxytocin induction/stimulation).

We noted the week of obtaining the final diagnosis of congenital anomaly as well as the week of pregnancy termination in each case. For final malformation verification all fetuses that were not liveborn were sent to autopsy and hi-

stopathological examinations. Liveborn children were assessed by neonatologist after birth. All malformations were grouped according to the affected organ system and divided on those with and without genetic cause.

All obtained data were statistically analyzed using methods of descriptive (number, percentage, mean, standard deviation) and analytical statistics and applying the SPSS 20 software. Significance of differences between categories of assessed parameters was examined by $\chi^2$ test. Correlations of fetal anomaly type and pregnancy outcome with patients' characteristics and medical history data were tested using Spearman's correlation.

Finally, we applied multiple logistic regression to investigate the predictors of occurrence of different types of congenital anomalies based on patients' characteristics and medical history data. Moreover, we performed binary logistic regression to investigate the predictors genetic anomalies based on patients' characteristics and medical history data.

## Results

Study included 773 pregnant women aged from 18 to 46 years. Data regarding patients' age, previous parity and the gestational week when the malformation was diagnosed are presented in Table 1. Majority of women did not have any hereditary or chronic illnesses as well as pregnancy complication in previous and investigated pregnancy (Table 2).

**Table 1**

**Descriptive general data of investigated patients**

| Parameters | Minimum–Maximum | Mean ± Standard deviation |
|---|---|---|
| Age (years) | 18.00–46.00 | 30.35 ± 6.35 |
| Previous parity (n) | 0.00–9.00 | 1.07 ± 1.30 |
| Live-born children up to now (n) | 0.00–6.00 | 0.63 ± 0.81 |
| Prior abortions (n) | 0.00–7.00 | 0.17 ± 0.61 |
| Prior miscarriages (n) | 0.00–4.00 | 0.23 ± 0.55 |
| Gestational month of miscarriage (n) | 0.00–7.00 | 2.30 ± 0.90 |
| Gestational week of malformation diagnosis (n) | 9.00–39.00 | 23.81 ± 7.05 |

**n - number of occurrences.**

**Table 2**

**Frequency of investigated parameters in examined patients**

| Parameters | Number (%) | $\chi^2$ | *p* |
|---|---|---|---|
| Diabetes mellitus in family | | | |
| no | 736 (95.2) | 2033.784 | 0.001 |
| mother | 25 (3.2) | | |
| father | 9 (1.2) | | |
| others | 3 (0.4) | | |
| Gestational diabetes | | | |
| no | 758 (98.1) | 714.164 | 0.001 |
| yes | 15 (1.9) | | |
| RhD incompatibility | | | |
| no | 695 (89.9) | 492.483 | 0.001 |
| yes | 78 (10.1) | | |
| RhD immunization | | | |
| no | 772 (99.9) | 769.005 | 0.001 |
| yes | 1 (0.1) | | |
| Infections during pregnancy | | | |
| no infections | 728 (94.2) | 3344.684 | 0.001 |
| toxoplasmosis | 6 (0.8) | | |
| rubella and/or varicella | 5 (0.6) | | |
| cytomegalovirus | 4 (0.4) | | |
| other viruses[1] | 13 (1.7) | | |
| intestinal bacteria | 13 (1.7) | | |
| other rare findings[2] | 5 (0.6) | | |
| Double test findings | | | |
| low risk | 590 (76.3) | 214.294 | 0.001 |
| high risk | 183 (23.7) | | |
| Triple test findings | | | |
| low risk | 371 (58.3) | 34.371 | 0.001 |
| high risk | 265 (41.7) | | |
| Genetic abnormalities | | | |
| no | 578 (74.8) | 1035.554 | 0.001 |
| syndrome Down | 107 (13.8) | | |
| other aneuploidies | 51 (6.6) | | |
| gene mutations/rearrangements | 37 (4.8) | | |

**[1]Other viruses – Parvo B 19, HBV – hepatitis B virus, HCV – hepatitis C virus, HPV– human papilloma virus, influenca and Zika viruses; [2]other rare findings – Ureaplasma, Mykoplasma and Chlamydia; RhD – rhesus D.**

**Table 3**

**Ultrasonography (US)/magnetic resonance (MR) and final findings of fetal congenital anomalies/malformations**

| Parameters | Number (%) | $\chi^2$ | *p* |
|---|---|---|---|
| US / MR findings of fetal anomalies and malformations and/or other pathologies potentially implying on anomalies | | | |
| no anomalies | 148 (19.1) | 580.142 | 0.001 |
| central nervous system | 226 (29.2) | | |
| neck and thorax | 41 (5.3) | | |
| cardiovascular | 85 (11.0) | | |
| gastrointestinal | 47 (6.1) | | |
| musculoskeletal | 38 (4.9) | | |
| urogenital | 57 (7.4) | | |
| other rare findings | 10 (1.3) | | |
| combined multiple | 70 (9.1) | | |
| intrauterine growth restriction | 26 (3.4) | | |
| Final findings of fetal anomalies and malformations | 197 (25.5) | 704.503 | 0.001 |
| central nervous system | 42 (5.4) | | |
| neck and thorax | 68 (8.8) | | |
| cardiovascular | 50 (6.5) | | |
| gastrointestinal | 38 (4.9) | | |
| musculoskeletal | 46 (6.0) | | |
| urogenital | 40 (5.2) | | |
| chromosomal without anatomy | 48 (6.2) | | |
| other rare findings | 244 (31.6) | | |

Significantly more women had low risk on screening test, both Double and Triple. However, Triple test seemed to be more reliable in our population for congenital malformation prediction as almost 40% of pregnancies were adequately recognized as in risk. US and MR as diagnostic tool for congenital malformations were very reliable as 74.3% of malformations were appropriately prenatally detected (Table 3). These imaging methods had the best results for assessment of central nervous system (CNS) anomalies. Still, in some cases no anomalies were visualized or only intrauterine growth restriction (IUGR) and abnormality in amniotic fluid volume were registered.

Nine different groups of fetal anomalies/malformations (according to organ system) were confirmed on the examinations upon pregnancy termination (Table 3). The most common once were malformations of the CNS, while majority of fetuses had combined multiple anomalies. Genetic cause for congenital anomalies was present in 25.2% of pregnancies (Table 2) out of which Down's syndrome was the most common.

When genetic abnormalities were analyzed we registered eight deletions, five duplications, six inversions, four translocations and 14 single gene polymorphysms. When aneuploidies were evaluated Turner's syndrome was registerd in eight case, Patau in eight cases, Edward's syndrome in 15 cases, Klinefelter's syndrome in seven cases, triple X in three cases, trisomies of chromosomes 8, 18 and 20 in one case each, mosaic in six cases, while in one case multiple trisomies were registered.

In patients with gestational diabetes mellitus we registered anomalies of the CNS in six cases, cardiovascular system (CVS) in two cases, gastrointestinal (GIT) in two cases (omphalocelea), while in one patient fetus had urogenital (kidney) anomalies. In four patients with gestational diabetes mellitus we registered multiple fetal anomalies out of which in two cases fetuses had combined cystic neck hygroma with abdominal tumefactions and in remaining two cases cystic neck hygroma was combined with mediastinal tumors and generalised fetal hydrops.

Pregnancy outcomes are presented in Table 4. Medical pregnancy abortion was performed in 71.8% of cases. Only 10.2% of pregnancies ended in term. Significantly more pregnancies were ended during the second trimester. In 63 women Caesarean section had to be performed due to obstetrical indications.

**Table 4**

**Pregnancy outcomes**

| Parameters | Number (%) | $\chi^2$ | *p* |
|---|---|---|---|
| Medical abortion | | | |
| no | 218 (28.2) | 146.920 | 0.001 |
| yes | 555 (71.8) | | |
| Curettage (I or II trimester) | | | |
| no | 455 (58.8) | 419.397 | 0.001 |
| yes | 318 (41.1) | | |
| Induced vaginal delivery | | | |
| no | 457 (59.1) | 408.290 | 0.001 |
| yes | 316 (40.0) | | |
| Caesarean section parva | | | |
| no | 712 (92.1) | 548.255 | 0.001 |
| yes | 63 (8.2) | | |
| Pregnancy termination time | | | |
| in term | 79 (10.2) | 734.389 | 0.001 |
| I trimester | 113 (14.6) | | |
| II/III trimester | 581 (75.2) | | |
| Live-born children | | | |
| no | 673 (87.1) | 424.746 | 0.001 |
| yes | 100 (12.9) | | |

In our study 12.9% (100 out of 773) of children had correctible malformations and therefore were successfully liveborn. Anomaly type and having liveborn children correlated negatively (ρ = - 0.075; $p = 0.037$). Among investigated fetuses liveborn children had all registered anomaly types. However, the majority of anomalies that were considered minor and/or tretable were those of gastrointestinal tract (mostly gastrochisis and omphalocela). The best outcome for children was obtained in case of gastroschisis omphalocele (26 out of 50 children were liveborn – 52%). Out of CNS anomalies liveborn children mostly had slight ventriculomegalia, while majority of minor urogenital anomalies in our sample were renal cysts. There were four cases of single heart anomalies that were successfully operated after delivery. Moreover, in our study there were also 16 cases of multiple anomalies that were treatable and these mostly included combined CVS anomalies. Contrary, only one child (2.4%) with malformations of the neck and thorax could be saved.

Patients age, findings of Double and Triple tests, genetic abnormalities and I trimester curettage as the pregnancy termination method correlated positively, while gestational week of diagnosis and vaginal method of delivery correlated negatively with the type of registered anomalies (Table 5). Combined multiple anomalies were more often registered in older women. These malformations were mostly on genetic basis and registered early by screening methods. Consequently, pregnancies with fetuses that had combined multiple anomalies in our sample were commonly terminated in the first trimester.

Having liveborn children with congenital anomalies correlated positively with having all previous children liveborn, gestational week of malformation diagnosis and the findings of US/MR, while it correlated negatively with patient's age, findings of Double and Triple tests, genetic abnormalities as well as the pregnancy termination time and type. So it can be seen that having healthy previous pregnancies and performing regular pregnancy check-ups that could allow early diagnosis of any gestational complications is the best way to ensure that even children with congenital anomalies can be live-born if their malformations are correctable. Conversely, genetic anomalies were the major cause of both spontaneous as well as medically induced pregnancy terminations.

Finally, we obtained a significant model for prediction of occurrence of different types of congenital anomalies based on patients' characteristics and medical history data (R = 0.412; adjusted $R^2 = 0.613$; F = 2.999; $p = 0.003$; constant = 0.724). According to our findings the most important predictors of having a child with combined multiple anomalies were mother's age (B = 0.183), while predictor of CNS anomalies was gestational diabetes (B = -2.0303). Moreover, we obtained a significant model for prediction of genetic anomalies based on patients' characteristics and medical history data (B = 0.704; Wald = 15.572; Nagelkerke $R^2 = 0.617$; $\chi^2 = 24.082$; $p = 0.004$; explained variance = 68.3%). The significant predictor was mother's age (constant = -5.073; B = 0.143).

**Table 5**
**Correlations of fetal anomaly type and pregnancy outcome with patients' characteristics and medical history data**

| Parameters | Anomaly type | Live-born children |
|---|---|---|
| Patients age | | |
| rho | **0.217** | **-0.138** |
| *p* | **0.001** | **0.001** |
| Previous parity | | |
| rho | 0.029 | -0.063 |
| *p* | 0.426 | 0.079 |
| Live-born children up to now | | |
| rho | -0.001 | **0.107** |
| *p* | 0.979 | **0.003** |
| Prior miscarriages number | | |
| rho | 0.061 | -0.014 |
| *p* | 0.091 | 0.704 |
| Gestational month of miscarriage | | |
| rho | -0.072 | -0.009 |
| *p* | 0.392 | 0.914 |
| Gestational week of malformation diagnosis | | |
| rho | **-0.198** | **0.169** |
| *p* | **0.001** | **0.001** |
| Diabetes mellitus in family | | |
| rho | -0.056 | -0.014 |
| *p* | 0.119 | 0.693 |
| Gestational diabetes | | |
| rho | -0.042 | 0.002 |
| *p* | 0.242 | 0.963 |
| RhD incompatibility | | |
| rho | 0.050 | -0.001 |
| *p* | 0.166 | 0.974 |
| RhD immunization | | |
| rho | 0.044 | -0.014 |
| *p* | 0.225 | 0.700 |
| Infections during pregnancy | | |
| rho | 0.031 | 0.051 |
| *p* | 0.392 | 0.154 |
| Double test findings | | |
| rho | **0.324** | **-0.160** |
| *p* | **0.001** | **0.001** |
| Triple test findings | | |
| rho | **0.145** | **-0.082** |
| *P* | **0.000** | **0.022** |
| Genetic abnormalities | | |
| Rho | **0.496** | **-0.162** |
| *P* | **0.001** | **0.001** |
| US/MR findings | | |
| Rho | **0.128** | **0.071** |
| *P* | **0.001** | **0.050** |
| Medical abortion | | |
| Rho | -0.018 | **-0.615** |
| *P* | 0.612 | **0.001** |
| Curettage | | |
| rho | **0.112** | **-0.323** |
| *p* | **0.002** | **0.001** |
| Induced vaginal delivery | | |
| rho | **-0.075** | **-0.219** |
| *p* | **0.036** | **0.001** |
| Caesarean section parva | | |
| rho | 0.033 | -0.013 |
| *p* | 0.363 | 0.724 |
| Pregnancy termination time | | |
| rho | 0.001 | **-0.416** |
| *p* | 0.972 | **0.001** |

**RhD – rhesus D; US – ultrasonography; MR – magnetic resonance.**
**Note: Statistically significant values are bolded.**

## Discussion

Worldwide investigations have shown that occurrence of congenital anomalies varies greatly among countries. The prevalence of congenital anomalies according to literature data ranges from as low as 1.07% in Japan and as high as 4.3% in Taiwan [8, 9]. Major anomalies, which significantly affect the development and quality of life of a human individual, are present in 2%–3% of newborn children, while another 2%–3% of malformations are diagnosed by the age of five [10, 11]. They are one of the main causes of childhood deaths (up to 20%–25% of cases). Contrary, minor anomalies (skin lesions, small ears or a narrow gap between the eyebrows) occur in about 15% of newborns, they do not affect health, but their presence can indicate at the same time the existence of some major malformations [1, 12]. Furthermore, frequency and structure of different types of congenital anomalies depend on the investigated population. Specific studies have registered the predominance of different congenital anomalies, however, based on all available literature data the most common ones are usually neurologic, cardiac, gastrointestinal and musculoskeletal malformations. Abnormalities on all other organ systems are less often reported [13, 14].

Differences found in congenital anomalies rates in different countries and studies could be based on actual variations among assessed populations or due to different anomalies definitions or study methods [7, 15]. Additionally, inclusion of stillbirths, prenatally diagnosed cases and pregnancy terminations increase significantly the overall prevalence of children with congenital anomalies. Moreover, in less developed countries there are no registries of children with malformations or the data are poorly documented and insufficient [13, 16]. Consequently, epidemiologists are often reluctant to present the total prevalence of congenital anomalies in certain countries and populations [9]. Nevertheless, congenital malformations, taken collectively, are fairly common, and account for a disproportionate share of adverse perinatal outcomes. Therefore, in the year 1979, a network of population-based registries, European Surveillance of Congenital Anomalies (EUROCAT) was made in order to conduct epidemiological surveillance of congenital anomalies in Europe [4]. Clinicians and researchers are encouraged to use data from this and other reliable population-based registries, while all countries should take participation in active registration and reporting of congenital anomalies [3].

In our study of prenatally diagnosed congenital malformations in the population from central Serbia the most common single organ system anomalies were registered on CNS, while numerous children also had multiple combined anomalies. Majority of these multiple anomalies were due to genetic syndromes (mostly Down syndrome). In our sample only 12.9% of children were liveborn. This was the first study in Serbian population that made prediction models for congenital anomalies based on patients' characteristics and medical history data. According to logistic regression the most important predictors of having a child with combined multiple anomalies were mother's age, while gestational diabetes was associated with CNS anomalies. The significant predictor of genetic anomalies was mother's age.

## Conclusion

In our sample from central Serbian referral tertiary clinic congenital anomalies of CNS were the most common single system anomalies, although malformations of all organ systems were registered. Majority of pregnancies had to be discontinued due to combined multiple anomalies caused by genetic disorders. Older mother's age and diabetes can imply on the high risk for fetal malformations. Regular pregnancy check-ups can allow early diagnosis of any gestational complications and ensure that even children with congenital anomalies can be liveborn if their malformations are correctable. Construction and regular updating of a detailed (including all patients data) congenital anomalies registry in Serbia is necessary and might help clinicians and enhance further investigations of this issue.

## Conflict of interest

Authors declare no conflict of interest. This study received no funding.

## REFERENCES

1. *Boyle B, Addor MC, Arriola L, Barisic I, Bianchi F, Csaky-Szunyogh M*, et al. Estimating Global Burden of Disease due to congenital anomaly: an analysis of European data. Arch Dis Child Fetal Neonatal Ed 2018; 103(1): F22–F28.
2. *Oliveira CI, Fett-Conte AC*. Birth defects: risk factors and consequences. J Pediatr Genet 2013; 2(2): 85–90.
3. *World Health Organization* (WHO). Congenital anomalies. Fact sheet; 2016 Available from: http://www.who.int/mediacentre/factsheets/fs370/en.
4. *European Surveillance of Congenital Anomalies* (EUROCAT). Reports and Papers. Available from: http://www.eurocat-network.eu/homepage
5. *Gazibara T, Kisic-Tepavcevic D, Dotlic J, Matejic B, Grgurevic A, Pekmezovic T*. Patterns of infant mortality from 1993 to 2007 in Belgrade (Serbia). Matern Child Health J 2013; 17(4): 624–31.
6. *Francine R, Pascale S, Aline H*. Congenital anomalies: prevalence and risk factors. Univ J Public Health 2014; 2(2): 58–63.
7. *Garne E, Khoshnood B, Loane M, Boyd P, Dolk H; EUROCAT Working Group*. Termination of pregnancy for fetal anomaly after 23 weeks of gestation: a European register-based study. BJOG 2010; 117(6): 660–6.
8. *Aloui M, Nasri K, Ben Jemaa N, Ben Hamida AM, Masmoudi A, Gaigi* SS, et al. Congenital anomalies in Tunisia: Frequency and risk factors. J Gynecol Obstet Hum Reprod 2017; 46(8): 651–5.
9. *Kirby RS*. The prevalence of selected major birth defects in the United States. Semin Perinatol 2017; 41(6): 338–44.
10. *St Louis AM, Kim K, Browne ML, Liu G, Liberman RF, Nembhard WN*, et al. Prevalence trends of selected major birth defects: A multi-state population-based retrospective study, United States, 1999 to 2007. Birth Defects Res 2017; 109(18): 1442–50.

11. *Nasri K, Ben Fradj MK, Hamdi Th, Aloui M, Ben Jemaa N, Nahdi S*, et al. Epidemiology of neural tube defect subtypes in Tunisia, 1991-2011. Pathol Res Pract 2014; 210(12): 944–52.
12. *Brodwall K, Greve G, Leirgul E, Klungsoyr K, Holmstrom H, Vollset SE*, et al. The five-year survival of children with Down syndrome in Norway 1994-2009 differed by associated congenital heart defects and extracardiac malformations. Acta Paediatr 2018; 107(5): 845–53.
13. *Sarkar S, Patra C, Dasgupta MK, Nayek K, Karmakar PR*. Prevalence of congenital anomalies in neonates and associated risk factors in a tertiary care hospital in Eastern India. J Clin Neonatol 2013; 2(5): 131–4.
14. *Dolk H, Loane M, Garne E*. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol 2010; 686: 349–64.
15. *WangY, Liu G, Canfield MA, Mai CT, Gilboa SM, Meyer RE*, et al. National Birth Defects Prevention Network. Racial/ethnic differences in survival of United States children with birth defects: a population-based study J Pediatr 2015; 166(4): 819–26.e1–2.
16. *Pangkanon S, Sawasdivorn S, Kuptanon C, Chotigeat U, Vandepitte W*. Establishing of National Birth Defects Registry in Thailand. J Med Assoc Thai 2014; 97 Suppl 6: S182–8.
17. *Sipek A, Gregor V, Sipek A Jr, Hudakova J, Horacek J, Klaschka J*, et al. Incidence of congenital heart defects in the Czech Republic-current data. Ceska Gynekol 2010; 75(3): 221–42. (Czech)
18. *Ben Halim N, Ben Alaya Bouafif N, Romdhane L, Kefi Ben Atig R, Chouchane I, Bouyacoub Y*, et al. Consanguinity, endogamy, and genetic disorders in Tunisia. J Community Genet 2013; 4(2): 273–84.
19. *Taruscio D, Baldi F, Carbone P, Neville AJ, Rezza G, Rizzo C*, et al. Primary Prevention of Congenital Anomalies: Special Focus on Environmental Chemicals and other Toxicants, Maternal Health and Health Services and Infectious Diseases. Adv Exp Med Biol 2017; 1031: 301–22.
20. *Garcia-Ferreyra J, Hilario R, Duenas J*. High percentages of embryos with 21, 18 or 13 trisomy are related to advanced paternal age in donor egg cycles. JBRA Assist Reprod 2018; 22(1): 26–34.
21. *Eriksen NB, Damm P, Mathiesen ER, Ringholm L*. The prevalence of congenital malformations is still higher in pregnant women with pregestational diabetes despite near-normal HbA1c: a literature review. J Matern Fetal Neonatal Med 2017; 27: 1–5.

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# The importance of physical treatment in children underwent craniosynostosis surgery in the first year of life

## Značaj habilitacionog tretmana kod dece operisane od kraniosinostoza u prvoj godini života

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**Abstract**

**Background/Aim.** Craniosynostosis is a condition that occurs intrauterine or develops in the infant period, and represents premature fusion of cranial sutures. This fusion of sutures limits the normal cranium development and leads to disorder in the phase of rapid growth and development of the brain. Creation of craniosynostoses is associated with an increased incidence of developmental delay during the breastfeeding period. Craniosynostoses are treated by surgery. The role of a physiatrist is to postoperatively assess psychomotor development and implement habilitation treatment. The aim of this study was to determine distribution of the type of craniosynostoses according to the age and gender of patients, effectiveness of habilitation treatment and to estimate the somatosensory evoked potential in the preoperative and postoperative period in children who underwent craniosynostosis surgery in the first year of life. **Methods.** The study was designed as a retrospective research. The data were collected from medical records of 51 children with craniosynostoses and delay in psychomotor development who underwent surgical intervention. The children included in this study, during follow-up, were involved in the habilitation treatment. **Results.** An early diagnosis and surgical intervention had a favorable effect on the development of motor function in children with craniosynostoses. The importance of stimulation treatment in the postoperative period was also proved for achievement of an adequate degree of motor development in children in relation to age. The results of our study confirmed the results obtained in previously published studies that the children who did not undergo surgery and start with the habilitation treatment immediately after it, had delay in psychomotor development of moderate degree. **Conclusion.** Habilitation treatment significantly reduced the deviations in psychomotor development of children with craniosynostoses if it started immediately after the surgical procedure.

**Key words:**
**craniosynostoses; skull; infant; psychomotor disorders; evoked potentials; somatosensory physical therapy modalities.**

**Apstrakt**

**Uvod/Cilj.** Kraniosinostoza je stanje koje nastaje intrauterino ili se razvija u odojačkom periodu, a predstavlja prerano srastanje kranijalnih sutura. Ovakvo srastanje sutura ograničava normalan razvoj kranijuma i dovodi do poremećaja u fazi brzog rasta i razvoja mozga. Nastanak kraniosonostoza povezan je sa povećanom incidencijom kašnjenja u razvoju tokom odojačkog perioda. Tretman kraniosinostoza je hirurški. Uloga fizijatra je da u postoperativnom periodu izvrši procenu psihomotornog razvoja i sprovede habilitacioni tretman. Cilj ovog rada je bio da se kod dece operisane od kraniosonostoza u prvoj godini života utvrdi distribuciju tipa kraniosinostoza prema uzrastu i polu deteta, utvrdi efikasnost habilitacionog tretmana i ispitaju somatosenzorni evocirani potencijali u preoperativnom i postoperativnom periodu. **Metode.** Sprovedena je retrospektivna studija, a podaci su prikupljeni iz medicinske dokumentacije 51 deteta kod kojih je postavljena dijagnoza kraniosinostoze i urađena hiruška intervencija, a kod kojih je ustanovljeno kašnjenje u motornom razvoju u odnosu na uzrast i tip kraniosinostoze. Deca obuhvaćena ovim istraživanjem su, tokom praćenja, bila uključena u habilitacioni tretman. **Rezultati.** Pokazano je da rano dijagnostikovanje i hirurška intervencija imaju

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povoljan efekat na razvoj motornih funkcija dece obolele od kraniosinostoze. Takođe je dokazan značaj stimulacionog tretmana u postoperativnom periodu na dostizanje adekvatnog stepena motornog razvoja deteta u odnosu na starosnu dob. Rezultati našeg istraživanja potvrđuju rezultate dobijene u ranije objavljeni studijama, da deca koja nisu operisana i uključena u habilitacioni tretman postoperativno, pokazuju kašnjenje u psihomotornom razvoju umerenog stepena.

**Zaključak.** Habilitacioni tretman značajno je smanjio odstupanja u psihomotornom razvoju dece sa kraniosinostozom, ukoliko je započet odmah posle hirurške intervencije.

**Ključne reči:**
**sinostoze; lobanja; novorođenče; psihomotorni poremećaji; evocirani potencijali, somatosenzorni; fizikalna terapija.**

## Introduction

Craniosynostosis is a condition that occurs intrauterine or develops in the infant period. It represents premature fusion of cranial sutures [1]. This fusion of sutures limits the normal cranium development and leads to disorder in the phase of rapid growth and development of the brain [2]. The prevalence of craniosynostosis in the United States is 10–16 per 10,000 live births, in the UK 4–5 per 10,000 live births, while in France is 4.7 per 10,000 live births [3]. In the Republic of Serbia, the official data have not been released yet. The clinical picture of craniosynostosis shows disrupted form of the skull (dyscrania), fontanelles disappear little earlier than it is normally, there is a ridge along the ossified suture. Craniosynostosis can be syndromic (Syndrome Apert and Syndrome Crouson) and nonsyndromic. According to the localization, craniosynostoses can be sagittal, metopic, coronal, lambdoid, and combined. According to the etiology, craniosynostoses can be primary, secondary and syndromic. According to the number of affected sutures, they can be a single (solitary) and multiple [4, 5]. Craniosynostoses are treated with surgery, by variety of approaches (frontoorbital improving or endoscopic) depending on the localization [6]. Creation of craniosynostoses is associated with an increased incidence of developmental delay during the breastfeeding period. Motor skills have proved especially vulnerable to damage during this developmental period [2]. Treatment of craniosynostosis is surgical. The role of a physician is to postoperatively assess psychomotor development and implement habilitation treatment. Stimulation treatment should make a favorable impact on the physical and mental development of the child who underwent craniosynostosis surgery in the first year of life, ie. during the breastfeeding period [7].

The aim of this study was to determine distribution of the type of craniosynostosis according to the age and gender of patients, effectiveness of habilitation treatment and to estimate somatosensory evoked potentials (SEP) in the preoperative and postoperative period in children who underwent craniosynostosis surgery in the first year of life.

## Methods

The study was designed as a retrospective research; the data were collected from medical records of 51 children with diagnosed craniosynostosis who underwent surgical intervention at the Department of Neurosurgery of the University Children's Hospital in Belgrade in the period from 2011 to 2013. Children had a delay in gross motor development in relation to the age and type of craniosynostosis. Delay in motor development in children was revealed by examination of physiatrists and neurosurgeons, who found that children had not reached a milestone in motor development for their age according to the Munich Development Scale [8]. This scale covers the monitoring of development segments during the first year of life like crawling, sitting, walking, grasping, perception, speech perception and social behavior. Severity of the clinical picture, ie. degree of central coordination disorder, was determined by the physiatrist in relation to the deviation from postural Vojta reactions [9]. The children included in this study, during follow-up, were involved in the habilitation treatment recommended by physiatrists and neurosurgeons.

Dependent variables monitored in the study were: age of a child, preoperative findings of a physiatrist, preoperative findings of SEP, findings of a physiatrist and SEP three and six months after craniosynostosis surgery, while the independent variable was the type of craniosynostosis. In order to apply statistical and econometric methodology, the data relating to the variables were coded as follows: gender: 1 – boy, 2 – girl; craniosynostosis type: 1 – syndromic, 2 – nonsyndromic; type of craniosynostosis: 1 – sagittal, 2 – lamboid, 3 – coronal, 4 – metopic and 5 – combined; findings of a physiatrist: 1 – without findings, 2 – findings of is normal, 3 – disorder of the central coordination of the lower degree (the deviation of the normal motor development ± one month), 4 – disorder of the central coordination of a moderate level (the deviation of the normal motor development 2–3 months), 5 – disorder of the central coordination of severe degree (deviation from the normal motor development up to 6 months); SEP findings: 1 – no finding, 2 – normal finding, 3 – disorder of the lower degree, 4 – disorder of moderate degree; genetic anomaly: 1 – does not exist, 2 – exists.

## Results

Our study tested and followed 51 children with craniosynostosis. Of all the respondents, 34 (66.7%) were boys and 17 (33.3%) were girls. The average age of the children expressed through the arithmetic mean was 169.08 days (50% of children were younger than 154 days, and other half of children was older than 154 days). Distribution of patients according to the type of craniosynostosis and gender is given in Table 1. Based on the data presented in Table 1 only one girl had the type 1 of craniosynostosis (syndromic). All other children, 50 of them, had the type 2 of craniosynostosis (nonsyndromic). Among them, 34 (66.7%) were boys and 17

(33.3%) were girls. Distribution of patients according to the type of craniosynostosis and age is shown in Figure 1. The patients were divided into three groups according to their age. The youngest patients, younger than 126 days, mostly suffered from nonsyndromic craniosynostosis type 1 and type 5 (9 or 17.6%, and 8 or 15.7%, respectively). Patients aged between 127 to 182 days mostly suffered from nonsyndromic craniosynostosis type 5 (6 or 11.8%). The same was in the third age group, over 183 days. Ten of them (19.6%) had nonsyndromic craniosynostosis type 5.

**Table 1**
**Distribution of patients based on the gender and the type of craniosynostosis**

| Type of craniosynostosis | Patients, n | | Total, n (%) |
|---|---|---|---|
| | boy | girl | |
| Syndromic | 0 | 1 | 1 (2) |
| Nonsyndromic | 34 | 16 | 50 (98) |
| Total, n (%) | 34 (66.6) | 17 (33.3%) | 51 (100) |

Distribution of patients with craniosynostoses according to the preoperative findings of a physiatrist related to the central coordination disorder degree is given in Table 2. It was shown that 17 (33.3%) patients had some type of nonsyndromic craniosynostosis but without preoperative finding of a physiatrist. Finding of a physiatrist for one patient was normal, 8 (15.7%) patients had disorder of the central coordination of the lower degree, 19 (37.3%) patients had disorder of the central coordination of the moderate degree and 6 (11.8%) of the patients had disorder of the central coordination of the severe degree. Totally, 33 (64.7%) of the patients with nonsyndromic craniosynostosis had disturbed central coordination of various degrees.

Distribution of patients with craniosynostosis according to the findings of a physiatrist related to the central coordination disorder degree three months after operation is given in Table 3. It was shown that 8 (15.7%) of the patients did not have finding of a physiatrist three months after surgical treatment (5 of them had combined type of craniosynostosis), 3 (5.9%) of the patients had normal finding of a physiatrist, 14 (27.5%) of the patients had disorder of the central coordination of the lower degree, 21 (41.2%) of the patients had disorder of the central coordination of the moderate degree, and 5 (9.8%) of the patients had disorder of the central coordination of the severe degree; a total of 40 (78.4%) of the children had some degree of the central coordination disorder three months after operation, mostly those with sagittal and combined type of craniosynostosis (14 and 18, respectively).

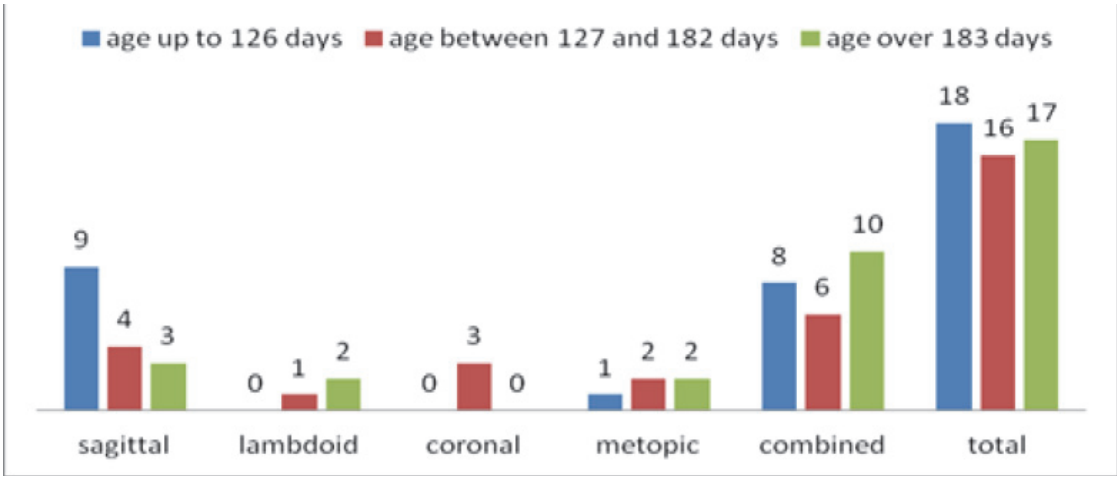


**Fig. 1 – Distribution of patients based on the age and the type of craniosynostosis.**

**Table 2**
**Distribution of patients based on the type of craniosynostosis according to preoperative findings of a physiatrist**

| Type of nonsyndromic craniosynostosis | Findings of a physiatrist | | | | | Total number |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| Sagittal | **5** | 0 | 3 | **6** | **2** | 16 |
| Lambdoid | 2 | 0 | 0 | 0 | 1 | 3 |
| Coronal | 0 | 0 | 0 | 2 | 1 | 3 |
| Metopic | 2 | 1 | 0 | 1 | 1 | 5 |
| Combined | **8** | 0 | **5** | **10** | 1 | 24 |
| Total number | **17** | **1** | **8** | **19** | **6** | **51** |

**Note: bolded values represent the highest number in a total sample of patients (n = 51)**
**Findings of a physiatrist: 1 – without finding, 2 – finding is normal, 3 – disorder of the central coordination of lower degree (deviation of the normal motor development ± one month), 4 – disorder of the central coordination of moderate degree (the deviation of the normal motor development 2–3 months), 5 – disorder of the central coordination of severe degree (deviation from the normal motor development up to 6 months).**

**Table 3**
**Distribution of patients based on the type of craniosynostosis according to the findings of a physiatrist three months after surgery**

| Type of craniosynostosis | Findings* of a physiatrist | | | | | Total number |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| Sagittal | 1 | 1 | **6** | **6** | **2** | 16 |
| Lambdoid | 1 | 1 | 0 | 0 | 1 | 3 |
| Coronal | 0 | 0 | 0 | 3 | 0 | 3 |
| Metopic | 1 | 0 | 0 | 3 | 1 | 5 |
| Combined | **5** | 1 | **8** | **9** | **1** | 24 |
| Total number | **8** | 3 | **14** | **21** | **5** | 51 |

**Note: bolded values represent the highest number in a total sample of patients (n = 51)**
***For explanation see under Table 2.**

Distribution of patients based on the type of craniosynostosis according to the findings of a physiatrist six months after the surgery is given in Table 4. It can be seen that six months after the operation, all the patients had findings of a physiatrist related to the central coordination disorder, mostly the lower and moderate degree (37 patients or 72.5%).

**Table 4**
**Distribution of patients based on the type of craniosynostosis according to the findings of a physiatrist six months after surgery**

| Type of craniosynostosis | Findings* of a physiatrist | | | |
|---|---|---|---|---|
| | 2 | 3 | 4 | Total number |
| Sagittal | 5 | 4 | **7** | 16 |
| Lambdoid | 1 | 1 | 1 | 3 |
| Coronal | 0 | 1 | 2 | 3 |
| Metopic | 1 | 3 | 1 | 5 |
| Combined | **8** | **13** | 3 | **24** |
| Total number | 15 | 22 | 14 | 51 |

**Note: bolded values represent the highest number in a total sample of patients (n = 51)**
***For explanation see under Table 2.**

There were no statistically significant differences in distribution of patients according to SEP findings in three different periods of time: preoperatively, three months and six months after the surgical treatment (Table 5).

## Discussion

Our study included 51 children, 34 (66.7%) boys and 17 (33.3%) girls, underwent craniosynostosis surgery in the first year of life. This gender distribution of children with craniosynostosis is in accordance with results of the longitudinal studies of American pediatric neurosurgeons, published in 2012 and 2015 [10, 11]. Namely, in those studies two-thirds of children who underwent craniosynostosis surgery were male.

Our results showed that average age of the children was 169.08 days. Previous studies suggest that surgery before the age of 6 months results in the improvement in long-term neurological outcome [12].

Distribution of our patients according to the type of craniosynostosis and gender is consistent with results reported in 2015 that 8% of all the patients affected by craniosynostosis suffer from nonsyndromic craniosynostosis [13]. Most our patients, 16 (31.4%) and 24 (47.1%) had nonsyndromic craniosynostosis type 1 and type 5, respectively.

Published data show that children with detected craniosynostosis usually undergo the surgery at the age of 4 to 16 months [2, 12]. This attitude of American neurosurgeons is different from the attitudes of our neurosurgeons. Our opinion is that the best period form performing the surgical treatment is the age of 0 to 6 months because the first year of life is a time when the habilitation treatment after surgery has the best effect on the of psychomotor development of the child. Craniosynostosis in elderly children cause damage of various cognitive functions (attention, speech, abstract thinking) and their recovery is incomplete after surgery, as evidenced by a number of longitudinal studies [10, 14].

The results after three months of the intervention showed that the number of patients with no findings of a physiatrist reduced from 33.3% to 15.7%. It is necessary that all children underwent the craniosynostosis surgery have physiatrist's examination and habilitation treatment as well. This is the only way that patients with craniosynostosis get the opportunity to achieve maximum functional recovery period [15].

**Table 5**
**Distribution of patients according to sensory evoked potential (SEP) findings, preoperatively, three months and six months after the surgery**

| SEP findings | Patients, n (%) | | |
|---|---|---|---|
| | preoperatively | 3 months after surgery | 6 months after surgery |
| No finding | 40 (78.4) | 39 (76.5) | 37 (72.5) |
| Normal | 0 (0) | 0 (0) | 3 (5.9) |
| Lower degree disorder | 3 (5.9) | 6 (11.8) | 8 (15.7) |
| Moderate degree disorder | 8 15.7 | 6 (11.8) | 3 (5.9) |
| Total | 51 (100) | 51 (100) | 51 (100) |

Six months after the operation, all the patients had findings of a physiatrist related to the central coordination disorder, mostly the lower and moderate degree.

All the subjects in this study were included in the postoperative habilitation treatment, which led to an evident clinical improvement six months after the intervention. Limitation of our research was the fact that it was not possible to use the scales for evaluation of psychomotor development such as the Psychomotor Development Index as well as the Bayley Scales of Infant Development, which would make physical treatment findings more accurate.

A small number of the SEP findings, regardless of the severity of the disorder and the type of craniosynostosis, show an inadequate diagnosis and monitoring of recovery of patients after the surgery. This result speaks about the need for education of physiatrists about the importance of neurophysiological testing the patients with craniosynostosis.

Our study analyzed the findings of somatosensory evoked potentials. Since the evoked potentials diagnose changes in the conductivity of the afferent fibers and maturation of CNS, the findings that are generated by the SEP represent a reliable parameter of general motor status, and, accordingly, it is expected that children with deviations in the SEP findings are on a continuous rehabilitation. Our research was carried out during the postoperative habilitation. It was shown that the abnormal SEP finding is an important diagnostic tool in assessing the planning and implementation of continuous habilitation. The real significance of neuropsychological testing would be evident if the analysis of all the types of evoked potentials in patients are carried out for a longer follow-up. On this manner an adequate monitoring of the recovery and development of CNS functions would be provided.

The preoperative findings of a physiatrist as well as those 3 months after the surgery did not differ. In both periods, most patients had the moderate degree of the central coordination disorder (19 patients preoperatively and 21 patients 3 months after the surgery). These results suggest that a longer interval of time is required for the physical therapy treatment, regardless of the severity of the clinical findings. Results relating to physical therapy cannot be detected after only three months of the treatment.

Analysis of findings of a physiatrist 6 months after the surgery showed that all the patients had that finding and that was no patient with the central coordination disorder of the severe degree. Besides, this analysis revealed that most of the patients (22 or 43.1%) had the mild impairment of the central coordination, 15 (29.54%) of the patients had normal finding, and 14 (27.4%) of the patients had the central coordination disorder of the moderate degree. These results were much better than those from the preoperative period and the three-months period after the surgery and are consistent with those from the literature [2]. On the other hand, these results show the effectiveness of postoperative habilitation treatment.

Analysis of the SEP findings showed that the most patients had no such a finding in all three period of time when clinical estimation of the patients was performed (78.4%, 76.5% and 72.5% of the patients in the preoperative period, three months after the surgery and six months after the surgery, respectively). Contrary to rhis, the preoperative findings of a physiatrist could not be found in 17 patients whereas 6 months after the surgery all the respondents had such the finding. Due to this we could not perform correlation analysis between clinical and SEP findings in our patients. In order to make the data comparable, it is necessary that a physiatrist at each stage of the treatment refer patients to the neurophysiological testing (preoperatively, three and six months after the surgery).

Regardless of mentioned limitations, our study showed that the early diagnosis and surgical intervention had a favorable effect on the development of motor function in children with craniosynostosis. The importance of habilitation treatment in the postoperative period was also proved for the achievement of an adequate level of motor development in children underwent craniosynostosis surgery in relation to age.

## Conclusion

If children with craniosynostosis begin habilitation treatment immediately after the surgical intervention, there is a significant reduction of the psychomotor development deviations.

## REFERENCES


1. *Melville H, Wang Y, Taub PJ, Jabs EW*. Genetic Basis of Potential Therapeutic Strategies for Craniosynostosis. Am J Med Genet A 2010; 152A(12): 3007–15.
2. *Da Costa AC, Anderson VA, Savarirayan R, Wrennall JA, Chong DK, Holmes AD* et al. Neurodevelopmental functioning of infants with untreated single – suture craniosynostosis during early infancy. Childs Nerv Syst 2012; 28(6): 869–77.
3. *Wall SA, Thomas GP, Johnson D, Byren JC, Jayamohan J, Magdum SA et al.* The preoperative incidence of raised pressure in non-syndromic sagittal craniosynostosis is underestimated in literature. J Neurosurg Pediatr 2014; 14(6): 674–81.
4. *Kajdic N, Spazzapan P, Velnar T*. Craniosynostosis-Recognition, clinical characteristics, and treatment. Bosn J Basic Med Sci 2018; 18(2): 110–6.
5. *Ruane EJ, Garland CB, Camison L, Fenton RA, Nischal KK, Pollack IF* at al. A Treatment Algorithm for Patients Presenting with Sagittal Craniosynostosis after the Age of 1 Year. Plast Reconstr Surg 2017; 140(3): 582–90.
6. *Paniagua B, Emodi O, Hill J, Fishbaugh J, Pimenta LA, Aylward SR*, at al. 3D of Brain Share and Volume After Cranuial Vault Remodeling Surgery for Craniosynostosis Correction in Infants. Proc SPIE Int Soc Opt Eng 2013; 8672: 86720V.
7. *Sze RW, Parisi MT, Sidhu M, Paladin AM, Ngo AV, Seidel KD et al.* Ultrasound screening of the lambdoid suture in the child with posterior plagiocephaly. Pediatr Radiol 2003; 33(9): 630–6.
8. *Hellbrugge TH, Coulin S, Reisa Bergezann E, Kohler G, Lajosi F, Schamberger R.* Munchener Funktionelle Entwicklungsdiagnostik. Munchen: Univesitat Munchen, Institut fur sociale Padiatric und Jugendmedizin; 1977. (German)

9. *Vojta V*. Die Zerebralen Bewegungsstörungen im Säuglingsalter. Stuttgart: Enke 1984. (German)
10. *Bellew M, Chumas P*. Long-term developmental follow up in children with nonsyndromic craniosynostosis. J Neurosurg Pediatr 2015; 16(4): 445–51.
11. *Ardalan M, Rafati A, Nejat F, Farazmand B, Majed M, El Khashab M*. Risk factors associated with craniosynostosis: a case control study. Pediatr Neurosurg 2012; 48(3): 152–6.
12. *Patel A, Yang JF, Hashim PW, Travieso R, Terner J, Mayes LC*, et al. The impact of age at surgery on long-term neuropsychological outcomes in sagittal craniosynostosis. Plast Reconstr Surg 2014; 134(4): 608e–17e.
13. *Governale LS*. Craniosynostosis. Pediatr Neurol 2015; 53(5): 394–401.
14. *Speltz ML, Collet BR, Wallace ER, Starr JR Cradock MM, Buono L*, et al. Intellectual and academic functioning of school-age children with single-suture craniosynostosis. Pediatrics 2015; 135(3): e615–23.
15. *Knight SJ, Anderson VA, Spencer-Smith MM, Da Costa AC*. Neurodevelopmental Outcomes in Infants and Children with Single-suture Craniosynostosis: a systematic review. Dev Neuropsychol 2014; 39(3): 159–86.

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CASE REPORTS


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# Penetrating neck injury with consequential thoracic complications managed with use of video-assisted thoracoscopic surgery – A case report

## Penetrantna povreda vrata sa posledičnim grudno-hirurškim komplikacijama rešenim primenom video-asistirane torakoskopije

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**Abstract**

**Introduction.** Penetrating injuries of the neck are potentially life-threatening conditions. They can cause injuries of larynx, trachea, esophagus and major blood vessels in this area. **Case report.** The patient was a 28-year-old male who was stabbed with broken glass penetrating the front side of the base of his neck. The patient had dyspnea and the wound was inflicted the night before admission to hospital. An otorhinolaryngologist found a stab wound in the region of the left basis of the neck. The wound was 2 cm long with no signs of bleeding and deep injuries of the anatomical structures of the neck. However, since left hemopneumothorax was clinically and radiologically apparent, drainage of the thorax was performed upon admission to the intensive care unit. Initially, 400 mL of hemorrhagic effusion was evacuated. However, 24 hours later the patient became hemodynamically unstable. It was an indication for video-assisted thoracoscopy (VATS). Therefore, VATS was used as a diagnostic method in order to determine the nature of the injury. Intraoperatively, we treated a laceration of pleuropulmonary adhesion which was continuously bleeding from the apex of the thoracic cavity. As a result, adequate surgical hemostasis was achieved. Furthermore, during the three-week postoperative period, thoracic tubes were placed due to the prolonged air leakage. A thoracic tube was placed laterally along with another one which was placed in intercostal space higher. After total reexpansion of the left lung, thoracic tubes were extracted, and the patient was discharged. **Conclusion.** Nowadays, VATS has become a highly important ultimate treatment of thoracic trauma. This minimally invasive method allows us to verify injury type and localization, to resolve it and further to follow-up evaluation of pathological changes in the lungs, pericardium, mediastinum, pleura and thoracic wall. In the case of stab wounds in the cervical region, any injuries of the lungs and pleura must be taken into consideration.

**Key words:**
**neck injuries; hemothorax; pneumothorax; drainage; thoracoscopy; minimally invasive surgical procedures.**

**Apstrakt**

**Uvod.** Penetrantne povrede vrata su vrlo ozbiljne povrede zbog mogućih lezija grkljana, dušnika, jednjaka i velikih krvnih sudova te regije. **Prikaz bolesnika.** Bolesnik, star 28 godina povređen je ubodom razbijenog stakla u predeo prednje strane baze vrata. Bolesnik se javio na pregled zbog otežanog disanja, a povreda je nastala noć uoči prijema. Prilikom pregleda otorinolaringologa konstatovana je ubodna rana u predelu baze leve strane vrata dužine oko 2 cm bez znakova aktivnog krvarenja i prisustva povreda dubljih anatomskih struktura vrata. Klinički i radiološki ova povreda manifestovala se levostranim hematopneumotoraksom. Nakon prijema u jedinicu intenzivne nege učinjena je torakalna drenaža, kojom je evakuisano inicijalno 400 mL hemoragičnog sadržaja. Nakon 24 časa došlo je do hemodinamske nestabilnosti bolesnika. To je bila indikacija za hiruršku intervenciju. Primenjena je video-asistirana torakoskopija (VATS) kojom je dijagnostikovana priroda povrede. Intraoperativno je uočena presečena pleuro-pulmonalna priraslica koja se nalazila u vrhu intratorakalnog prostora odakle je kontinuirano krvarilo. Urađena je hirurška hemostaza. Tokom daljeg lečenja, koje je ukupno trajalo tri nedelje, morali smo zbog prolongirane aerostaze da plasiramo još jedan torakalni dren lateralnije i za jedan interkostalni prostor više od prethodnog. Nakon postignute kompletne reekspanzije levog pluća drenovi su izvađeni i bolesnik je otpušten iz klinike. **Zaključak.** VATS danas zauzima sve više mesta u definitivnom zbrinjavanju torakalne traume. Ovom minimalno invazivnom metodom u mogućnosti smo da lokalizujemo i

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utvrdimo vrstu povrede, zbrinemo iste i dalje pratimo evaluaciju patoloških promena pluća, perikarda, medijastinuma, pleure i zida grudnog koša. Kod ubodnih rana vrata moramo misliti i isključiti i povrede pluća i pleuralnih prostora.

**Ključne reči:**
**vrat, povrede; hematotoraks; pneumotoraks; drenaža; torakoskopija; hirurgija, minimalno invazivne procedure.**

## Introduction

Penetrating neck injuries (PNIs) are very serious conditions that can jeopardize patient's life due to the anatomical position of vital organ structures within a relatively small and unprotected anatomical region [1]. PNIs are observed in 10% of all trauma patients and carry a 3–6% mortality rate [2,3]. The most common causes of intentional PNIs are stab injuries and missile injuries from firearms. On the other hand, accidental PNIs are most often due to falls on sharp objects, such as sticks or glass. Regardless of a cause, it is important to determine the mechanism of penetration because of the extent of tissue damage and possible treatment options [2,3].

Both anatomically and clinically, the neck is divided into the posterior and anterior triangle with the anterior neck triangle being subsequently classified into three horizontal zones of injury (from zone I to III) for PNIs [4]. Although it has been reported that zone II injuries (between the angle of the mandible and the cricoid cartilage) are the most common (50–80% of all PNIs), injuries to zone I (between the clavicles and the cricoid cartilage) carry the highest mortality due to vascular or visceral injuries and high-risk surgical exploration [3, 5–8]. Vascular injuries are the most frequent complications, occurring in 25% of PNIs, and are associated with a 50% mortality rate [2]. Although different blood vessels can be affected, injuries of common carotid artery are rare due to the elasticity and wall thickness of this blood vessel [2].

Previous studies reported rare incidence of aerodigestive injuries as potential complications of PNIs (Table 1) [3, 5, 8]. However, Demetriades et al. [7], reported the incidence of hemopneumothorax in 40 out of 223 patients (17.9%) with PNIs. However, it is important to emphasize that the mortality of the traumas that include aerodigestive injuries is between 10–20% [3, 5–8]. Additionally, other reported complications of PNIs are: thrombophlebitis of jugular vein, mediastinitis, sepsis, stenosis of the airways, and posttraumatic fistula [1,2].

Symptoms of PNIs may range from visible bleeding, dyspnea, dysphagia, stridor, pain, focal neurological deficits to a fatal hemorrhagic shock. Diagnostic methods of PNIs include: standard otorhinolaryngological examination, chest radiography, neck and thorax multislice computed tomography (MSCT), Doppler of blood vessels of the neck, as well as the endoscopic methods: laryngoscopy, bronchoscopy, and esophagoscopy [8–10].

Video-assisted thoracoscopy surgery (VATS) has become a standard diagnostic and therapeutic modality in thoracic surgery. It has been described as a useful method for the diagnosis and management of thoracic injuries, including the complications of PNIs [11, 12].

The aim of this case report was to present a patient with a PNI of zone I and consequential thoracic complications successfully resolved by the application of VATS.

## Case report

The patient was a 28-year-old male who was stabbed with broken glass penetrating the front side of the neck (zone I injury) the night before admission to our clinic. The next day the patient was examined at the Emergency Department in charge of surgical admissions at the Military Medical Academy in Belgrade. A linear 2 cm laceration was noted in the zone I of the neck with no active bleeding. There was an interruption of skin and subcutaneous tissue to the muscle layer (Figure 1). An otorhinolaryngologist performed rhinoscopy, oropharyngoscopy and indirect laryngoscopy to rule out any injuries of the ear, nose and throat region. Due to complaints of chest pain and dyspnea, a chest radiography (Figure 2) and MSCT of the thorax were taken. They revealed pneumomediastinum and hemopneumothorax on the left, as well as subcutaneous emphysema (Figure 3).

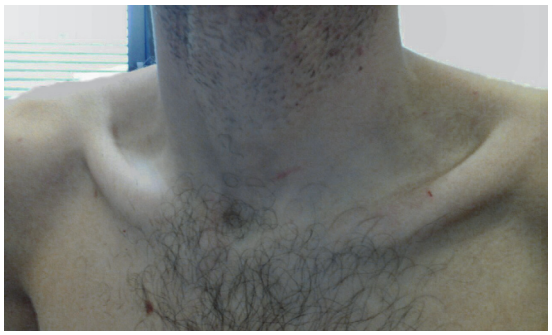

**Fig. 1 – Location of the neck wound.**

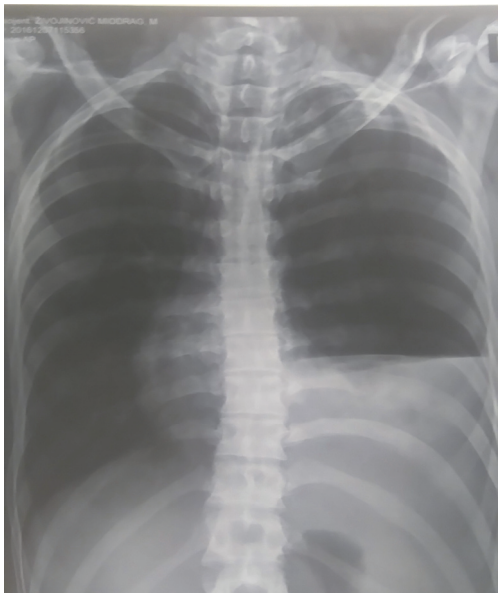

**Fig. 2 – Radiography of the lung and heart at the time of admission.**

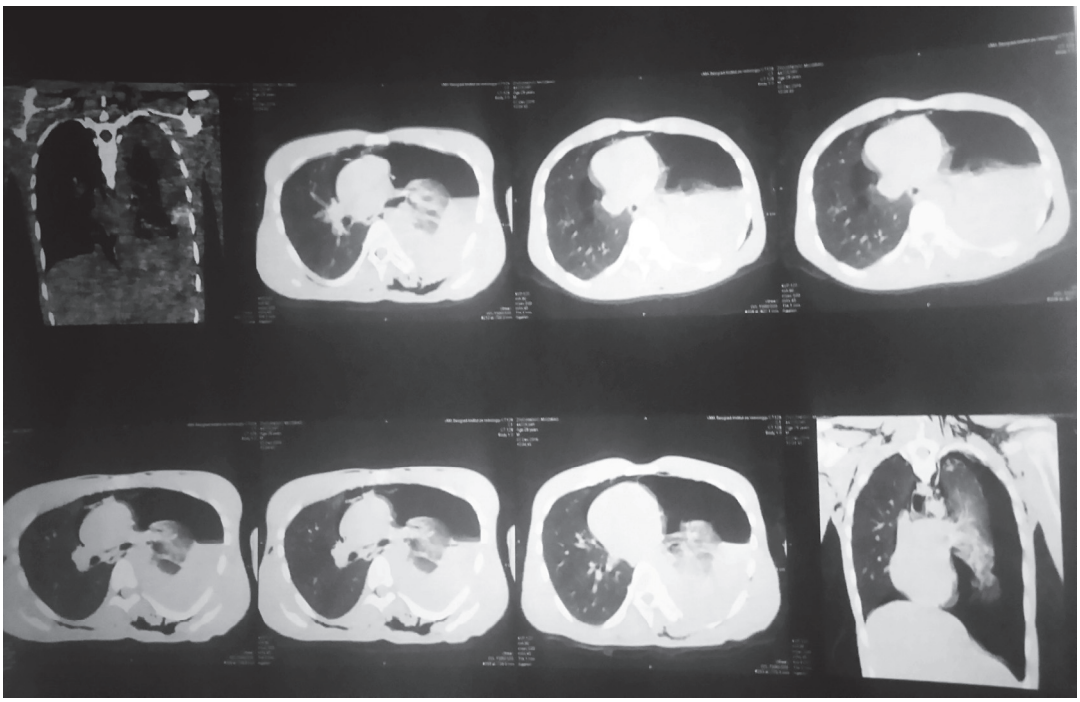

**Fig. 3 – Multisliced computed tomography (MSCT) of the thorax at the time of admission.**

The patient was admitted to the surgical intensive care unit, where he underwent thoracic tube placement. By the next morning he had drainage of 400 mL of hemorrhagic fluid. The patient remained hemodynamically stable and the following day he was referred to the Clinic for Chest Surgery of the Military Medical Academy. During the afternoon hours his blood pressure dropped as well as his hemoglobin and hematocrit levels. Under the circumstances, a VATS exploration was performed under general anesthesia. Two incisions were made on the left side of the thoracic wall − one in the VIII intercostal space at the posterior axillary line, and the other one in the IV intercostal space at the anterior axillary line (Figure 4).

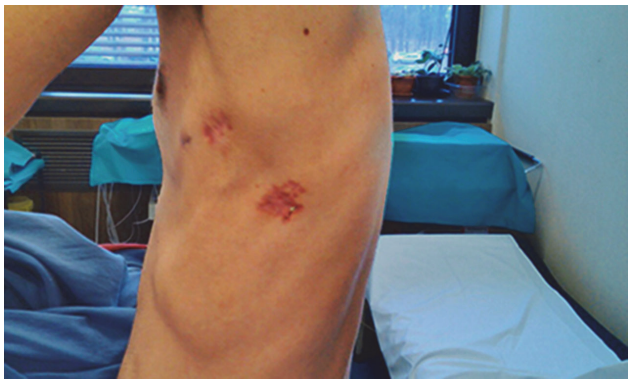

**Fig. 4 – The sites where ports and thoracic tubes were placed.**

During the procedure we noticed bleeding from an adhesion in the apex of the left pleural cavity with no lung lesions and/or bullous changes. Surgical hemostasis was performed, which led to the patient becoming hemodynamically stable. Due to delayed lung reexpansion, another thoracic tube was placed ten days after the VATS procedure, which resulted in prolonged hospitalization. As control radiography showed total reexpansion of the left lung, the tubes were removed after the clamping probe (Figure 5). The patient was discharged in good general condition after 22 days of hospitalization.

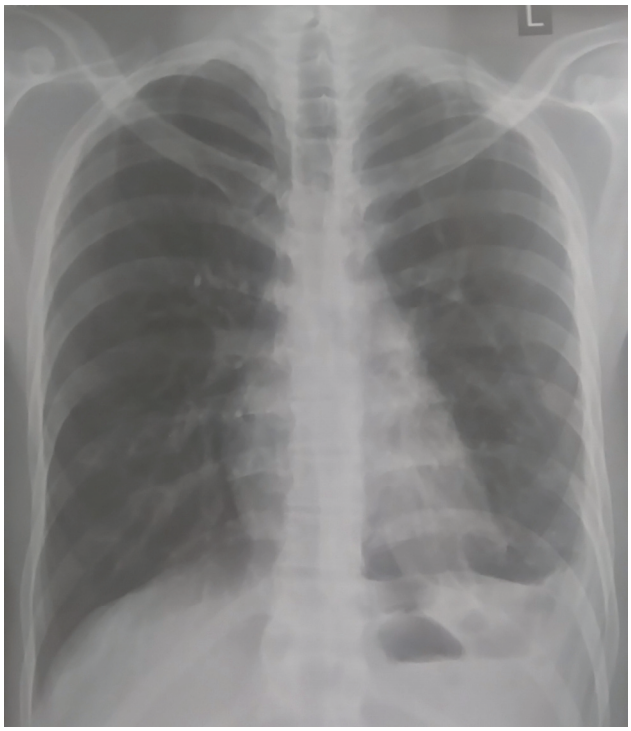

**Fig. 5 – Radiography of the lung and heart at the time of discharge.**

## Discussion

Based on the scientific literature, PNIs are observed in 10% of all trauma patients [2, 3]. Due to the vital organs present in this region and their complex anatomical relationships, these injuries require a multidisciplinary approach. Therefore, a prompt intervention of the entire medical team is of the highest importance for an adequate management of PNIs (e.g. vascular, visceral and neurological injuries) and prevention of fatal complications [2, 3].

The initial assessment of PNIs is still one of the biggest controversies in trauma surgery [7]. In this case report, we did not use routine surgical exploration of the neck although, in the past, it was obligatory and represented the standard treatment for PNIs, irrespective of signs or symptoms [13, 14]. The major argument for this is a potential possibility to miss occult life-threatening injuries. In most cases, zone II injuries are surgically explored because they are more accessible and easy to explore. On the other hand, zone I or III injuries are usually evaluated by several imaging methods prior to surgery, since they are difficult to manage [7, 15]. This led to a high rate of negative neck exploration (30–80%) and significantly associated morbidity (50%) [16]. Although the zoning system of PNI classification was the most commonly used in everyday clinical practice, it is important to emphasize that the external hole often does not correlate with the internal injury [15].

Contemporary protocols for assessing and treating patients with PNIs are based on the patient's hemodynamics and airway status, together with a thorough physical examination. Recent studies suggest selective non-operative management (SNOM) of PNIs, [5–8] which was also applied in our patient. It is based on thorough clinical examination and additional investigations as a safe and reliable way to exclude clinically significant injuries. Roepke et al. [15] recommend that computed tomography angiography should be performed additionally in hemodynamically stable patients who are with no hard signs of injury. The benefits of the SNOM approach have been confirmed with high sensitivity (93–95%) and a negative predictive value of 97% [17, 18]. On the other hand, in hemodynamically unstable patients with hemoptysis, hematemesis, arterial bleeding, rapidly expanding hematoma, subcutaneous emphysema, hoarseness or painful swallowing, a prompt evaluation under anesthesia that might include laryngoscopy, esophagoscopy, bronchoscopy or even surgical exploration is recommended [5–8, 15].

Although pharyngoesophageal injuries were not presented in our case, it is important to emphasize that their overlooking is among the most frequent pitfalls in penetrating neck trauma, as clinical signs are not always obvious. Any delays in treating them may lead to major morbidity or even death [6, 8]. Esophageal injuries are uncommon and difficult to detect in the early stages due to inconspicuous clinical findings. If the diagnosis is made within 24 h, more than 90% of patients survive; otherwise, the survival rate drops quickly [19]. Although esophageal injuries are more common than pharyngeal injuries, the latter ones are usually obvious on presentation and intraoral examination, whereas the diagnosis of occult injuries of the hypopharynx could easily be missed during a simple clinical examination. However, the management of pharyngeal injuries is usually conservative unless there is a major facial bone that needs debridement or edema causing airway obstruction [6, 8].

In the presented case report, zone I PNI caused bleeding from an adhesion in the apex of the left pleural cavity, which was successfully treated with VATS. What was unique about the injury of this young male was that the cutting wound on the neck did not look serious during the first examination. Nevertheless, his parietal pleura was injured but no lesions were detected on aerodigestive structures. Regarding the injuries which could quite possibly cause hemopneumothorax or bleeding of unknown location within the pleural cavity, VATS is a preferable diagnostic and therapeutic procedure [11, 12]. Although a physical examination and plan chest radiography are the essential diagnostic tools in chest trauma, the use of VATS is recognized as an important diagnostic tool, especially in the cases with unknown source of hemothorax. It is safe, less invasive, potentially as effective as thoracotomy, tolerated better than thoracotomy, and with fewer postoperative complications compared to thoracotomy [20, 21].

Landreneau et al. [22] presented how 23 cases of retained hemothorax were successfully managed by using VATS, which is in agreement with our case. In addition, in a retrospective analysis of 121 case reports of patients operated for open chest trauma, Samiatina and Rubikas [23] reported that VATS could be an alternative intervention to urgent thoracotomy. VATS was used in 33 cases and other 88 patients were operated through thoracotomy incision. They stated that drain presence in the pleural cavity and duration of postoperative treatment after VATS were significantly shorter compared to urgent thoracotomy. They also reported significantly less consumption of non-narcotic analgesics in the group of patients treated with VATS compared to the patients managed by urgent thoracotomy [23].

VATS requires developed surgical skills, physicians' experience and a well- equipped facility. An additional requirement for VATS use is that the patient's condition must allow one-sided lung ventilation under general anesthesia. There are also several contraindications for use of VATS: hemodynamic instability, massive hemothorax, suspected cardiac injuries, etc. Furthermore, the most common complication of VATS is transient hypoxemia or reversible arrhythmia while more serious and frequent complications are reported in patients with malignances [11, 12, 20, 21].

## Conclusion

The management of PNIs requires a multidisciplinary team of physicians who will be included in the diagnostics and treatment depending on the complexity of the involved anatomical structures. Although visceral complications are uncommon, a thorough physical examination along with additional investigations should further reduce a possibility of their development. VATS is a safe, less invasive and reliable method for resolving visceral complications of PNIs in the chest.

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## REFERENCES


1. *Demetriades D, Asensio JA, Velmahos G, Thal E.* Complex problems in penetrating neck trauma. Surg Clin North Am 1996; 76(4): 661–83.
2. *Bryant AS, Cerfolio RJ.* Esophageal trauma. Thorac Surg Clin 2007; 17(1): 63–72.
3. *Mahmoodie M, Sanei B, Moazeni-Bistgani M, Namgar M.* Penetrating neck trauma: review of 192 cases. Arch Trauma Res 2012; 1(1): 14–8.
4. *Roon AJ, Christensen N.* Evaluation and treatment of penetrating cervical injuries. J Trauma 1979; 19(6): 391–7.
5. *Nason RW, Assuras GN, Gray PR, Lipschitz J, Burns CM.* Penetrating neck injuries: analysis of experience from a Canadian trauma centre. Can J Surg 2001; 44(2): 122–6.
6. *Thoma M, Navsaria PH, Edu S, Nicol AJ.* Analysis of 203 patients with penetrating neck injuries. World J Surg 2008; 32(12): 2716–23.
7. *Demetriades D, Theodorou D, Cornwell E, Berne TV, Asensio J, Belzberg H,* et al. Evaluation of penetrating injuries of the neck: prospective study of 223 patients. World J Surg 1997; 21(1): 41–7; discussion 47–8.
8. *Van Waes OJ, Cheriex KC, Navsaria PH, van Riet PA, Nicol AJ, Vermeulen J.* Management of penetrating neck injuries. Br J Surg 2012; 99(Suppl 1): 149–54.
9. *Inaba K, Branco BC, Menaker J, Scalea TM, Crane S, DuBose JJ,* et al. Evaluation of multidetector computed tomography for penetrating neck injury: a prospective multicenter study. J Trauma Acute Care Surg 2012; 72(3): 576–83; discussion 583–4; quiz 803–4.
10. *Burgess CA, Dale OT, Almeyda R, Corbridge RJ.* An evidence based review of the assessment and management of penetrating neck trauma. Clin Otolaryngol 2012; 37(1): 44–52.
11. *Milanchi S, Makey I, McKenna R, Margulies DR.* Video-assisted thoracoscopic surgery in the management of penetrating and blunt thoracic trauma. J Minim Access Surg 2009; 5(3): 63–6.
12. *Ahmed N, Jones D.* Video-assisted thoracic surgery: state of the art in trauma care. Injury 2004; 35(5): 479–89.
13. *Golueke PJ, Goldstein AS, Sclafani SJ, Mitchell WG, Shaftan GW.* Routine versus selective exploration of penetrating neck injuries: a randomized prospective study. J Trauma 1984; 24(12): 1010–4.
14. *Meyer JP, Barrett JA, Schuler JJ, Flanigan DP.* Mandatory vs selective exploration for penetrating neck trauma. A prospective assessment. Arch Surg 1987; 122(5): 592–7.
15. *Roepke C, Benjamin E, Jhun P, Herbert M.* Penetrating Neck Injury: What's In and What's Out? Ann Emerg Med 2016; 67(5): 578–80.
16. *Apffelstaedt JP, Müller R.* Results of mandatory exploration for penetrating neck trauma. World J Surg 1994; 18(6): 917–9; discussion 920.
17. *Azuaje RE, Jacobson LE, Glover J, Gomez GA, Rodman GH Jr, Broadie TA,* et al. Reliability of physical examination as a predictor of vascular injury after penetrating neck trauma. Am Surg 2003; 69(9): 804–7.
18. *Gonzalez RP, Falimirski M, Holevar MR, Turk B.* Penetrating zone II neck injury: does dynamic computed tomographic scan contribute to the diagnostic sensitivity of physical examination for surgically significant injury? A prospective blinded study. J Trauma 2003; 54(1): 61–4 ; discussion 64–5.
19. *Smakman N, Nicol AJ, Walther G, Brooks A, Navsaria PH, Zellweger R.* Factors affecting outcome in penetrating oesophageal trauma. Br J Surg 2004; 91(11): 1513–9.
20. *Lang-Lazdunski L, Mouroux J, Pons F, Grosdidier G, Martinod E, Elkaïm D,* et al. Role of videothoracoscopy in chest trauma. Ann Thorac Surg 1997; 63(2): 327–33.
21. *Ben-Nun A, Orlovsky M, Best LA.* Video-assisted thoracoscopic surgery in the treatment of chest trauma: long-term benefit. Ann Thorac Surg 2007; 83(2): 383–7.
22. *Landreneau RJ, Keenan RJ, Hazelrigg SR, Mack MJ, Naunheim KS.* Thoracoscopy for empyema and hemothorax. Chest 1996; 109(1): 18–24.
23. *Samiatina D, Rubikas R.* Video-assisted thoracoscopic surgery as an alternative to urgent thoracotomy following open chest trauma in selected cases. Medicina (Kaunas) 2004; 40 Suppl 1: 134–8.


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# Post-traumatic stress disorder psychotic subtype or comorbid psychotic disorder and evaluation of military service ability

## Podtip post-traumatskog stresnog poremećaja ili komorbidni psihotični poremećaj i procena sposobnosti za vojnu službu

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**Abstract**

**Introduction.** Recent studies have shown that diagnostic differences in the opinion whether some case is a psychotic subtype of posttraumatic stress disorder (PTSD) or a comorbid psychotic disorder still exist. In a case of mental disorders, a specific nature of military environment requires a detailed evaluation of abilities for military service (MS). **Case report.** A 34-year old male noncommissioned officer (NCO) showed symptomatology of PTSD (according to the Diagnostic and Statistical Manual of Mental Disorders – DSM-IV) after experiencing a traumatic event in peacetime conditions. In addition to experiencing trauma as an adult, the patient was also exposed to early-age trauma, when his father committed suicide. After a pharmacotherapy and cognitive behavioral therapy treatment, he was remitted and returned to his duty. Triggered by new stress caused by unfavorable environmental factors (occupational environment), psychotic phenomenology appeared. After two years of psychiatric treatment, patient was evaluated unfit for MS. **Conclusion.** Early-age trauma and/or PTSD are predispositions for a comorbid psychotic disorder, while the diagnostic entity of psychotic subtype of PTSD requires further research. Evaluation of MS abilities in patients with psychotic disorder based on our clinical experience, will require a psychiatric treatment for at least two years, which is in accordance with a research conducted in the British Army.

**Key words:**
**stress disorders, post-traumatic; psychotic disorders; comorbidity; military personnel; drug therapy; recurrence; professional competence.**

**Apstrakt**

**Uvod.** Dosadašnja istraživanja ukazuju da još uvek postoje dijagnostička razmatranja da li je u pitanju psihotični podtip posttraumatskog stresnog poremećaja (PTSP) ili komorbidni psihotički poremećaj (KPP). Specifičnost vojne sredine, zahteva kod svakog mentalnog poremećaja brižljivu evaluaciju kapaciteta za obavljanje vojne službe (VS). **Prikaz bolesnika.** Prikazan je podoficir, star 34 godine, koji je nakon mirnodopskog traumatskog događaja ispoljio simptomatologiju PTSP (prema Dijagnostičkom i statističkom priručniku za mentalne poremećaje – DSM IV). Pored traume u odraslom dobu, bolesnik je bio izložen i traumatizaciji u ranom detinjstvu, zbog suicida oca. Nakon tretmana lekovima i kognitivno-bihevioralnom psihoterapijom, postignuta je remisija i bolesnik je vraćen na svoju dužnost. Nakon novog stresa, uslovljenog nepovoljnim faktorima radne sredine, došlo je do ispoljavanja i psihotične fenomenologije. Nakon dvogodišnjeg psihijatrijskog lečenja, bolesnik je ocenjen "Nesposoban" za VS. **Zaključak.** Rana trauma i/ili PTSP su predisponirajući faktori za KPP, dok dijagnostički entitet – PTSP psihotični podtip, zahteva dalje istraživanje. Procena sposobnosti za VS kod bolesnika sa psihotičnim poremećajem, na osnovu našeg iskustva, nameće potrebu za, najmanje, dvogodišnjim psihijatrijskim lečenjem, što je u skladu sa istraživanjem sporovedenim u britanskoj armiji.

**Ključne reči:**
**stresni poremećaji, posttraumatski; psihotički poremećaji; komorbiditet; vojni kolektiv; lečenje lekovima; recidiv; sposobnost, radna.**

### Introduction

Recent studies point to association between PTSD and psychotic disorders, but there are still diagnostic differences in opinion whether this is a subtype of PTSD or a comorbid psychotic disorder [1]. Namely, noticeably higher PTSD rates were recorded in patients with psychotic disorder (30%) than in overall population (7.8%) [2]. On the other hand, about 10%

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of PTSP patients also have a psychotic experience [1], while other studies show significantly higher incidence (15–64%) [3]. The explanation for this may lie in the possible overlap of genes associated with PTSD, and genes which carry an increased risk of developing psychotic disorders, schizophrenia in particular [4, 5].

Comorbidity of PTSD and psychotic disorders is best researched in war veterans. Evidence gained in the war years on the territory of former Yugoslavia showed that as many as 40% of war veterans reported some kind of psychotic symptoms, mainly delusions and hallucinations. Most of them didn't experience any bizarre hallucinations, only the hallucinations related to the traumatic event itself [6]. Similar results regarding war veterans with psychotic symptoms are found in studies conducted in America. There was, also, a high correlation between the intensity of PTSD and psychotic symptoms [7, 8].

Having in mind the biopsychosocial approach, it is evident that some personality traits (immaturity, neuroticism, ambivalence, insecurity, passive-dependent traits), as well as unfavorable circumstances of social milieu of patient's life and work (lack of support, lack of understanding, mockery, added pressures, subsequent imposition of a sense of responsibility and guilty conscience) are factors which could predispose development and persistence of symptoms of PTSD and comorbid disorders [9].

Responsibility of military psychiatrists in the process of evaluation of capability for military service (MS) is significant because of the specific nature of military occupation and the military environment in which a patient should be reintegrated. An officer is an educator, teacher and instructor of his younger colleagues and soldiers and his authority needs to be based on expert knowledge, human capacity and inclination towards correct leadership and command. Also, the officer needs to be capable of working as a team player and carrying out guard service duties (one of the most demanding combat tasks in peacetime). A specific nature of military environment which includes military discipline, particular language used in commands, specific symbolism, emphasized ceremonialism, specific occupational and private environment, specific evaluation and assessment criteria, life and work within a group and separation from the family additionally complicate and create difficulties in evaluating professional MS working ability in active military personnel (AMP) [10, 11].

**Case report**

We presented a 34-year old male patient, noncommissioned officer (squad commander, NCO) displaying symptomatology of PTSD and psychosis as well as the process of evaluation of professional military service (PMS).

The patient started with the psychiatric treatment three weeks after the traumatic experience. Trauma was acquired in peacetime conditions, namely the patient was in the civilian environment, in the evening, after his working hours, when he was physically assaulted by a group of strangers and was hit by a dull object in the head (he didn't lose consciousness, had nausea or vomited). Since the event, the patient has been thinking about what could have happened, has been concerned for his life, has been having sleep disturbances (dreaming about the traumatic event), has been irritable, tense, prone to losing his temper quickly, having headaches, retreated from social situations, has been apathetic and avoided talking about the trauma or leaving the house without his spouse. Details from patient's personal anamnesis which were to be noted – he was married with two children, genetic burden was present, the father committed suicide when he was 10. Before the traumatic event, patient had never consulted a psychiatrist and was described as a responsible and diligent NCO in his unit and by the military psychologist (mandatory hetero-anamnestic data from the unit if an AMP).

Psychiatric treatment began, three weeks after the trauma, in outpatient conditions (Department for Mental Health of the Clinic for Psychiatry, Military Medical Academy). During the outpatient treatment, the following diagnostic procedures were performed: somatic and neurological examination, computed tomography (CT) scan of the head and electroencephalography (EEG) results were normal, the results of routine blood (a complete blood count – CBC, and biochemical serum analysis) and urine tests, as well as thyroid gland hormones concentration [thyroid-stimulating hormone (TSH), free thyroxine (FT4), triiodothyronine (T3), anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies], all were in the normal range. The patient received antidepressant (paroxetine 20 mg/day), anxiolytic (alprazolam 1 mg/day), hypnotic therapy (zolpidem 5 mg/day), and cognitive-behavioral therapy. After a few months of therapy, along with a one month long sick leave, the patient was remitted and returned to the unit as capable, but was exempt from his guard service duty and on-call MS in the following three-month period.

In the months to follow his remission, for a total duration of a year after the trauma, the patient was treated in outpatient conditions at the Department for Mental Health of the Clinic for Psychiatry, Military Medical Academy in Belgrade. He had check-ups every month, with gradual reduction of medicament therapy, complete remission and the return to adequate behavior in professional, social and family environment.

Upon returning to the unit, the patient suffered a new stress. He was informally accused by his superior officers of being responsible of an event that happened in his absence and was verbally pressured into admitting guilt. One year after the first trauma (i.e. the physical assault), and shortly after the new stress, the patient was again experiencing intense traumatic experience ruminations, sleep deprivation and nightmares; he was irritable, retreated from social situations, avoided talking about the trauma, had difficulty being a functional family member and was professionally dysfunctional. During a premature and irregular check-up at the Department for Mental Health of the Clinic for Psychiatry, Military Medical Academy, PTSD relapse was evident, but with psychotic symptoms now present.

Delusions regarding relations and persecutions, as well as auditory hallucinations were present; elements of these psychotic symptoms were associated with traumatic experi-

ence. Due to the deterioration of his mental condition, one year after the first trauma, the patient was hospitalized for treatment. Cognitive model of PTSD in our patient is shown in Figure 1.

Hospital treatment, for the first time, included an atypical antipsychotic (risperidone 4 mg/day), and it was continued with an antidepressant (sertraline 50 mg/day), mood stabilizer (valproate 1,000 mg/day) and anxyolitic (alprazolam 1 mg/day), which reduced PTSD and psychotic symptomatology. In the period of hospitalization, the Mini-International Neuropsychiatric Interview for diagnosing psychotic disorders was applied [12]; posttraumatic stress disorder was diagnosed by the use of the Clinician Administered PTSD Scale (CAPS) [12], according to the Diagnostic and Statistical Manual of Mental Disorders – DSM-IV [13, 14], and exposure to early-age trauma was diagnosed through the Childhood Trauma Questionnaire (short review of the six early traumatic experiences: death, divorce, violence, sexual abuse, illness or others) [15].

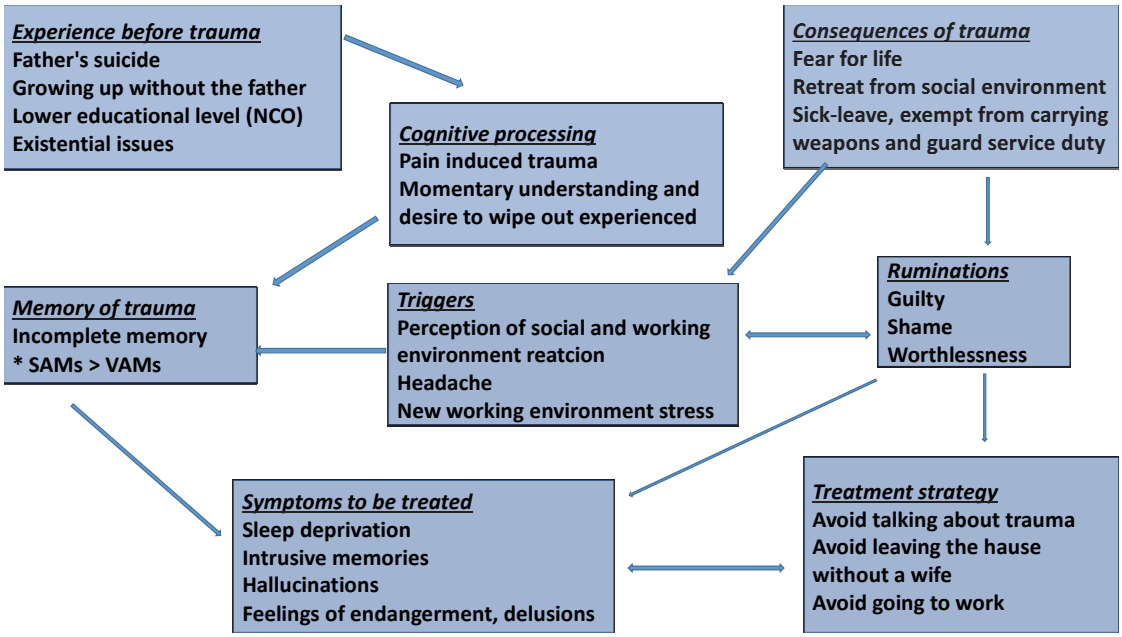


**Fig. 1 – Cognitive model of presented post-traumatic stress disorder (PTSD), adapted by Ehlers and Clark, 2000. [11].**

***Memories are encoded in SAM (Situationally Accessible Memories) instead of VAM (Verbally Accessible Memories) system, which prevents cognitive assessment and eventual overcoming the traumatic experience.**
**NCO – non-commissioned officer.**

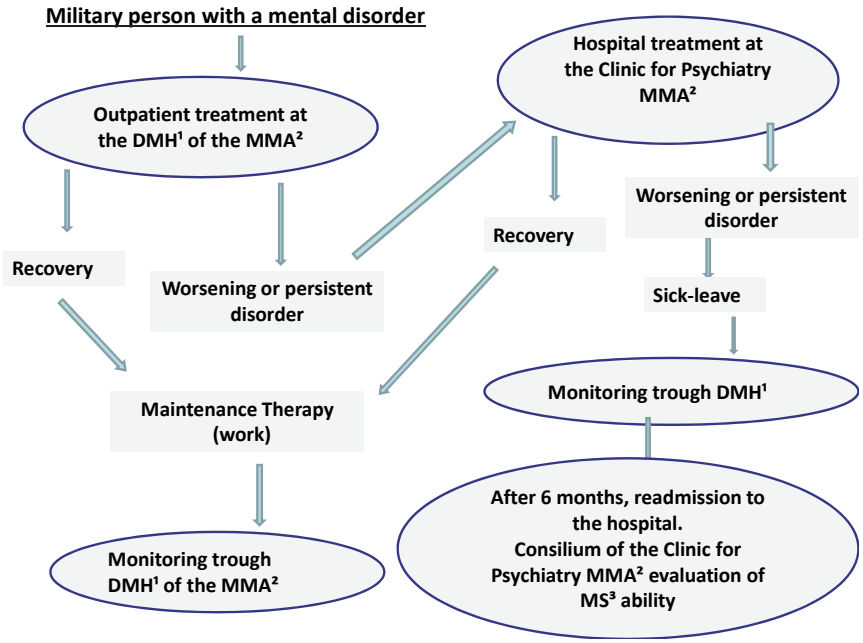


**Fig. 2 – Evaluation of military service capabilitiesn of a military person with a mental disorder.**
**¹DMH – Department of Mental Health; ²MMA – Military Medical Academy; ³MS – Military Service.**

Having in mind that he is AMP, a specific nature of his occupation and the fact that he was receiving antipsychotic therapy treatment (risperidone 6 mg/day) the outpatient treatment was continued, and he was declared unable to work until further. The dose of antipsychotics was increased during outpatient treatment, given that after the end of the hospital treatment (risperidone 8 mg/day) and leaving the protected environment, delusions intensified. The patient was granted extended sick-leave with regular psychiatric check-ups. In the following period, the smallest provocative circumstance of family and social functioning (e.g. change in common patient activities, need for unplanned obligations, family events), especially those related to his occupational environment (military organization) resulted in a more intense feeling of endangerment and psychotic symptomatology. For the second time the patient was admitted for hospital treatment (2 years after the traumatic experience). The Consilium of the Clinic for Psychiatry evaluated him "unfit" for MS. MS evaluation procedure is presented in Figure 2. The following schematic representation is the result of a long-standing experience of psychiatrists of the Clinic for Psychiatry, Military Medical academy in Belgrade, which was acquired in working with AMP having mental disorders, which also includes assessing their abilities for MS.

## Discussion

Previous studies do not give clear instructions on diagnose and treatment of PTSD with psychotic symptoms. To the best of our knowledge, none of the previous cases in our community, comprised a precise evaluation of a patient with PTSD and psychotic symptoms and the MS ability evaluation.

This case showed a noncommissioned officer with PTSD phenomenology manifestations became acute after a traumatic experience.

A year after the trauma, as a result of unfortunate events (occupational environment), there was a relapse of PTSD, along with psychotic symptoms, and elements of these psychotic experiences were associated with traumatic experience. Psychotic symptoms could be manifested several months, sometimes even several years after the traumatic experience. Association between psychotic elements and trauma shows that traumatic experience and/or PTSD could be predispositions for psychotic disorder [16]. With regard to physical abuse, up to 53% of patients with PTSD experience positive psychotic symptoms [17]. Studies show that childhood trauma is a risk factor of psychosis and that psychotic patients with a cognitive model of childhood trauma are more inclined to substance abuse, PTSD, depression, anxiety [18, 19], i.e., early-age trauma is a risk factor of psychosis and PTSD [20]. In our patient's case, early-age trauma (father's suicide) and PTSD could be predisposing factors for comorbid psychotic disorder.

Opinions regarding the existence of diagnostic entity of PTSD psychotic subtype are conflicting. While some authors support the idea of this PTSD subtype [21, 22], others deny it [23]. It is adulthood trauma that could be responsible for the existence of this PTSD subtype [21], and in this sense our patient fits into this diagnostic entity. However, we still cannot say for sure this is a psychotic subtype of PTSD having in mind that further phenomenological, biological and epidemiological studies of this diagnostic entity are to be conducted.

Moreover, occurrence of psychotic symptoms in patients with PTSD requires the use of antipsychotics, which adapts the approach to PTSD treatment. Studies show that antipsychotic monotherapy lasting 4-6 weeks (fluphenazine, olanzapine, risperidone, quetiapine) diminishes symptoms in patients with PTSD who show resistance to a conventional antidepressant treatment [24]. The study of prescribing off-label antipsychotics to war veterans in USA showed that up to 41.8% antipsychotics were prescribed specifically to patients with PTSD with psychotic symptoms. Moreover, most often prescribed antipsychotics were quietiapine (42.9%), risperidone (21.2%) and olanzapine (7.2%) [25], but the efficiency in the treatment showed also clozapine, amisulpride, fluphenazine [24]. A conventional treatment implies the use of selective serotonin reuptake inhibitors (SSRI) with 60% response rate. However, only 20–30% of patients achieve full remission [25]. The positive effects of antidepressant therapy are manifested in the reduction of sleep disturbances, intrusiveness and aggression, but they can also have positive effects on psychotic symptoms appearing alongside PTSD.

The patient presented in this report was treated by sertraline (a SSRI), risperidone (an atypical antipsychotic) and a mood stabilizer. Reduction of PTSD phenomenology and psychotic symptomatology was achieved, but with a regular use of antipsychotic and antidepressant therapy. Each attempt to withdraw antipsychotic therapy in the course of a two-year follow-up resulted in psychotic relapse.

On the other hand, there is the military's frontline psychiatry doctrine which is particularly applicable in the time of peace. This implies fast return of an AMP to the unit. This is why it is important to properly evaluate the risks of an AMP with a psychotic disorder and in a pharmacotherapy treatment returning to his unit. A study conducted in the British Army showed 48 cases of non-affective psychosis and 14 cases of schizophrenia among AMP in the course of 4 years. Only 8 patients, i.e., 16.7% were still in service after a two-year follow-up [26]. We must say that, to the best of our knowledge, this was the only research conducted in the army that showed a concise evaluation of MS ability among active military personnel with a psychotic disorder. Our patient's MS ability evaluation is in accordance with that of the British Army; it was carried out after a two-year follow-up. Having in mind patient's continuous antipsychotic therapy, his daily contact with weapons, as well as his guard service duty (potentially provocative emergency situations), he was estimated unfit for PMS.

## Conclusion

Early-age trauma and/or PTSD are predisposing factors for comorbid psychotic disorder, while diagnostic entity of psychotic subtype of PTSD requires further research. Presence of psychotic symptoms in patients with PTSD requires the combined use of antidepressants and antipsychotics

which adapts the approach to PTSD treatment. Evaluation of MS abilities in patients with psychotic disorder, based on our clinical experience, requires a psychiatric treatment for at least two years, which is in accordance with a research conducted in the British Army. Thereby, a specific nature of MS organization, duties of the patient, his mental disorder and pharmacotherapy used, additionally complicate and cause difficulties for military psychiatrists in evaluating professional military service ability.

## REFERENCES


1. *OConghaile A*, *DeLisi LE*. Distinguishing schizophrenia from posttraumatic stress disorder with psychosis. Curr Opin Psychiatry 2015; 28(3): 249–55.
2. *Buckley PF*, *Miller BJ*, *Lehrer DS*, *Castle DJ*. Psychiatric comorbidities and schizophrenia. Schizophr Bull 2009; 35(2): 383–402.
3. *Braakman MH*, *Kortmann FA*, *van den Brink W*. Validity of 'posttraumatic stress disorder with secondary psychotic features': a review of the evidence. Acta Psychiatr Scand 2009; 119(1): 15–24.
4. *Duncan LE, Ratanatharathorn A, Aiello AE, Almli LM, Amstadter AB, Ashley-Koch AE,* et al. Largest GWAS of PTSD (N=20 070) yields genetic overlap with schizophrenia and sex differences in heritability. Mol Psychiatry 2018; 23(3): 666–73.
5. *Jablensky AV*, *Kalaydjieva LV*. Genetic epidemiology of schizophrenia: phenotypes, risk factors, and reproductive behavior. Am J Psychiatry 2003; 160(3): 425–9.
6. *Spiric Z*. Ratna psihotrauma kod srpskih veterana. Beograd: Čigoja štampa; 2008. (Serbian)
7. *David D, Kutcher GS, Jackson EI, Mellman TA*. Psychotic symptoms in combat-related posttraumatic stress disorder. J Clin Psychiatry 1999; 60(1): 29–32.
8. *Hamner MB, Frueh CB, Ulmer HG, Arana GW*. Psychotic features and illness severity in combat veterans with chronic posttraumatic stress disorder. Biol Psychiatry 1999; 45(7): 846–52.
9. *Milosavljevic M, Drakulic B, Perunicic I, Lecic Tosevski, D*. Procena faktora rizika za posttraumatski stresni poremećaj kod ratnih veterana bivše Jugoslavije. Psihijatrija danas 2011; 43(2): 141–53. (Serbian)
10. *Dedic G*. Prilagodjavanje vojnika na vojnu sredinu. Beograd: Novinsko-izdavački centar Vojska; 2001.
11. *Ehlers A*, *Clark DM*. A cognitive model of posttraumatic stress disorder. Behav Res Ther 2000; 38(4): 319–45.
12. *Sheehan DV*, *Lecrubier Y*, *Sheehan KH*, *Amorim P*, *Janavs J*, *Weiller E*, et al.. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998; 59(Suppl 20): 22–33; quiz 34–57.
13. *Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS* et al. The development of a Clinician-Administered PTSD Scale. J Traumatic Stress 1995; 8(1): 75–90.
14. *Bell CC*. Diagnostic and statistical manual of mental disorders: DSM-IV. JAMA 1994; 272(10): 828–9.
15. *Pennebaker JW, Susman JR*. Disclosure of traumas and psychosomatic processes. Soc Sci Med 1998; 26(3): 327–32.
16. *Bleich A*, *Moskowits L*. Post traumatic stress disorder with psychotic features. Croat Med J 2000; 41(4): 442–5.
17. *Kilcommons AM*, *Morrison AP*. Relationships between trauma and psychosis: an exploration of cognitive and dissociative factors. Acta Psychiatr Scand 2005; 112(5): 351–9.
18. *Larkin W*, *Read J*. Childhood trauma and psychosis: evidence, pathways, and implications. J Postgrad Med 2008; 54(4): 287–93.
19. *Mihaljevic M, Zeljic K, Soldatovic I, Andric S, Mirjanic T, Richards A,* et al. The emerging role of the FKBP5 gene polymorphisms in vulnerability stress model of schizophrenia: further evidence from a Serbian population. Eur Arch Psychiatry Clin Neurosci 2017; 267(6): 527–39.
20. *Powers A*, *Fani N*, *Cross D*, *Ressler KJ*, *Bradley B*. Childhood trauma, PTSD, and psychosis: Findings from a highly traumatized, minority sample. Child Abuse Negl 2016; 58: 111–8.
21. *Lindley SE*, *Carlson E*, *Sheikh J*. Psychotic symptoms in posttraumatic stress disorder. CNS Spectr 2000; 5(9): 52–7.
22. *Braakman MH, Kortmann FA, van den Brink W*. Validity of 'posttraumatic stress disorder with secondary psychotic features': a review of the evidence. Acta Psychiatr Scand 2009; 119(1): 15–24.
23. *Gaudiano* BA, *Zimmerman* M. Evaluation of evidence for the psychotic subtyping of post-traumatic stress disorder. Br J Psychiatry 2010; 197(4): 326–7.
24. *Pivac N, Kozaric-Kovacic D*. Pharmacotherapy of treatment-resistant combat-related posttraumatic stress disorder with psychotic features. Croat Med J 2006; 47(3): 440–51.
25. *Alexander* W.. Pharmacotherapy for Post-traumatic Stress Disorder In Combat Veterans. Focus on Antidepressants and Atypical Antipsychotic Agents. P T. 2012; 37(1): 32–8.
26. *Turner MA*, *Finch PJ*, *McKechanie AG*, *Kiernan MD*, *Hawksley OJ*, *Wadhwani S*, et al. Psychosis in the British Army: a 2-year follow-up study. Mil Med 2006; 171(12): 1215–9.


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CASE REPORT


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# Iatrogenic pulmonary fat embolism after surgery in a patient with fatty liver

## Jatrogena masna embolija pluća posle hirurške intervencije kod bolesnika sa masnom jetrom

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**Abstract**

**Introduction.** Fat embolism refers to the presence of fat globules in the lung parenchyma and its peripheral circulation. Obstruction of the lung vessels by fat emboli can lead to acute *cor pulmonale* when the compensatory capabilities of the pulmonary vasculature are exceeded. **Case report.** We presented a case of a 78-year old man who suffered a dissection of abdominal aortic aneurysm. Urgent surgical procedure was performed and aneurysm replaced with aorto-bifemoral bypass grafting using a Dacron graft. Despite the procedure the patient died the following day. The autopsy revealed that the cause of death was hypovolemic shock. There were no bone fractures (also no fractures of ribs and sternum from cardiopulmonary resuscitation) or injuries of the subcutaneous fat tissue or other organs (besides those from the surgery). However, additional autopsy findings included fatty liver change, small liver hemorrhages (confirmed microscopically), as well as a presence of fat droplets in the hepatic veins, as well as in the pulmonary vessels, i.e. pulmonary fat embolism [confirmed with hematoxylin/eosin (H/E), and Sudan III staining], which could be the contributing cause of death. **Conclusion.** The presented case indicates that pulmonary (or even systemic) fat embolism should be considered as the possible iatrogenic cause of unexpected and unexplained death in the cases where elective surgical procedures were performed in patients with fatty liver change. Pathologists must be aware of this possibility, since it is not easily recognized on routine H/E staining, and some of the special staining technique should be applied.

**Key words:**
**embolism, fat; aneurysm, ruptured; postoperative complications; pulmonary embolism; fatty liver; diagnosis, differential; death.**

**Apstrakt**

**Uvod.** Masna embolija je prisustvo masnih kapi u plućnoj i perifernoj cirkulaciji. Kada se prevaziđu kompenzatorne mogućnosti plućne cirkulacije, njena opstrukcija masnim kapima može dovesti do akutnog plućnog srca. **Prikaz bolesnika.** Prikazan je muškarac, star 78 godina kod koga je došlo do disekcije aneurizme trbušne aorte. Učinjena je hitna hirurška intervencija – aorto-bifemoralno premošćenje dakronskim graftom. Uprkos primenjenim merama lečenja smrtni ishod nastupio je sledećeg dana. Obdukcijom je ustanovljeno da je uzrok smrti hipovolemijski šok. Obdukcijom nisu ustanovljeni prelomi kostiju (takođe, ni prelomi grudne kosti, niti rebara, koji su mogli nastati prilikom reanimacije) ili povrede potkožnog masnog tkiva ili drugih organa (izuzimajući organe na kojima je izvršena hirurška intervencija). Međutim, nađena je i bolest masne jetre, sitna supkapsularna krvarenja (potvrđena mikroskopskim pregledom), kao i prisustvo masnih kapi u hepatičkim venama, kao i krvnim sudovima pluća, tj. ustanovljena je masna embolija pluća [potvrđena hematoksilin-eozin (H/E) i Sudan III bojenjem], što je moglo biti doprinoseći uzrok smrti. **Zaključak.** Prikazani slučaj pokazuje da masna embolija pluća ili čak sistemska masna embolija mora biti uzeta u obzir kao mogući jatrogeni uzrok neočekivane smrti bolesnika sa masnom jetrom, potvrgnutih elektivnom hirurškom zahvatu. Patolozi moraju biti svesni ove činjenice, pošto masnu emboliju nije lako prepoznati na rutinskim H/E bojenjima, zbog čega se moraju primeniti neka od specijalnih tehnika bojenja.

**Ključne reči:**
**embolija, masna; aneurizma, ruptura; postoperativne komplikacije; pluća, embolija; jetra, masna infiltracija; dijagnoza, diferencijalna; smrt.**

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## Introduction

A presence of fat droplets in the lung circulation presents a pulmonary fat embolism. With the obstruction of the sufficient percent of pulmonary vessels by the fat emboli, with the exceeding of its compensatory capabilities, an acute right heart failure may develop [1–3]. Commonly, the lung fat embolism after sustained trauma is a subclinical event: droplets of fat are sucked into the venous system at a site of fracture and then get stuck in the pulmonary circulation. Once entering the lungs, fat globules might enter the systemic circulation, reaching different organs. The clinical manifestation of systemic fat embolism, primarily with progressive respiratory distress and a deterioration in central nervous system function is called fat embolism syndrome [2]. Although most commonly associated with trauma [1–3], there are some described cases of non-trauma related fat embolism [4–10]. We presented one such case, most probably caused iatrogenically, in a setting of previously existing fatty liver change.

## Case report

A 78-year-old man was admitted to the hospital, after being transferred from a regional medical center with the computed tomography (CT) confirmed rupture of abdominal aortic aneurysm. The diameter of aneurysm was 87 × 79 mm and the rupture was localized on the right lateral side of the abdominal aorta, just below the renal arteries. The urgent surgical procedure was performed: open transperitoneal resection of the ruptured part of the aortic aneurysm, which was replaced with aorto-bifemoral bypass grafting using a Dacron graft. During the entire course of the operation, the patient was hypotensive. Several hours after the procedure, the patient died with the clinical signs of hemorrhagic shock.

The autopsy was performed the following day. The macroscopic examination showed signs consistent with hemorrhagic shock, as well as signs of the described surgical procedure. The signs of severe atherosclerosis were most prominent in the aorta, and slightly less in coronary arteries. The lungs were livid and heavy (total lung weight was about 1,600 g), while the liver showed signs of fatty change, as well as small subcapsular bleedings on its surface. There were no bone fractures (and also no fractures of ribs and sternum from cardiopulmonary resuscitation) or injuries of the subcutaneous fat tissue or other organs (besides those from the surgery). Microscopic examination [hematoxylin/eosin (H/E) staining] of the liver showed moderate to severe fatty change in hepatocytes, but also small subcapsular fresh hemorrhages (Figures 1a and 1b). Examination of the lungs, however, revealed the possible presence of fat droplets in the pulmonary vessels, i.e. the pulmonary fat embolism (Figure 2a). Therefore, additional Sudan III-staining was performed on samples taken from the lungs, as well as the brain, kidneys and liver. The histological findings of frozen sections of the lungs confirmed the pulmonary fat embolism: red sausage-shaped or rounded, multiple, disseminated fat emboli were present in every microscopic field (Figures 2b, 2c and 2d). These findings corresponded with the moderate, second grade lung fat embolism [1]. Fat droplets were not found in the brain and kidneys. On the other hand, this special staining confirmed fatty change in the hepatocytes, but also showed the presence of multiple fat globules in middle caliber hepatic veins (Figures 1c and 1d). The main cause of death was attributed to hemorrhagic shock due to rupture of aortic aneurysm, while pulmonary fat embolism was the contributing factor.

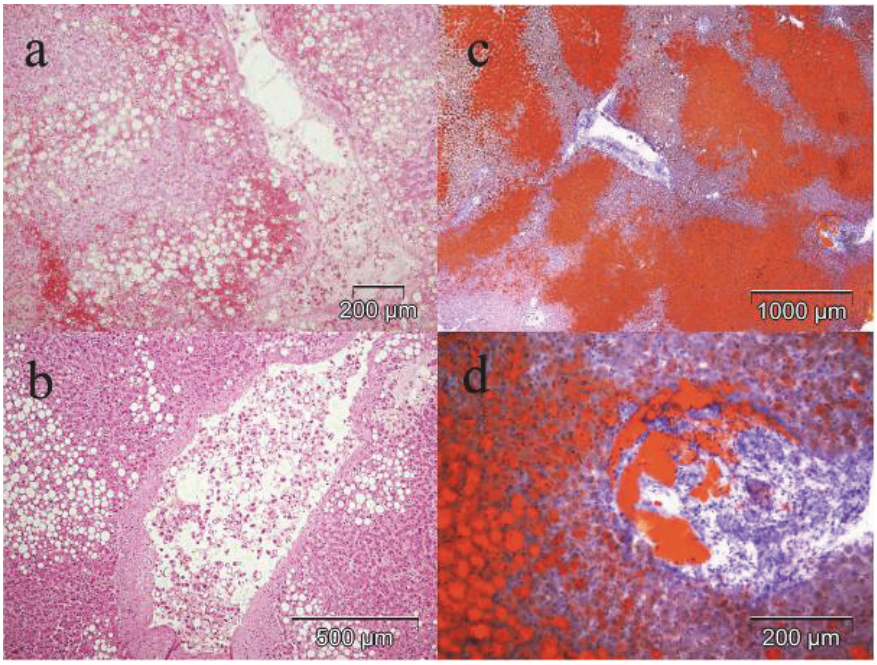


**Fig. 1 – Liver: a, b) Fatty changed liver with small tissue hemorrhages due to injury during the surgical procedure [hematoxylin/eosin (H/E) staining]; c, d) Note the presence of fat globules in the hepatic veins (frozen Sudan III-stained sections), visible both in H/E and Sudan III stains.**

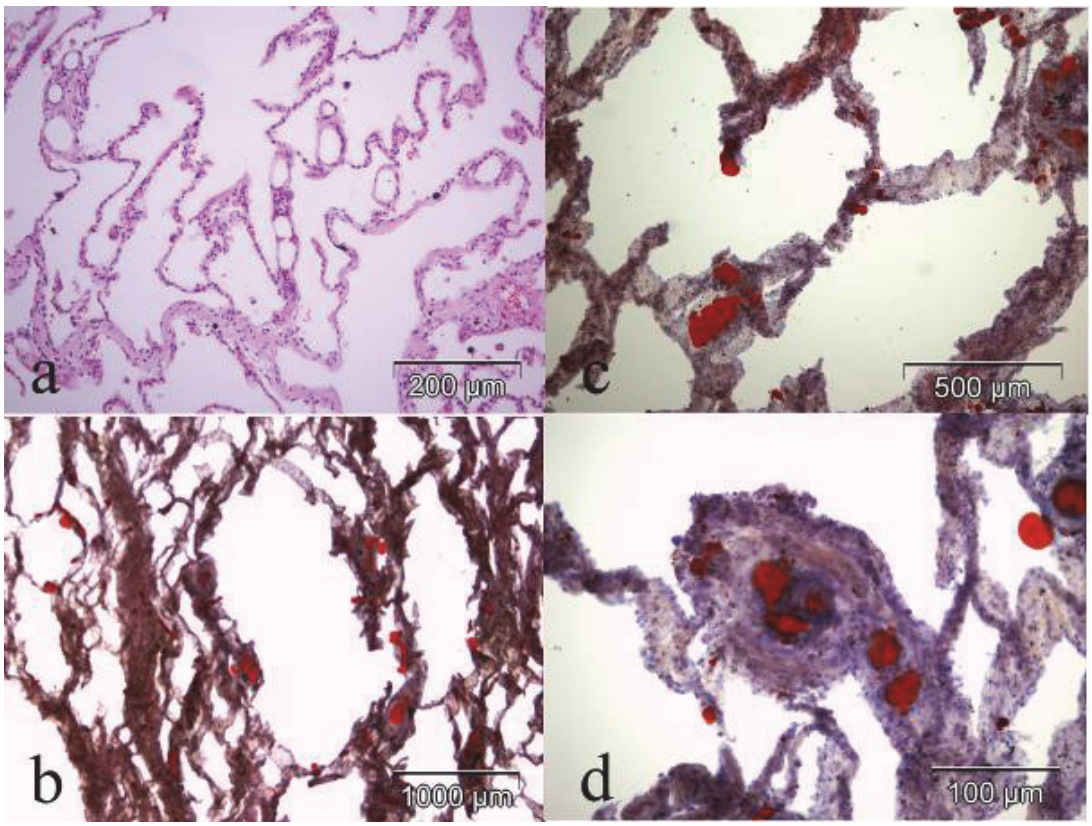


**Fig. 2 – a) Hematoxylin/eosin (H/E) staining of the lungs shows the presence of fat globules in small pulmonary vessels; b, c, d) Frozen Sudan III-stained sections of the lungs show red, drop, sausage- and branching-shaped fat emboli in pulmonary vessels.**

## Discussion

Fat embolism is most commonly associated with trauma (i.e. fractures) [1–3], however there is relatively small number of described cases of fat embolism in the absence of trauma, associated with alcoholic or steroid-induced fatty liver, acute hepatic necrosis or diabetes [4–8], and only a few papers of postoperative fat embolism [9]. One of studies found quite surprising incidence of fat embolism in cases of sudden death – in 34 out of 65 cases [10].

Fat embolism represents the mechanical blockage of blood vessels by circulating fat globules. Lungs are most commonly affected organ, but fat embolism also might affect organs such as the brain, retina, and skin [11]. Systemic fat embolism syndrome is defined as clinical manifestation of systemic fat embolism [2], and it could be potentially fatal complication of trauma or surgical procedure. Typical occurrence is 12h to 72 h after the surgery, presenting with progressive respiratory insufficiency, consciousness disorders and petechiae. Different neurological disorders may be present, ranging from mental confusion to altered level of consciousness, and also transient and reversible disorders manifesting as generalized convulsions and focal deficits [11].

There are several theories that explain the pathophysiological features of fat embolism. So called infloating theory, or the traditional view of fat embolism explains that fat is physically pushed into the veins after trauma, most typically after fracture of long bones [1]. Lipase theory explains that trauma causes an elevation of plasma lipase titer, which then destabilizes circulating fats by de-emulsification, saponification and mobilizing lipid stores. A second biochemical theory invokes the possible histotoxic effects of free fatty acids from bone marrow. Finally, shock and coagulation theory is based on noting that many patients who develop post-traumatic fat embolism are also hypovolemic. Hypovolemia leads to a slowing down of circulation with "slugging" of blood components and forming of micro-aggregates in the lungs. Tissue trauma worsens this by damaging vessels' intimal layer causing platelet activation. Fat from bone marrow then might provide a possible adherence surface for the activated platelets [1].

In the presented case, in the absence of fractures or extensive necrosis of fat tissue, the lung fat embolism could be explained by some of the latter theories. However, both gross and microscopic findings of small subcapsular hemorrhages in the liver, together with the presence of fat globules in hepatic veins and fatty changed hepatocytes indicated a somewhat different mechanism. Most probably, the mechanical pressure on the fatty changed liver during the urgent surgical procedure of abdominal aorta, led to liver injury with small hemorrhages and release of fat from injured hepatocytes. Fat emboli from hepatocytes then entered venous circulation, and finally led to the pulmonary fat embolism which could additionally exacerbate the existing hypovolemic shock. Al-

though undoubtedly the main cause of death in the presented case was hypovolemic shock due to abdominal aortic aneurysm rupture, iatrogenic pulmonary fat embolism could be considered as additional, contributory cause of death.

There is yet no specific treatment for either the pulmonary embolism or fat embolism syndrome [11]. However, it is essential that support measures, especially in treating adult respiratory distress syndrome, such as oxygen therapy or mechanical ventilation, have to be undertaken in more severe cases.

**Conclusion**

The presented case indicates that iatrogenic pulmonary (or even systemic) fat embolism should be considered as the possible cause of unexpected and unexplained death in the cases where elective surgical procedures were performed in patients with fatty liver change. The pathologists must be aware of this possibility, since it is not easily recognized on routine H/E staining, and some of the special staining technique should be applied.

## R E F E R E N C E S


1. *Mellor A, Soni N.* Fat embolism. Anaesthesia 2001; 56(2): 145–54.
2. *Nikolić S, Živković V, Babić D, Djonić D, Djurić M.* Systemic fat embolism and the patent foramen ovale – a prospective autopsy study. Injury 2012; 43(5): 608–12.
3. *Nikolić S, Živković V, Strajina V, Djonić D, Popović V, Djurić M.* Lung fat embolism in a body changed by putrefaction: a hip fracture ante-mortem in origin – case report. Med Sci Law 2012; 52(3): 178–80.
4. *Durlacher SH, Meier JR, Fisher RS, Lovitt WV Jr.* Sudden death due to pulmonary fat embolism in persons with alcoholic fatty liver. Am J Pathol 1954; 30: 633–4.
5. *Kent SP.* Fat embolism in diabetic patients without physical trauma. Am J Pathol. 1955; 31(3): 399–403.
6. *Hill RB Jr.* Fatal fat embolism from steroid-in-duced fatty liver. N Engl J Med 1961; 265: 318–20.
7. *Hulman G.* Fat macroglobule formation from chylomicrons and non-traumatic fat embolism. Clin Chim Acta 1988; 177(2): 173–8.
8. *Schulz F, Trübner K, Hildebrand E.* Fatal fat embolism in acute hepatic necrosis with associated fatty liver. Am J Forensic Med Pathol 1996; 17(3): 264–8.
9. *Schulz F. Tsokos M.* Fatty liver and fat embolism. A case report. Rechtsmedizin 2004; 14(6): 463–6. (Luxembourgish)
10. *Kim PJ, Pollanen MS.* Osmium impregnation detection of pulmonary intravascular fat in sudden death: A study of 65 cases. J Forensic Leg Med 2012; 19(4): 201–6.
11. *Costa AN, Mendes DM, Toufen C, Arrunátegui G, Caruso P, de Carvalho CR.* Adult respiratory distress syndrome due to fat embolism in the postoperative period following liposuction and fat grafting. J Bras Pneumol 2008; 34(8): 622–5. (English, Portuguese)


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CASE REPORT


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# Malignant melanoma metastasis in the ileum – two case reports

## Metastaza malignog melanoma u ileum

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**Abstract**

**Introduction.** Malignant melanoma is a tumor that develops from melanocytes, so this type of tumor can occur in any part of body containing the cells. Melanoma is less common than other skin tumors. It is very aggressive type of skin tumor with very early metastases and accounts for 75% of cases of death due to skin tumor. In the initial phase the tumor growth is horizontal. Early detection of changes is very important. Over time, as the tumor gets the vertical growth phase, occurrence of metastases depends on the depth of invasion (Breslow). Malignant melanoma metastasizes to regional lymph nodes, but also in the liver, lung, brain and almost any place that can be populated by the hematogenous route. **Case report.** We reported two cases of patients who were urgently surgically treated in the Emergency Center of Vojvodina, Serbia, with the clinical signs and symptoms of ileus. In both cases, the ileum resection was performed with terminal ileostomy. Histological analysis was performed and through morphology and immunohistochemical profile, in both cases, the diagnosis was metastatic malignant melanoma in the ileum. **Conclusion.** In patients with diagnosed malignant melanoma of the skin with symptoms of abdominal pain and/or anemia, application of modern imaging techniques is imperative in order to obtain an early diagnosis of gastrointestinal metastases of this tumor, because the rapid detection and radical resection may contribute to the overall survival of these patients.

**Key words:**
**diagnosis; immunohistochemistry; ileus; melanoma; neoplasm metastasis; surgical procedures, operative; treatment outcome.**

**Apstrakt**

**Uvod.** Maligni melanom je tumor koji se razvija od melanocita, ćelija koje proizvode pigment melanin, te se ovaj tip tumora može razviti u bilo kom delu tela koji sadrži ove ćelije. Melanom je ređi od drugih tumora kože, ali predstavlja uzuzetno agresivan tip tumora kože koji vrlo rano daje metastaze i pripisuje mu se oko 75% slučajeva smrtnog ishoda zbog tumora kože. U početnoj fazi tumor ima horizontalan rast, te je vrlo značajno što ranije dijagnostikovanje promene. Vremenom tumor dobija i vertikalnu fazu rasta, te upravo od dubine invazije (Breslow) zavisi pojava metastaza. Tumor metastazira ne samo u regionalne limfne čvorove, već i u jetru, pluća, mozak i skoro svako mesto koje može biti naseljeno hematogenim putem. **Prikaz bolesnika.** U radu su prikazana dva bolesnika koja su hitno hirurški zbrinuta u Urgentnom centru Vojvodine zbog kliničkih simptoma i znakova ileusa. U oba slučaja urađene su resekcije ileuma sa terminalnom ileostomijom. Učinjena je patohistološka analiza i na osnovu imunohistohemije i morfološke slike u oba slučaja je postavljena dijagnoza metastaze malignog melanoma u ileum. **Zaključak.** Kod bolesnika kojima je dijagnostikovan maligni melanom kože sa simptomima bolova u stomaku i/ili anemijom, primena modernih imidžing tehnika je imperativ u cilju postavljanja rane dijagnoze gastrointestinalnih metastaza tog tumora. Brza detekcija i radikalna resekcija mogu doprineti dužini preživljavanja ovih bolesnika.

**Ključne reči:**
**dijagnoza; imunohistohemija; creva, opstrukcija; melanom; neoplazme, metastaze; hirurgija, operativne procedure; lečenje, ishod.**

## Introduction

Malignant melanoma is a tumor that develops from melanocytes, so this type of tumor can occur in any part of body containing the cells. Melanoma is less common than other skin neoplasm, but it is the most serious type of skin tumor and accounts for 75% of cases of death due to skin tumors. This neoplasm is more common in people whose

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family history provides information about the existence of skin tumors [1, 2], prominent moles, astrocytoma, pancreatic cancer, changes in previously normal naevus, as well as information about exposure to the sun, especially early in life. In females, the most common localization of melanomas are legs, while in men those are usually present on the back [3, 4]. Melanomas of the skin are usually clinically asymptomatic, but the most important clinical signs are change in color or size of pigmented lesions. The main clinical warning signs we can see using alphabet of melanoma – Asymmetry, Border (edges), Color, Diameter (≥ 6 cm), Evolution [5]. There are four basic types of the growth of malignant melanoma: the superficial spreading malignant melanoma (in about 75% of cases), nodular melanoma, lentigo malignant melanoma and acral lentiginous melanoma [6]. It is crucial to detect this change in the early phases of melanoma. Malignant melanoma shows the initial inclination of the radial growth. Radial growth is horizontal growth within the dermis (*in situ*) and the superficial layers of the dermis when the cells have the ability to metastasize and there is no evidence of angiogenesis [7, 8]. Over time, the growth acquires a vertical component, melanoma grows down into the deeper layers of the dermis [7, 8]. Based on the thickness of the invasive vertical growth phase measured in millimeters is what determines the likelihood of metastasis. According to the Breslow there are five stages of depth of malignant melanoma in the skin: stage I - depth is less or equal of 0.75 mm; stage II - depth is between 0.76–1.5 mm; stage III - depth is between 1.51–2.25 mm; stage IV - depth of tumor is between 2.26–3.0 mm; stage V - depth is greater than 3 mm [9]. Other indicators of metastatic potential are density of lymphatics, the number of mitosis and ulceration over the tumor. The tumor metastasizes to regional lymph nodes, but also in the liver, lung, brain and almost any place that can be populated by the hematogenous route [10]. Treatment of malignant melanoma is a surgical removal of the tumor. Sentinel lymph node biopsy provides additional information about the biological aggressiveness of the tumor [11, 12]. According to international guidelines histopathological analysis of the sentinel node (the first lymph node on the way of lymph after tumor) helps in decision to avoid early unnecessary elective lymphadenectomy. Early elective lymphadenectomy did not show better survival after surgery of malignant melanoma, but surely it can make a patient more difficult to recover [13, 14]. Although most of the lesions occur in the skin, they can also be found in other rare localities, including oral genital mucosa, esophagus, meninges, eye, etc. If the tumor is smaller and comprises a thin layer of skin, by surgical removal of the tumor, chances for cure are definitely great. Since the probability of the recurrence of malignant melanoma depends on the depth of invasion of cancer cells (in such cases they are also spread), the treatment includes surgical removal of the tumor, as well as chemotherapy, radiotherapy and immunotherapy [3, 4].

## Case report

### Case I

Male patient, aged 43, was presented in the Emergency Room of Vojvodina, Serbia with the clinical picture of ileus. The patient had abdominal pain and bloating feeling, lack of gas and stool for a period of 4 days, and repeatedly vomited. He denied other symptoms, as well as diseases of the importance of heredity. After clinical examination, laboratory and radiological examinations, the ileus diagnosis was made, and urgent surgical treatment was indicated. The Laboratory of the Centre for Pathology and Histology received the surgical specimen. Small bowel length of 20 cm, on which the mucosa was changed in size 5 x 3 x 1 cm, dark brown, with wide base attached to the intestinal mucosa (Figure 1).

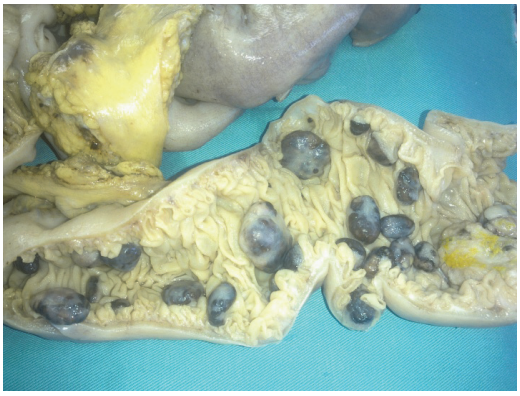
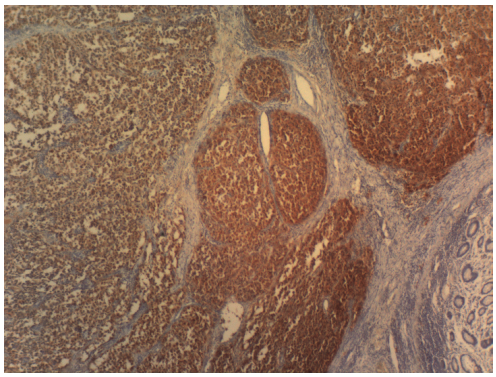
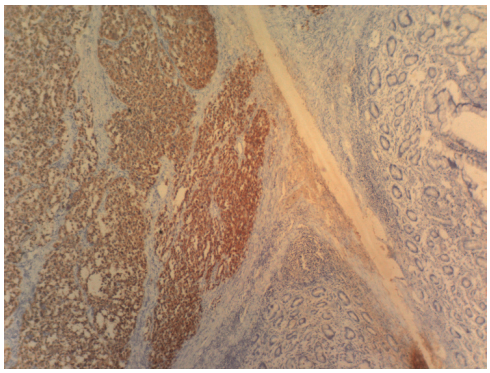


**Fig. 1 – Macroscopic appearance of changes in the small intestinal mucosa (the first patient).**

**A)**





**B)**

**Fig. 2 – Immunohistochemical examination of the surgical specimen (the first patient): A) S100; ×100; B) HMB45 stain, ×400**

Detailed histopathological examination verified the polygonal and spindle cells hyperchromatic and vesicular nuclei, prominent acidophilic nucleoli in places, medium abundant acidophilic cytoplasm arranged in solid beach and partly in the swirling structure with fibrotic-vascular stroma. Described tumor infiltrating all layers of the wall of the ileum extending to the surrounding connective and fatty tissue, and in some places were present areas of necrosis. Special immunohistochemical stainings were made: S-100 + (Figure 2A), Melan A +, HMB + (Figure 2B), AE1/AE3 -, Desmin -, Vimentin + / -, and based on histological and immunohistochemical description of the tumor, the diagnosis of metastatic malignant melanoma in the ileum was made. In the examined lymph nodes, which were received with a portion of the small intestine, the reactive changes were found.

**Case II**

The patient, aged 34 years, was admitted in the Emergency Room of Vojvodina, Serbia with abdominal pain which lasted for a month. Disease history stated that there was a surgery of malignant melanoma on his right hand when metastases in the lymph nodes were found. He denied the existence of diseases of importance to heredity. Having applied clinical, laboratory and radiological examinations, the patient was hospitalized in the Semintensive Department of Energency Room (ER) of Vojvodina with clinical signs of ileus. The Laboratory of the Centre for Pathology and Histology received the surgical specimen. Small bowel length of 25 cm, on which the mucosa had vegetative change dimensions 6 x 3 x 5.5 cm, of a yellowish color, which impressed on the serial sections that infiltrated the intestinal wall. By detailed histopathological examination of preparations, atypical polygonal and round cells were observed with hyperchromatic and vesicular nuclei, some bizarre-looking with two nucleoli and in places with prominent acidophilic nucleoli. Tumor cells were spread into the alveolar structure, papillary formations and the less solid beaches, whereby the surface of the tumor was mainly ulcerated and permeated with necrotic tissue. The tumor stroma was abundant and connective-vascular. Tumor infiltrated the mucosa, submucosa, and muscle layer of the wall of the small intestine. Special immunohistochemical stainings were made: Vimentin + + Melan A + (Figure 3A), HMB45 (Figure 3B), CK7 -, CK20 -, AE1/AE3 -, S100 + (Figure 3C), Tropomyozin -, Desmin -, and diagnosis of metastatic melanoma in the small intestine was made. In the examined lymph nodes, which were received with a portion of the small intestine, the reactive changes were found.

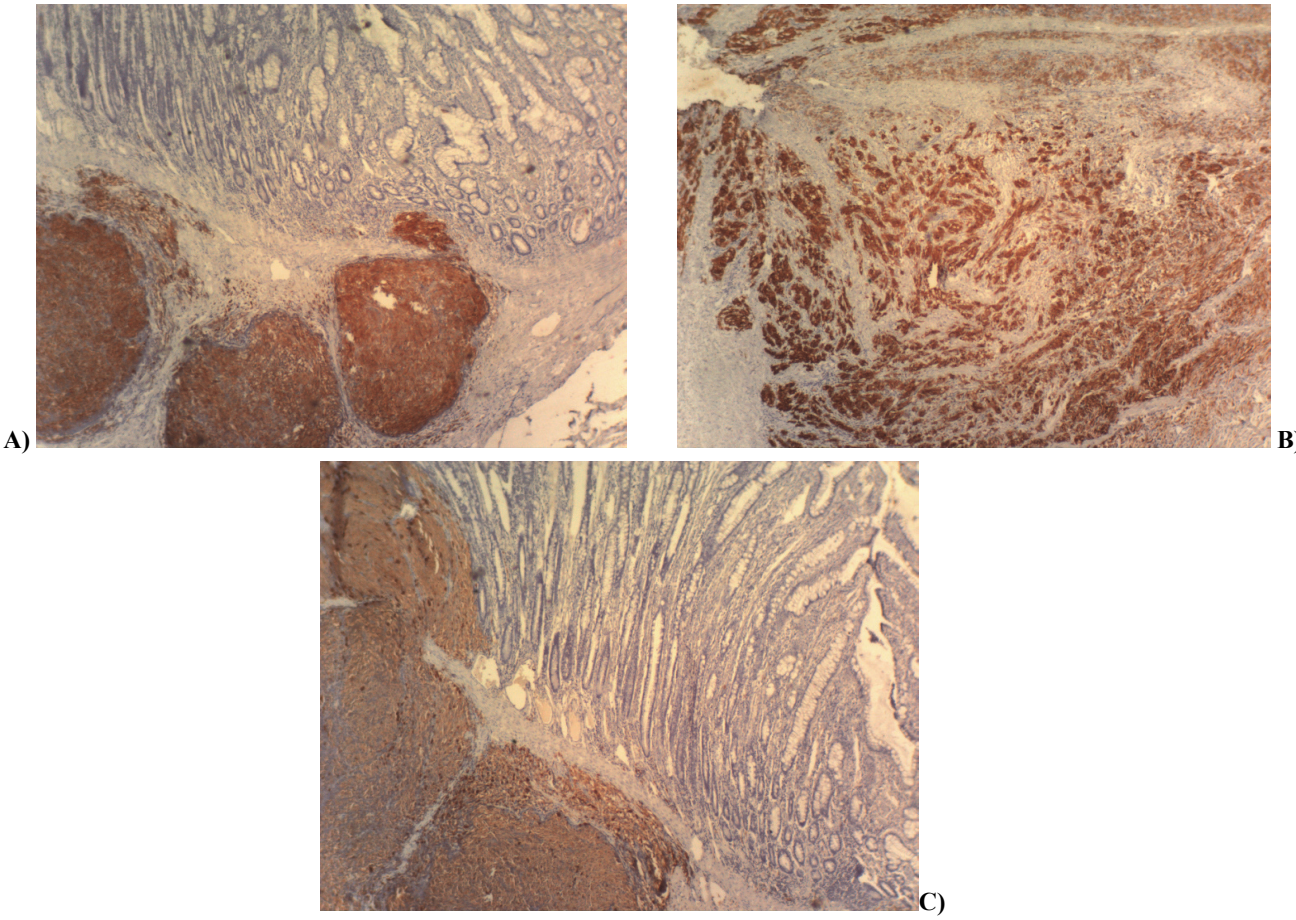



**Fig. 3 – Special immunohistochemical stainings of the surgical specimen (the second patient): A) Melan A stain, ×400; B) HBM45 stain, ×400; C) S100 stain; ×400.**

## Discussion

Primary malignant melanoma of the gastrointestinal tract is extremely rare, compared to the metastasis of this tumor in the small intestine [15]. Malignant melanoma counts 1% to 3% of all malignant lesions of the gastrointestinal tract [16], and as noted above, the majority of these tumors are metastatic spread of the primary tumor, mainly from the skin, but can also have the origin from the retina, the anus or nail plate [17]. Routine barium mash-ray examination and computed tomography (CT) examinations have limited options, but fluorodeoxyglucose (FDG) positron emission tomography (PET) CT imaging can demonstrate the existence of metastatic malignant melanoma in the small intestine. PET CT imaging is highly sensitive identification of changes in human body which is carried out by measuring the biochemical activity of tumor cells that is extremely high in malignant tumors. Metastatic disease may be suspected in all patients with gastrointestinal complaints and a personal history of previous treatment of malignant melanoma of the skin [15]. The period between diagnosis of primary malignant melanoma and identification of metastases in the gastrointestinal tract varies from 2 to 180 months, and most of them are the findings at the autopsy [18]. Melanoma of the small intestine is often asymptomatic. If there are symptoms, they could be vague and nonspecific, and may be very different – from chronic pain in the abdomen (17%–64%), the occult or manifest bleeding (26%–84%) and body weight loss (10%–47%) [18]. As a nonspecific clinical picture, it is necessary to exclude other causes of abdominal discomfort for the safety of diagnosing metastases of malignant melanoma. Sometimes, melanoma of the small intestine is manifested with the clinical picture of the emergency state due to intestinal obstruction or intussusceptions and, rarely, intestinal perforation. Up to now only six cases of the intestinal perforation and metastases of malignant melanoma have been described [19–23], and 20 cases of minor intussusceptions of the small intestine [24]. Metastatic malignant melanoma is usually polypoid, multinodular, and depending on the size can cause obstruction, as in our case. The primary localization is most common previously diagnosed skin lesion. In 4%–12% of the intestinal metastasis of melanoma, it is impossible to determine the nature and localization of primary lesion [25]. Gutman et al. [26] published a study of indications for surgical treatment of metastatic malignant melanoma of the gastrointestinal tract. Half of the patients in this study were considered for elective surgical treatment, and 22% of patients required urgent surgical treatment for intestinal obstruction or overt gastrointestinal bleeding. Olila et al. [27] have published the results of their research which show statistically significant prolongation of survival in surgically treated patients particularly those who had complete resection. Surgical treatment provides better survival compared to pharmacological or palliative ones [24–31]. Kadakia et al. [32] published the results of studies in which 70% of anemia were present, as well as acute bleeding from the upper gastrointestinal tract, which was present in 50% of observed cases with metastasis of malignant melanoma in the gastrointestinal tract, while the others registered abdominal pain (60%), intestinal obstruction (47%), nausea and vomiting (41%), and gastrointestinal bleeding (30%). Abdominal masses were recorded in only 10% of patients. The histological picture of malignant melanoma showing different relations of spindle cells and areas of epithelial proliferation with large nuclei and abundant eosinophilic cytoplasm [33]. Tumor cells may indicate melanin pigment within the stromal macrophages, but they can be completely amelanotic. Immunohistochemical staining for melanoma that does not depend on the melanin pigment includes: vimentin, S 100 protein and the more specific HMB 45 and melan A. Melan A antibody is high specific for melanocytic tumors in the skin, with positivity in benign and malignant tumors, but in neoplasm in intestine it shows high specificity for malignant melanoma. In patients who died due to metastatic spread of melanoma the intestinal metastases were found in 43.5% to 86.3% of the cases.

## Conclusion

Primary malignant melanoma of the small intestine occurs rarely. It is always metastatic lesion of treated or untreated skin melanoma. Diagnosis is often very late set, and these patients are considered for urgent surgical treatment. Due to high incidence of gastrointestinal metastases in patients with a personal history of malignant melanoma of the skin, manifested as abdominal pain and/or anemia, the use of modern imaging techniques is imperative in order to obtain an early diagnosis of gastrointestinal metastases of this tumor and timely undertake radical surgical resection in order to improve overall survival in this group of patients. Among diagnostic methods, histopathological analysis is golden standard in diagnosis of malignant melanoma.

## REFERENCES


1. *Fallah M, Pukkala E, Sundquist K, Tretli S, Olsen JH, Tryggvadottir L*, et al. Familial melanoma by histology and age: joint data from five Nordic countries. Eur J Cancer 2014; 50(6): 1176–83.
2. *Begg CB, Hummer A, Mujumdar U, Armstrong BK, Kricker A, Marrett LD,* et al. Familial aggregation of melanoma risks in a large population-based sample of melanoma cases. Cancer Causes Control 2004; 15(9): 957–65.
3. *Jerant AF, Johnson JT, Sheridan CD, Caffrey TJ*. Early detection and treatment of skin cancer. Am Fam Physician 2000; 62(2): 357–68, 375–6, 381–2.
4. Kanavy HE, Gerstenblith MR. Ultraviolet radiation and melanoma. Semin Cutan Med Surg 2011; 30(4): 222–8.
5. *Kumar V, Abbas AK, Fausto N, Mitchell RN*. Robbins basic pathology. 8th ed. New York: Elsevier Inc; 2007.
6. *Atanackovic M*. Patologija. Beograd: Medicinski fakultet Univerziteta u Beogradu; 2003.
7. *Wat H, Salopek TG*. Revisiting vertical growth phase and regression for thin melanoma. J Am Acad Dermatol 2017; 76(1): e27.

8. *Betti R*, Agape *E, Vergani R, Moneghini L, Cerri A*. An observational study regarding the rate of growth in vertical and radial growth phase superficial spreading melanomas. Oncol Lett 2016; 12(3): 2099–102.
9. *Breslow A*. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970; 172(5): 902–8.
10. *Gassenmaier M, Eigentler TK, Keim U, Goebeler M, Fiedler E, Schuler G*. Serial or Parallel Metastasis of Cutaneous Melanoma? A Study of the German Central Malignant Melanoma Registry. J Invest Dermatol 2017; 137(12): 2570–7.
11. *Faries MB, Thompson JF, Cochran AJ, Andtbacka RH, Mozzillo N, Zager JS,* et al. Completion dissection or observation for sentinel-node metastasis in melanoma. N Engl J Med 2017; 376(23): 2211–22.
12. *Leiter U, Stadler R, Mauch C, Hohenberger W, Brockmeyer N, Berking C,* et al. Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): a multicentre, randomised, phase 3 trial. Lancet Oncol 2016; 17(6): 757-67.
13. *Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Elashoff R, Essner* R, et al. Sentinel-node biopsy or nodal observation in melanoma. N Engl J Med 2006; 355(13): 1307–17.
14. *Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Nieweg OE, Roses DF,* et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. N Engl J Med 2014; 370(7): 599–609.
15. *Kruse J, Heath R*. Melanoma metastatic to the gastrointestinal tract. Am Fam Physician 1990; 41(1): 165–8.
16. *Blecker D, Abraham S, Furth EE, Kochman ML*. Melanoma in the gastrointestinal tract. Am J Gastroenterol 1999; 94(12): 3427–33.
17. *Atmatzidis KS, Pavlidis TE, Papaziogas BT, Papaziogas TB*. Primary malignant melanoma of the small intestine: report of a case. Surg Today 2002; 32(9):831– 3.
18. *Wysocki WM, Komorowski AL, Darasz Z*. Gastrointestinal metastases from malignant melanoma: report of a case. Surg Today 2004; 34(6): 542–6.
19. *Tsilimparis N, Menenakos C, Rogalla P, Braumann C, Hartmann J*. Malignant melanoma metastases as a cause of small bowel perforation. Onkoligie 2009; 32(6): 356–8.
20. *Alwhouhayb M, Mathur P, Al Bayaty M*. Metastatic melanoma presenting as a perforated small bowel. Turk J Gastroenterol 2006; 17(3): 223–5.
21. *Brummel N, Awad Z, Frazier S, Liu J, Rangnekar N*. Perforation of metastatic melanoma of small bowel with simultaneous gastrointestinal stromal tumor. World J Gastroenterol 2005; 11(17): 2687–9.
22. *Cholakov O, Tsekov KH, Beliaev O, Stefanov P, Cherveniakov A*. Disseminated malignant melanoma, complicated with upper-digestive hemorrhage. Khirurgiia (Sofiia) 2003; 59(3): 52–3.( Bulgarian)
23. *Klausner JM, Skornick Y, Lelcuk S, Baratz M, Merhav A*. Acute complications of metastatic melanoma to the gastrointestinal tract. Br J Surg 1982; 69(4): 195–6.
24. *Mucci T, Long W, Witkiewicz A, Mastrangelo MJ, Rosato EJ, Berger AC*. Metastatic melanoma causing jejunal intussusception. Journal of Gastrointestinal Surgery 2007; 11(12): 1755–7.
25. *Baniukiewicz A, Zaremba-Woroniecka A, Wróblewski E, Łaszewicz W*. Przerzutowy czerniak złośliwy żołądka jako przyczyna krwawienia z przewodu pokarmowego. Opis przypadku. Gastroenterologia Polska 2000; 7: 61–3. (Polish)
26. *Gutman H, Ben-Ami E, Shapira-Frommer R, Schachter J*. Multidisciplinary management of very advanced stage III and IV melanoma: Proof-of-principle. Oncol Lett 2012; 4(2): 307–10.
27. *Ollila DW, Essner R, Wanek LA, Morton DL*. Surgical resection for melanoma metastatic to the gastrointestinal tract. Arch Surg 1996; 131(9): 975–9; 979–80.
28. *Godlewski J, Kuciel-Lisieska G, Tenderenda M, Troska P, Widawski T*. Przerzuty czerniaka do narządów przewodu pokarmowego. Nowotwory 2009; 59: 96–8. (Polish)
29. *Kycler W, Łaski P, Porzegowski M, Teresiak M*. Chirurgiczne leczenie przerzutów do odległych narządów wprzebiegu czerniaka – opis przypadku. Wspolczesna Onkologia 2006; 4: 175–8. (Polish)
30. *Essner R, Lee JH, Wanek LA, Itakura H, Morton DL*. Contemporary surgical treatment of advanced-stage melanoma. Arch Surg 2004; 139(9): 961–6; discussion 966–7.
31. *Pirisi M, Leutner M, Grazioli S, Bartoli EG*. A woman with anemia and a small-bowel obstruction. CMAJ 2004; 170(5): 788.
32. *Kadakia CS, Parker A, Canales L*. Metastatic tumors to the upper gastrointestinal tract: endoscopic experience. Am J Gastroenterol 1992; 87(10): 1418–23.
33. *Bender GN, Maglinte DD, McLarney JH, Rex D, Kelvin FM*. Malignant melanoma: Patterns of metastasis to the small bowel, reliability of imaging studies, and clinical relevance. Am J Gastroenterol 2001; 96(8): 2392–400.

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I N M E M O R I A M

**Dr Ljiljana Vučković Dekić, naučni savetnik**
**(1943–2019)**
**Lekarka–ombudsman ali i „vitez za kulturu”**

U mnoštvu lekara uvek se izdvajaju oni koji svoju privrženost nauci ispoljavaju uz izraženu kolegijalnost, nesebičnost i dečiju radost kad se objave rezultati istraživanja u časopisu, izraženi patriotizam i humanost. O takvim osobinama, po svemu, bolje prosuđuju oni koji osobu posmatraju sa strane.

Dr Ljiljanu Vučković Dekić poznavala sam poslednje dve decenije sarađujući na planu dobre naučne prakse i ocene naučnog poštenja pri publikovanju, predstavljanja tema iz onkologije i naučno–istraživačkog rada na preventivnim seminarima nacionalnog Instituta „Batut”, tesne saradnje oko okupljanja naučnog odbora za „Batutove dane” 2013. sa temom „Rak u Srbiji”, okupljanja urednika medicinskih časopisa i saradnje sa njima, uređivanja lista „Lekar” ali i zajedničkog organizovanje niza događaja iz domena humanosti. Čini se da je humanost nerazdvojna i da predstavlja nadgradnju tema iz oblasti nauke i međusobne saradnje lekara, organizacija i institucija.

Spoj nauke, humanosti, umetnosti i kulture delovao je zajednički kroz rad i život lekarke Ljiljane Vučković Dekić. Kroz radni vek izrasla je u naučnog radnika koji se prepoznaje i lep je primer uspeha žena lekara u nauci u nacionalnom institutu za rak i naučnoj zajednici Srbije. Da, ona je zaista bila pravi pedagog, profesor. Okupljala je kolege, podsticala i radovala se njihovom napredovanju.

Dr Ljiljana Dekić, kao član Akademije medicinskih nauka Srpskog lekarskog društva (AMN SLD), rukovodila je grupom „Preventivna medicina”. Kao član Sekcije za humanost, umetnost i kulturu Društva lekara Vojvodine SLD našla je puta i načina da da prilog za obnovu biste prve srpske lekarke Drage Ljočić, a bila nam je čast da ona, kao žena lekar, otkrije njenu bistu. I tom prilikom pokazala je visoku svest i širinu svojstvenu intelektualcima.

Predsednik Sekcije koju pominjemo, dr Vladimir Jokanović, i sam član AMN SLD i „Preventivne grupe”, u intervjuu na isteku 2019. godine (30. decembra ) nabrojao je osobine pravih intelektualaca (https://www.youtube.com/watch?v=10YxMZD5Pfo&t=2738s). Intelektualac je, rekao je, školovan čovek, kulturan, civilizovan ali mora biti i kreativan – tek onda je intelektualac, koji razume ovaj svet i menja ga. Tužna je prilika ali je privilegija da to glasno kažem. Po svemu, dr Ljiljana Vučković Dekić, naučni savetnik, je pripadala pravim intelektualcima u Srbiji.

Posećivale smo spomenike podignute u čast medicinskih misija, najviše ženama, u Mladenovcu, Kragujevcu, Vranju, Vrnjačkoj Banji. Nije propuštala mogućnost da iskaže zahvalnost nekadašnjim dobrotvorima, što je odlika njene visoke svesti a za film „1915. godina” odmah mi je rekla „da je svaka naša porodica imala nekoga ko je prešao Albaniju”. Da li se patriotizam uči ili nasleđuje, pitam se.

O podvigu srpske vojske koja je na frontu Slivnice u borbama sa bugarskom vojskom prekinula dejstva i propustila humanitarnu pomoć dr Ljilja nije htela da propusti priliku da samnom to napiše, ilustruje i trajno zabeleži u Vojnosanitetskom pregledu–„Akt humanosti”. Poruka o veličini humanosti srpske vojske trajno je zabeležena na vidnom mestu u Međunarodnom crvenom krstu u Ženevi pa nije čudo što smo se upustile u dokumenta o osnivanja Crvenog krsta u Srbiji. Pisale smo i govorile i o dr Vladanu Đorđeviću i pronašle priliku da o tome čuju i pacijenti Instituta za onkologiju i radiologiju Srbije. Po svemu, dr Ljiljana Dekić je poticala iz srpske porodice koja je negovala tradiciju i uvažavala prošlost.

Dr Ljiljana nas je okupljala za koncerte nacionalnog ansambla narodnih igara i pesama „Kolo". Posebno je volela koreografije: „Vranjanska svita", „Dubočke kraljice"i „Trojno". Kako li je samo uživala kad o tome razgovaramo i sa koliko volje borila poslednjom snagom da gleda makar još jedan njihov koncert. Malo pred time uspela je da ode u Kragujevac i održi predavanje u svom velikom stilu a očevici procenjuju da nije nigde pogrešila. Volela je Kragujevac, medicinare, bila na otvaranju čitaonice na fakultetu imena dr Elizabet Ros, ali i na malom engleskom groblju gde je ova lekarka sahranjena sa dvema medicinskim sestrama. Kao žrtve pegavca u Velikom ratu ostale su zauvek u Srbiji. U svemu što je radila bilo je puno ljubavi i posvećenosti.

Koliko je samo volela Institut za onkologiju Vojvodine u Sremskoj Kamenici, prof. dr Vladimira Baltića i prof. dr Gordanu Bogdanović. Kako i ne bi kad je profesor osnovao Institut ali i „Studeničku akademiju" posvećenu novim saznanjima u onkologiju u zemlji i svetu, a dr Gordana Bogdanović nastavila uređivanje časopisa Instituta koji je on osnovao. Zato je i moje predavanje „O Studeničkoj akademiji" u Institutu „Batut" koje sam umesto njih pripremila za temu „Rak u Srbiji" bilo njima u čast. Dr Ljiljana me je naučila da se kolega na kolegu oslanja. Ja sam je samo sledila. Pogledom sa strane mogla sam dosta toga jasno da vidim i zapazim jer sam dugo „bila prisutna".

Dr Ljiljana Vučković Dekić ostaje da živi sa nama i u nama!

**Dr Slavica Žižić Borjanović**

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

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

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

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

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

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

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

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

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

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Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

**Skraćenice i akronimi**

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

**Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp**