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## RCRI VS. GSCRI IN SIX-MONTHS PREDICTION OF MYOCARDIAL INFARCTION AND CARDIAC ARREST OCCURRENCE

Mladjan Golubović<sup>1,2</sup>, Velimir Perić<sup>1</sup>, Milan Lazarević<sup>1,2</sup>, Tomislav Kostić<sup>2,3</sup>,  
Dragana Stokanović<sup>2</sup>, Miodrag Djordjević<sup>2,4</sup>, Vesna Marjanović<sup>2,5</sup>, Dragan J. Milić<sup>1,2</sup>

The aim of the study was to compare prognostic abilities of Revised Cardiac Risk Index (RCRI) and Geriatric Sensitive Cardiac Risk Index (GSCRI) for myocardial infarction (MI) and cardiac arrest (CA) occurrence during 180 days in patients older than 65 years scheduled for major elective vascular surgery. MICA occurrence was noted in 16 (11.1%) patients. Both myocardial infarction and cardiac arrest were of equal frequency, each in 8 patients (24.2%). MICA occurrence was associated both with higher RCRI ( $p < 0.001$ ) and GSCRI scores ( $p < 0.001$ ). Multivariate analysis of binary logistic and Cox regression models determined better predictor ability of GSCRI score. Each unit of GSCRI score was associated with 1.2 times ( $p < 0.05$ ) greater risk of MICA.

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**Key words:** RCRI, GSCRI, myocardial infarction, cardiac arrest, vascular surgery

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### Introduction

Preoperative risk stratification is a strategy of clinical doctors with the goal of preventing cardiac events (1). Myocardial infarction (MI) and cardiac arrest (CA) are major adverse cardiac events that carry highest mortality (2). By 2050, the population over the age of 65 will reach almost 90 million in the United States (3). Elective vascular procedures belong to the subset of high risk surgeries based frequency of occurrence of the MI and CA, which is greater than 5% after 30 days (4). Given that elderly patients are more susceptible to cardiac

adverse events (5), their prediction is crucial in peri-operative management. Aim of the study was to compare prognostic abilities of Revised Cardiac Risk Index (RCRI) and Geriatric Sensitive Cardiac Risk Index (GSCRI) for MICA occurrence during 180 days in patients older than 65 years scheduled for major elective vascular surgery.

### Materials and methods

During the three-year period (2017-2019), the study included 144 patients (men:women = 2:1) with average age of 70 years. Essential inclusion criteria were major elective vascular surgery and age above 65 years. Patients were followed for 180 days after the procedure. All participants underwent balanced endotracheal anesthesia and detailed preoperative examination which included an extensive clinical and medical history evaluation. Outcomes of interests were myocardial infarction and cardiac arrest. We have implemented the following definitions:

1) MI - indicative electrocardiogram changes and new onset cardiac troponin I elevation greater than three times the upper reference limit and

2) CA - absence of heart mechanical response as effect of ventricular fibrillation, pulseless ventricular tachycardia and pulseless electric activity. The study was approved by Ethics Committee of the Faculty of Medicine, University of Niš and directed at the Clinic of Cardiovascular Surgery, University Clinical Center Niš, in consonance with the principles of the Declaration of Helsinki. All participants signed an informed consent before inclusion.

**Statistical analysis**

Statistical Package for Social Sciences (SPSS 21.0; Chicago, IL, USA) was used for data analysis. Quantitative variables were presented as means with SDs or medians with interquartile ranges. Student's t-test and Mann-Whitney U-test were implemented. Prediction of myocardial injury and cardiac arrest were identified by univariate and multivariate binary logistic and Cox-regression analysis. A p-value less than 0.05 was considered to be a measure of statistical significance.

**Results**

During the 180 days after surgical procedure, MICA was noted in 16 (11.1%) patients. Ten of these patients (62.5%) had MICA in the first month. Eleven patients (68.8%) had only one event, in two

patients two events were observed, and three events in three patients. Three patients (2.1%) died during the follow-up. Both myocardial infarction and cardiac arrest were of equal frequency, each in 8 patients (24.2%).

MICA occurrence was associated both with higher RCRI ( $p < 0.001$ ) and GSCRI scores ( $p < 0.001$ ) (Table 1).

ROC curve analysis has shown statistically significant power of RCRI and GSCRI in discriminating patients with and without MICA. Discriminatory ability of both scores was with  $AUC > 0.7$  (Table 2).

Multivariate analysis of binary logistic and Cox regression model were determined better predictor ability of GSCRI score. Each unit of GSCRI score was associated with 1.2 times ( $p < 0.05$ ) greater risk of MICA (Tables 3, 4).

**Table 1.** Correlation of RCRI and GSCRI with occurrence of MICA six months after the procedure

Risk score	With MICA	Without MICA	P value
RCRI	2.44 ± 1.21	1.25 ± 1.16	3.852 (0.000)*
RCRI (%)	11.29 ± 3.43	7.52 ± 3.85	3.741 (0.000)*
GSCRI	7.7 (2.8-12.1)	1.9 (0.3-7.2)	3.497 (0.000)†

\*- t-test, †- Z-test

MICA-myocardial infarction and cardiac arrest; RCRI-Revised Cardiac Risk Index; GSCRI-Geriatric Sensitive Cardiac Risk Index

**Table 2.** Discriminative ability of risk scores

Variable	Area (95% CI)	P value	Cut-off	Sensitivity (%)	Specificity (%)
RCRI	0.769 (0.655-0.882)	0.000	2.0	87.5	66.4
RCRI (%)	0.762 (0.650-0.874)	0.001	10.1	87.5	66.4
GSCRI	0.767 (0.666-0.868)	0.001	1.7	100.0	44.5

CI-confidence interval; RCRI-Revised Cardiac Risk Index; GSCRI-Geriatric Sensitive Cardiac Risk Index

**Table 3.** Binary logistic regression model of six-months MICA occurrence

Variable	Univariate analysis - OR (95% CI)	P value	Multivariate analysis- OR (95% CI)	P value
RCRI (%)	1.249 (1.095-1.424)	0.001		
RCRI ( $\geq 2$ or $\geq 10.1\%$ )	13.837 (3.007-63.668)	0.001		
GSCRI	1.157 (1.054-1.270)	0.002	1.367 (1.068-1.750)	0.013

OR-odds ratio; CI-confidence interval; RCRI-Revised Cardiac Risk Index; GSCRI-Geriatric Sensitive Cardiac Risk Index

**Table 4.** Cox regression model of six-months MICA occurrence

Variable	Univariate analysis - OR (95% CI)	P value	Multivariate analysis- OR (95% CI)	P value
RCRI	1.801 (1.285-2.525)	0.001	2.340 (0.733-7.469)	0.151
RCRI (%)	1.212 (1.078-1.363)	0.001		
RCRI ( $\geq 2$ or $\geq 10.1\%$ )	11.829 (2.687-52.075)	0.001		
GSCRI	1.130 (1.046-1.220)	0.002	1.251 (1.048-1.493)	0.013

OR-odds ratio; CI-confidence interval; RCRI-Revised Cardiac Risk Index; GSCRI-Geriatric Sensitive Cardiac Risk Index



## Discussion

RCRI is a simple and most commonly tested method for risk assessment of all surgical patients proposed by ESC/ESA (4). This score system is not reliable in prediction of major adverse cardiac events in vascular surgery patients (6). There are several obstacles in analysis of the clinical applicability of RCRI. In the original study, four major cardiovascular complications were selected as outcomes of interests: ventricular fibrillation or primary cardiac arrest, myocardial infarction, pulmonary edema and complete heart block. The definitions of these complications of that time differ from today's. For example: The diagnosis of pulmonary edema was made on the basis of radiograms, and myocardial infarction on the basis of detection of MB fraction of creatine kinase (7). In this study, we used high sensitivity TnI assay which is more specific and sensitive for diagnosis of MI (8).

ROC curve analysis showed AUC for RCRI of 0.762 (0.650 - 0.874) which represented a slightly smaller area compared to the original study (0.774 ± 0.032). Unexpectedly high values of AUC we explain by monitoring only two outcomes of interest as well as the lower prevalence of abdominal aortic aneurysm reparations (24%), since in the original study, RCRI showed unsatisfactory predictive characteristics in these patients (7).

GSCRI is a newly created risk score index for the geriatric population. Multivariate analysis of both binary logistic and cox regression models detected predictive significance of GSCRI over RCRI score. In our study conducted in geriatric vascular patients, we obtained almost identical values of AUC (0.767 vs. 0.76) as in the original study which included general geriatric cohort (9). We consider this to be a high value, since it is very difficult to achieve a value over 0.8 with a single score, especially in a group of high-risk patients such as geriatric vascular patients. The reasons for better predictive abilities of GSCRI in relation to RCRI can be found in:

- 1) a dichotomy was avoided in most variables in the formation of GSCRI;
- 2) lower cut-off values of creatinine were set in GSCRI (132 µmol/L vs. 176.8 µmol/L);
- 3) ASA score was implemented (10).

Davenport et al. reported that ASA score is one of the most important independent predictors for cardiac morbidity in general and vascular surgery. In the same group of predictors, there are also variables of age and type of surgery that were implemented in the GSCRI.

## Conclusion

MICA occurrence was associated both with high RCRI and GSCRI scores. GSCRI has better ability to predict MICA occurrence during six-months period.

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## RCRI VS. GSCRI U ŠESTOMESEČNOM PREDVIĐANJU POJAVE INFARKTA MIOKARDA I SRČANOG ZASTOJA

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Cilj studije je uporediti prognostičke sposobnosti revidiranog indeksa srčanog rizika (RCRI) i gerijatrijski osjetljivog indeksa srčanog rizika (GSCRI) za infarkt miokarda (MI) i srčani zastoj (CA) tokom 180 dana, kod bolesnika starijih od 65 godina, pripremljenih za veliku elektivnu vaskularnu hirurgiju. Pojava MICA evidentirana je kod 16 (11,1%) bolesnika. I infarkt miokarda i srčani zastoj su bili jednake učestalosti, kod 8 bolesnika pojedinačno (24,2%). Pojava MICA povezana je sa visokim vrednostima RCRI ( $p < 0,001$ ) i GSCRI skora ( $p < 0,001$ ). Multivarijantna analiza binarnog logističkog i Koks-regresionog modela označila je veću prediktorsku sposobnost GSCRI skora. Svaka jedinica GSCRI skora povezana je sa 1,2 puta većim rizikom za MICA.

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**Ključne reči:** RCRI, GSCRI, miokardni infarkt, srčani zastoj, vaskularna hirurgija

## COHERENCE SCORES AND COHERENCE LEVEL: MODIFICATION EFFECTS OF HYDRO-AROMATHERAPY AND MINDFULNESS MEDITATION ON PATIENTS SUFFERING FROM INFLAMMATORY RHEUMATIC DISEASES

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Heart rate variability (HRV) and coherence score (CS) or coherent heart rhythm pattern are a validated method to establish autonomic nervous system (ANS) activity.

The aim of this research was to assess the modifications of CSs in a sample of patients suffering from inflammatory rheumatic diseases during their physical treatment and rehabilitation based on natural factors as well as hydro-aromatherapy and meditation.

This study included 44 Norwegian patients with confirmed diagnosis of inflammatory rheumatic diseases during four weeks of physical treatment and rehabilitation at the Institute "Dr Simo Milošević" Igalo, Montenegro. They were divided into two groups: (1) experimental (n = 22) and (2) control (n = 22). All the patients received the standard protocol based on natural healing mud and mineral water. However, the patients in the experimental group had additional hydro-aromatherapy (mineral water enriched with essential oils of lavender, orange and lemon) and meditation. The emWave Pro machine measured CS before and after the treatment. There were three coherence levels (CL): low, medium and high. Low reflected the amount of time in which there was no wavelike activity, medium some wavelike activity, and high a considerable amount of wavelike activity in the HRV tracing.

After the treatment, the CS was found to be significantly higher in the experimental group than in the control group ( $p < 0.015$ ). Simultaneously, the high CL was significantly higher in the experimental group ( $p < 0.026$ ), and the low CL was significantly lower in the control group ( $p < 0.017$ ). Comparison of the obtained CLs between the experimental and control groups after treatment showed that only the medium CL was statistically different ( $p < 0.009$ ).

Standard protocol at the Institute Igalo with the addition of hydro-aromatherapy and meditation significantly improved the ANS activity of patients suffering from inflammatory rheumatic diseases.

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**Key words:** coherence score, coherence levels, inflammatory rheumatic diseases

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### Introduction

Diseases, especially of a chronic nature, such as chronic inflammatory rheumatic diseases (CIRDs), often cause major changes in the way a person functions and affect all aspects of life (1). The sense of coherence (SOC) concept was initially used to describe a person's capacity to respond to stressful

situations, such as chronic illness (2). It was subsequently replaced by physiological coherence (PC), a term that describes the degree of order, harmony and stability in rhythmic activities within living systems over any given time period (3).

In the human body, the heart is the most consistent and dynamic generator of rhythmic information patterns, which are measured as heart rate (HR) on a beat-to-beat basis. HR fluctuations result from the complex nonlinear interactions of several physiological systems. HR variability (HRV) is thus considered a measure of neurocardiac function, which reflects the heart-brain interactions and autonomic nervous system (ANS) dynamics (4, 5). In this complex network, the vagal nerve (VN) plays a central role, being the longest cranial nerve in the human body, containing about 75% of the parasympathetic (PSY) fibres. Of these VN fibres, 80-90% are afferent, and they generate the appropriate

autonomic responses via central reflex pathways (6). Simultaneously, the VN efferent fibres dominate in cardiac control (7). Therefore, HR best reflects the relative balance in the ANS dynamics (8).

Autonomic imbalance (AI), defined as an increased HR and a decreased HRV, has attracted the attention of researchers. According to published studies, AI is associated with increased morbidity and mortality in patients with various diseases (9), and it has been implicated as a key underlying feature of several inflammatory arthritides (10). The association of AI with the onset of rheumatoid arthritis (RA) could also be important for other immune-mediated inflammatory diseases (11). To further understand this phenomenon, AI has been defined as an immunomodulatory or inflammatory reflex. In this reflex, activation of the VN plays an integral role in the downregulation of inflammation (12). However, decreased VN function precedes the development of several risk factors for cardiovascular disease (13).

It seems that the inflammatory or immunomodulatory reflex, an integral aspect of the pathogenesis of inflammatory diseases mediated by the immune system, may be altered or modified by neuromodulation for the benefit of the patient (14). Mindfulness meditation (MM) is one of the non-invasive neuromodulation modalities aimed at enhancing parasympathetic tone (15). Recent well-controlled studies have indicated that MM training may reduce the concentration of inflammatory markers, such as C-reactive protein (CRP) and interleukin 6 (IL-6) (16).

Published studies have shown that aromatic oils (AOs) can also serve as stimulus for the non-invasive neuromodulation of the immunomodulatory reflex. Through the olfactory stimulus, AOs have various effects on physiological functions (17), especially the ANS (18). Their beneficial effects are not limited to only the olfactory system because they can enter the bloodstream by absorption through the skin and nasal and/or oral mucous membranes. In addition, as lyophilic substances, they can reach the brain. Consequently, AOs cause broad-spectrum effects, including brain wave activity and the normalisation of cortisol, dopamine and norepinephrine levels, as well as the CD4/CD8 lymphocyte ratio (19-22).

The aim of this research was to assess the modification of coherence score (CS) and coherence level (CL) in a sample of patients suffering from inflammatory rheumatoid diseases during their physical treatment and rehabilitation based on natural factors as well as hydro-aromatherapy and meditation.

## Materials and methods

This study included 44 Norwegian patients with confirmed diagnosis of CIRD recruited by the Section for Climate Therapy, Oslo University Hospital, Norway, and subjected to four weeks of physical treatment and rehabilitation at the Institute "Dr Simo Milošević" Igalo, Montenegro in August and

September 2018. The CIRD implied following diagnosis: rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA). They were divided into two groups:

- (1) experimental (n = 22) and
- (2) control (n = 22).

The groups did not differ in average age and disease duration (50y vs. 55y; 14y vs. 17y). All the patients included in the study were in remission of the underlying disease with low disease activity based on clinical and laboratory parameters. During treatment at the Institute, all the patients were taking prescribed rheumatological therapy. So, there were no contraindications for the use of the standard protocol, based on natural healing mud and mineral water. Natural healing mud is characterized by high mineral contents and biologically active substances extracted from Igalo Bay. It is applied on certain body parts (except at the fore part of the thorax-heart and head) at a temperature of 42-45 °C. Mineral water is of natural origin from several springs and in its content is dominated by minerals and bioactive substances. Water is used when heated at 36-38 °C.

The patients in the experimental group had additional hydro-aromatherapy, which comprised mineral whirlpool bath enriched with essential oils of lavender, orange and lemon, every other day, as well as 20 min of mindfulness-type meditation five days per week.

The emWave Pro device was used to measure the CSs before and after the four weeks of treatment. As a heart-rate monitoring system, the device used a pulse sensor (on the subject's earlobe) and breath pacer (on the subject's clothes) plugged into a computer, collected pulse data and translated coherence information into graphics displayed on computer screen. The device gave readings with regard to date, time, session duration, coherence score, average heart rate, challenge level and coherence rate. During each session measurements were continuously monitored and were displayed as numbers, coloured bars, and curves.

Physiologically, the coherence state was reflected in the presence of a smooth sine-wave-like pattern in the HRV tracing. The total CSs ranged as follows: basic (0.5-1), good (1-2), very good (2-3) and excellent (+3). There were three levels of CS: low, medium and high. A low CS reflected the amount of time during which there was no wavelike activity, medium indicated some wavelike activity, and high reflected a considerable amount of wave-like activity in the HRV tracing (22). Testing on the emWave Pro machine took 15 min in an air-conditioned room and in silence. Patients took a sitting position. The device was set to a mid-level challenge for testing. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Montenegro.

The results were statistically analysed in IBM SPSS Statistics, Version 23.0 software. The analyses included descriptive statistics and the paired t-test. A p value of < 0.05 was considered statistically significant.

## Results

The analysed groups of patients were matched by age and disease duration. However, females were more represented in both groups (experimental group, 60.9%; control group, 73.9%). The standard protocol significantly reduced HRs in the control group ( $t = 2.631$ ;  $p < 0.016$ ), but not in the experimental group (Table 1).

The average CSs in the experimental group after four weeks of treatment were significantly higher ( $t = 2.663$ ;  $p < 0.015$ ) than those in the con-

trol group. However, the differences in the CS ranges obtained before and after treatment were not statistically significant (Table 2).

The following results were obtained in the groups after analysing the impact of the treatment on the CS levels. First, high CS levels significantly increased in the experimental group ( $t = 1.926$ ;  $p < 0.026$ ); second, low CS levels significantly decreased in the control group ( $t = 2.795$ ;  $p < 0.017$ ); third, the medium CS levels were significantly lower in the experimental group than in the control group ( $t = 2.923$ ;  $p < 0.009$ ) (Table 3).

**Table 1.** Heart rates before and after treatment in the experimental and control groups (mean  $\pm$  SD)

HR (mean $\pm$ SD)	Before treatment	After treatment	p <
Experimental group (n = 22)	74.86 $\pm$ 11.62	73.76 $\pm$ 10.87	n.s.
Control group (n = 22)	77.00 $\pm$ 13.54	72.10 $\pm$ 11.04	0.016

**Table 2.** Coherence scores and Coherence score ranges before and after treatment in the experimental and control groups

Experimental group (n = 22)	Before treatment	After treatment	p <
Coherence score (mean $\pm$ SD)	0.9619 $\pm$ 0.2941	1.2524 $\pm$ 0.7075	0.015
Coherence range (%)			
Basic	50	25	n.s.
Good	50	60	n.s.
Very good	0	10	n.s.
Excellent	0	5	n.s.
Control group (n = 22)	Before treatment	After treatment	p <
Coherence score (mean $\pm$ SD)	1.0200 $\pm$ 0.4034	1.2850 $\pm$ 0.6327	n.s.
Coherence range (%)			
Basic	63.16	47.37	n.s.
Good	36.84	36.84	n.s.
Very good	0	10.53	n.s.
Excellent	0	5.26	n.s.

**Table 3.** Coherence score levels before and after treatment in the experimental and control groups (mean value  $\pm$  SD)

Experimental group (n = 22)	Before treatment	After treatment	p <
Low	46.9048 $\pm$ 16.2077	40.3810 $\pm$ 21.2402	n.s.
Medium	42 $\pm$ 11.9121	39.2857 $\pm$ 9.8242	n.s.
High	11.0952 $\pm$ 7.2381	20.3333 $\pm$ 21.5414	0.026
Control group (n = 22)	Before treatment	After treatment	p <
Low	43.9500 $\pm$ 18.4604	32.25 $\pm$ 18.4872	0.017
Medium	40.4 $\pm$ 12.0499	48.35 $\pm$ 15.9317	n.s.
High	15.85 $\pm$ 11.2216	19.5 $\pm$ 20.1847	n.s.

## Discussion

We investigated the effectiveness of hydroaromatherapy (HAT) and MM, as well as physical treatment and rehabilitation, on the possible non-invasive neuromodulation of ANS in patients with established CIRD. This study was carried out at the Institute Igalo, which is known for its natural factors and more than 70 years of experience in the physical treatment and rehabilitation of patients with rheumatological diseases. Hence, the focus was on the determination of physiological stress or HRV as a simple noninvasive measurement of autonomic balance through changes in CS levels.

It has long been postulated that ANS, particularly the PSY, modulates the immune system in chronic inflammatory diseases. It also has antinociceptive effects (23). Indeed, the PSY maintains the homeostasis of the psycho-neuroendocrine-immune system through the afferent (to the hypothalamus, central ANS, limbic system, thalamus and cortex) and efferent (cholinergic anti-inflammatory) pathways (24).

According to the literature, AOs have a powerful neuromodulatory effect. Furthermore, their ingredients have shown significant pharmacological activities, including anti-inflammatory properties (25), such as the reduction of oxygen and nitrogen radicals and the expression of proinflammatory cytokines, during chronic inflammation (26). Thus, lavender oil was confirmed to suppress inflammation and to restore antioxidative enzyme activity (27). Orange and lemon oils have central and peripheral antinociceptive effects in addition to their anti-inflammatory effect (28). In our study, aromatic oils of lavender, orange and lemon were applied during HAT, which, in addition to transcutaneous absorption, have significant effects through the nasal mucosa.

Another important neuromodulation tool, MM, was used in this study. Recent well-controlled studies have indicated that MM training could reduce inflammatory markers, such as CRP and IL-6 (29), cortisol, blood pressure, HR and tumour necrosis factor alpha (TNF  $\alpha$ ) (30). Despite the growing popularity of MM, the neurobiological mechanisms by which MM might affect stress are not well understood (31).

Previous studies have indicated that improving the coherence state may lead to improvements in patients suffering from CIRD (8). Thus, four weeks of treatment by HAT and MM, in addition

to the standard rehabilitation protocol, resulted in significantly improved CS and its levels. Our goal was to understand the possible neuromodulatory mechanisms of HAT and MM by analysing CS and its levels. HAT and MM, along with the standard rehabilitation protocol, led to a significant increase in the high CS level. At the same time, standard rehabilitation protocols alone, based on natural factors, significantly reduced the low CS level. According to the literature, the low CS level reflects hormonal (e.g., renin-angiotensin-aldosterone activity) and thermoregulatory effects on the heart. A medium CS level is modulated by the sympathetic (SY) and PSYs, and it is associated with baroreceptor activity. A high CS level is modulated by the PSYs, and it is related to respiration (32-34). This means that HAT and MM, with the standard protocol, enhances PSY activities, whereas the sole use of the standard protocol enhances SY activities, including HR. Furthermore, a statistically significant decrease in the CS levels in the experimental group indicates that HAT and MM have significant effects on PSY activity.

This pilot study has highlighted the importance of activating the innate healing mechanism underlying symptom suppression through the non-invasive neuromodulation of the ANS in order to improve the CS levels. Thus far, the importance of the innate healing mechanism has not been fully appreciated (22).

Despite recent advances in treatment optimization using biological and immunomodulatory therapies in rheumatologic patients, their use has been accompanied by significant side effects and an increase in the prevalence of comorbidities. The common protocols for physical treatment and rehabilitation are aimed at increasing mobility, reducing pain and, thus, improving quality of life. CIRD is characterized by disorders of the immune system, as well as oxidative and metabolic stress, which affect the overall CS levels.

The results of this study indicate that HAT and MM, along with the natural factors at the Institute Igalo, significantly improved ANS activity, especially PSY, in patients suffering from inflammatory rheumatoid diseases.

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EFEKTI HIDROAROMATERAPIJE I MEDITACIJE PUNE SVESNOSTI  
KOD BOLESNIKA SA UPALNIM REUMATOIDNIM BOLESTIMA**Vjeroslava Slavić<sup>1</sup>, Ivan Lakićević<sup>2</sup>, Jadranka Glišić<sup>1</sup>, Danijela Randelović<sup>1</sup>, Gordana Rajović<sup>1</sup><sup>1</sup>Institut "Dr Simo Milošević" Igalo, Igalo, Crna Gora<sup>2</sup>Black Dog Institut Randwick, Randwick, AustralijaKontakt: Vjeroslava Slavić  
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Nedavno su varijabilnost otkucaja srca (*HRV*) i koherentni rezultat (*CS*) ili koherentan obrazac srčanog ritma prihvaćene kao validirane metode za uspostavljanje aktivnosti autonomnog nervnog sistema (*ANS*).

Cilj ovog istraživanja je proceniti modifikacije *CS*-a u uzorku bolesnika koji pate od upalnih reumatoidnih bolesti, tokom njihovog fizikalnog lečenja i rehabilitacije, na osnovu prirodnih faktora, kao i hidroaromaterapije i meditacije.

Ovo istraživanje obuhvatilo je 44 norveška bolesnika sa potvrđenom dijagnozom inflamatornih reumatoidnih bolesti, tokom četiri nedelje fizikalnog lečenja i rehabilitacije u Institutu „Dr Simo Milošević“ Igalo, Crna Gora. Bolesnici su podeljeni u dve grupe: (1) eksperimentalna ( $n = 22$ ) i (2) kontrolna ( $n = 22$ ). Svi bolesnici dobili su standardni protokol zasnovan na prirodnom lekovitom blatu i mineralnoj vodi. Međutim, bolesnici u eksperimentalnoj grupi imali su dodatnu hidroaromaterapiju (mineralna voda obogaćena eteričnim uljima lavande, narandže i limuna) i meditaciju. Mašina emVave Pro merila je *CS*, pre i posle tretmana. Postojala su tri nivoa koherentnosti (*CL*): niski, srednji i visoki. Nizak nivo koherentnosti odražavao je količinu vremena u kojem nije bilo talasne aktivnosti, srednji nivo imao je neku talasnu aktivnost, a visoki nivo koherentnosti imao je značajnu količinu talasne aktivnosti u praćenju *HRV*-a.

Nakon tretmana, utvrđeno je da je *CS* značajno veći kod bolesnika iz eksperimentalne grupe nego kod bolesnika iz kontrolne grupe ( $p < 0,015$ ). Istovremeno, visoka *CL* bila je značajno veća kod bolesnika iz eksperimentalne grupe ( $p < 0,026$ ), a niska *CL* bila je značajno niža kod bolesnika iz kontrolne grupe ( $p < 0,017$ ). Poređenje dobijenih *CL* između eksperimentalne i kontrolne grupe nakon tretmana pokazalo je da je samo *CL* medijuma statistički različito ( $p < 0,009$ ).

Standardni protokol u Institutu Igalo uz dodatak hidroaromaterapije i meditacije značajno je poboljšao aktivnost *ANS* bolesnika koji pate od upalnih reumatoidnih bolesti.

*Acta Medica Medianae 2021;60(4):09-15.***Ključne reči:** ocena koherentnosti, nivoi koherentnosti, inflamatorne reumatoidne bolesti

## FEATURES OF ENDOCERVICAL ADENOCARCINOMA IN RELATION TO SQUAMOUS INTRAEPITHELIAL LESIONS AND SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

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Cervical adenocarcinoma poses a huge diagnostic and therapeutic problem due to its endocervical localization and specific etiopathogenesis. Aims of the paper were: to establish characteristics of cervical adenocarcinoma in comparison to squamous cell type, to assess the validity of current diagnostic methods in cervical adenocarcinoma diagnostics, and to determine the best combination of diagnostic procedures that reduce the percentage of false negative results and demonstrate high sensitivity in cervical adenocarcinoma diagnostics. This was a prospective study comprising 180 patients operated for high-grade cervical intraepithelial neoplasia II and III, adenocarcinoma in situ and cervical carcinoma at the Clinic of Gynecology and Obstetrics, University Clinical Center Niš.

The objective of our study was especially focused on patients with a histological diagnosis of adenocarcinoma. All the patients filled out a questionnaire prior to surgery and samples for human papilloma virus (HPV) testing were collected from all of them. HPV typing was performed at the Public Health Institute Niš, by using PCR method for detecting oncogenic HPV types and genotypes. HPV result was statistically significantly more commonly associated with adenocarcinoma. NILM finding was statistically significantly more commonly associated with AC pathohistological finding, while other cytological findings were statistically significantly more commonly associated with other PH results. Normal colposcopic finding was more commonly associated with AC pathohistological finding. Irregular bleeding finding in relation to defined groups of pathological finding is statistically significant and demonstrates medium strength of association. Irregular bleedings are statistically significantly more common in adenocarcinoma in comparison to severe forms of squamous changes.

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**Key words:** endocervical adenocarcinoma, diagnostics, HPV testing, colposcopic finding, irregular bleeding

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### Introduction

Cervical adenocarcinoma poses a huge diagnostic and therapeutic problem due to its endocervical localization and specific etiopathogenesis. The incidence rate of this histological type of cervical cancer is lower compared to that of a squamous type, accounting for 5 to 15%. Still, the latest epidemiological data reveal that the incidence rate of cervical adenocarcinoma has been increasing lately, especially in women under 40 years of age (1).

The incidence of squamous type of cervical carcinoma is significantly reduced due to exfoliative cytology and organized screening. On the other hand, since the sensitivity of cytology in detecting adenocarcinoma is low, the incidence rate of this histological type remains the same or is increasing.

The latest data of a Swedish trial in relation to the period of introducing cytology into cervical carcinoma screening programme reveal that the incidence rate of adenocarcinoma increased from 7% to

25.7%. The greatest increase of adenocarcinoma incidence, three-fold higher, was recorded in younger patients (2).

The entity of endocervical adenocarcinoma is a heterogeneous group comprised of different histological types.

The current World Health Organization (WHO) classification of endocervical carcinoma was first published in 2014 as a novel classification. Usual-type endocervical adenocarcinoma is the most common subtype that is mostly HPV positive, less aggressive and with better prognosis in comparison to other subtypes.

The second most common subtype is mucinous adenocarcinoma, including gastric, intestinal, and signet-ring cell type. They are less commonly HPV positive, more aggressive and with poor prognosis. Among them, gastric type distinguishes as the most aggressive and HPV negative (3).

Unlike HPV+ usual-type adenocarcinoma with disease-free survival (DFS) of 74%, in gastric type it is twice as low, with DFS of 38%. Also, in comparison to usual-type, gastric type has a propensity for rapid extracervical spread. So, at the time of established diagnosis in 60% of cases the disease stage is higher than stage II, unlike usual-type where the disease is detected in stage I in over 80% of cases (4).

Besides gastric type, clear cell and mesonephroid adenocarcinoma distinguish in the HPV negative group.

Out of other adenocarcinoma subtypes, the following ones are noteworthy: villoglandular carcinoma, endometrioid carcinoma, clear-cell carcinoma, serous carcinoma, mesonephroid carcinoma, and some other less common types, such as mixed neuroendocrines.

Villoglandular type has a better prognosis, it is HPV positive, as most of mucinous carcinomas are, it is more common in younger patients. Serous and clear cell types demonstrate HPV positivity in about 30%, whereas endometrial type is HPV positive in only 13% (5).

Aims of the paper were: to establish characteristics of cervical adenocarcinoma in comparison to squamous cell type, to assess the validity of current diagnostic methods (cytology, colposcopy, HPV typing) in cervical adenocarcinoma diagnostics, and to determine the best combination of diagnostic procedures that reduce the percentage of false negative results and demonstrate high sensitivity in cervical adenocarcinoma diagnostics.

### Patients and methods

This was a prospective study comprising 180 patients operated for high-grade cervical intraepithelial neoplasia II and III, adenocarcinoma in situ, and cervical carcinoma at the Clinic of Gynecology and Obstetrics, University Clinical Center Niš. The objective of our study was especially focused on patients with a histological diagnosis of adenocarcinoma. Our study enrolled 21 of them. The remaining

159 patients had a diagnosis of intraepithelial and invasive changes in the squamous epithelium.

All the patients filled out a questionnaire prior to surgery and samples for HPV testing were collected from all of them. Human papilloma virus typing was performed at the Public Health Institute in Niš, by using PCR method for detecting oncogenic HPV types and genotypes. All the results were statistically processed and presented in tables. A chi square test for independence ( $\chi^2$ ) was used for statistical analysis between categorical variables, and the Phi coefficient was used to measure the strength of association between variables. Statistically significant correlation was present in case of  $p < 0.05$ .

### Results

HPV- result was statistically significantly more commonly associated with AC (adenocarcinoma) pathohistological finding, for  $p < 0.05$ .

By comparing cytological and pathohistological results, it can be seen that NILM finding was statistically significantly more commonly associated with AC pathohistological finding, for  $p < 0.001$ , while other cytological findings were statistically significantly more commonly associated with other PH results for  $p < 0.05$ .

It is of statistical significance that normal colposcopic finding was more commonly associated with AC pathohistological finding for  $p < 0.05$ .

Irregular bleeding finding in relation to defined groups of pathological finding is statistically significant and demonstrates medium strength of association ( $c^2 = 14.114$ ;  $p < 0.001$ ;  $\phi = -0.336$ ). This analysis shows that irregular bleedings are statistically significantly more common in adenocarcinoma in comparison to more severe forms of squamous changes ( $p < 0.001$ ).

Anamnestic data on positive family history of malignancy was statistically significantly more commonly associated with AC pathohistological finding for  $p < 0.001$ .

### Discussion

From Table 1 it can be seen that the greatest percentage of adenocarcinomas is HPV positive (71.40% of them). On the other hand, if the percentage of HPV negative patients is analyzed, it can be observed that the percentage of these findings is higher in AC than in other squamous lesions (28.6:8%). This difference is statistically significant.

Literature data reveal similar results. Most cervical adenocarcinomas are HPV positive, primarily HPV 18 and 45, in about 77%, while HPV 16 positivity is about 15%. Unlike adenocarcinoma, squamous cell type is most commonly HPV 16, 31, 33 positive (6). The reason for such a distribution should also be searched in diversity of a phylogenetic tree of the virus itself and in the type and origin of epithelium, that is in local viral tropism. In comparison to squamous cell carcinomas, the percentage of HPV positive adenocarcinomas is a bit lower. Statistically, slightly over 10% of endocervical

adenocarcinomas is HPV negative (7). One of the reasons of such a distribution is in aforementioned heterogeneity of adenocarcinoma subtypes. Some of

these subtypes are HPV negative, with different etiopathogenesis and risk factors.

**Table 1.** HPV test results in adenocarcinoma and other squamous lesions

PH result	HPV positive-negative		Total	p
	HPV-	HPV+		
AC	6	15	21	0.004
	28.60%	71.40%	100.00%	
Others	12	138	150	
	8.00%	92.00%	100.00%	
Total	18	153	171	

Owing to understanding of these characteristics in relation to human papilloma virus infection, HPV test may serve as a good parameter in differential diagnosis of various cervical adenocarcinoma subtypes. Immunohistochemical markers, primarily p16 INK and CEA, as well as a range of a panel of other markers, predominantly Ki-67, may also be useful in achieving better subtypes differentiation. HPV positive adenocarcinoma types, such as usual-type, are thus p16 INK positive, and differentiation marker CEA negative, unlike HPV negative ones, including gastric type, that are p16INK negative and CEA markedly positive. In contrast to gastric type, clear cell carcinoma is CEA negative, and that can also be helpful in making a differential diagnosis (8). Immunohistochemical marker p53 can be useful in differential diagnosis as well, since the values of this marker are most commonly elevated in HPV negative adenocarcinomas, such as gastric and intestinal ones, unlike usual HPV positive type with this marker being positive in only 3% of cases (9).

Cervical adenocarcinoma is difficult to diagnose. Standard diagnostic procedures, cytology and colposcopy, demonstrate the greatest percentage of false-negative results in this histological type of cervical carcinoma. Precancerous changes on the glandular epithelium are defined as adenocarcinoma in situ AIS, or glandular intraepithelial neoplasia GIN.

Since the epithelium is single-layered, differentiation of the stage and severity is difficult both to pathologist and cytologist. In classifying the results, a cytologist cannot rely on differentiation principle and epithelium thickness involvement, but his/her cytologic impression is based on the principle of severity of atypical cells and three-dimensional cell arrangement into specific pseudoglandular forms, acinus, nuclear protrusion, feathering, and alike. Such diagnostic parameters increase subjective expression of a cytologist, thus significantly decreasing validity in adenocarcinoma detection. On the other hand, a problem in cytological diagnostics is also cell collecting and harvesting, considering their fragility and easy loss of cytoplasm. A special problem is inadequate and insufficient sampling as a consequence of a cotton swab sampling. All these problems have resulted in epidemiological data indicating that cytological screening introduction has not significantly reduced the incidence of cervical adenocarcinomas (10).

Table 2 shows data on cytological findings of patients operated for AC and squamous lesions. It can be seen from the Table, and it is also statistically confirmed, that a higher percentage of false-negative cytological results is noted in the group of patients with AC (85:13%).

**Table 2.** Cytological finding in adenocarcinoma (AC) and other squamous lesions

PH results	Cytological finding				PH result
	NILM	ASCUS, LSIL	HSIL, IC	Total	
AC	18	3	0	21	< 0.001
	85.70%	14.30%	0.00%	100.00%	
Others	21	69	69	159	
	13.20%	43.40%	43.40%	100.00%	
Total	39	72	69	180	

Cytology sensitivity in detecting AIS and invasive adenocarcinoma found in literature data also shows lower values in the range from 44 to 63% (11).

In that sense, a growing number of studies points out the necessity of introducing HPV typing into cervical carcinoma screening. Unlike cytology, HPV test statistically significantly increases adenocarcinoma detecting sensitivity. The latest studies show that HPV screening primarily increases adenocarcinoma detection for more than a two-fold in relation to cytology (12).

HPV test may not only have a diagnostic, but also a predictive, prognostic value. So, a positive HPV 18 and 45 test, when cytology finding shows no changes, may be the first indicator of developing adenocarcinoma in these patients in the next 10 years (13).

Fortunately, adeno-changes are mostly associated with squamous ones, in about 70% of cases, so they are detected incidentally while testing squamous epithelial lesions. Cytological finding in adeno-changes are suspicious of adenocarcinoma in only 25% of cases, while in 64% of cases a pathological cytological finding of adenocarcinoma done by a cytologist is described as a squamous lesion (14).

Colposcopic diagnostics is also a huge diagnostic problem in adenocarcinoma. Standard colposcopic images of AW epithelium, punctuation and mosaic are not necessarily present in adeno-changes. Colposcopic image may show only enlargement of the cervical volume, blood vessels atypia,

confluence and changes in papillae colour in the form of small islands on cylindrical epithelium. If the process is present in endocervical crypts, the crypt opening itself may be edematous in the form of a cuff around the gland opening, or as "skip" lesions with no colposcopic manifestations. The proximity of transformation (transitional) zone regarding clinical manifestations of adeno-changes severity is not important, since a lesion may be focal, far from the transformation zone, but it can still be an advanced form of adenocarcinoma. Each endocervical crypt opening here may present a separate transformation zone. Transformation zones type 3 are a special problem since this transitional zone cannot be seen.

All the above mentioned problems in colposcopic screening of adeno-changes are illustrated in Table 3. Unlike squamous lesions where colposcopy reveals about 44% findings with less severe histological stage, in the case of AC that percentage is 71%.

Anamnestic data on irregular vaginal bleeding may be a useful diagnostic parameter. Table 4 illustrates that more than 50% of patients with AC had irregular bleeding as a symptom, and that was the reason they reported to their gynecologists who established the diagnosis. Neoangiogenesis accompanies cervical carcinogenesis and is defined as the formation of new blood vessels that are fragile, without exactly the same components as normal blood vessels. This very fragility is one of the symptoms of irregular bleeding.

**Table 3.** Colposcopic finding in AC and other squamous lesions

PH result	Colposcopy			p
	Normal finding	Pathological finding	Total	
AC	15	6	21	0.03
	71.40%	28.60%	100.00%	
Others	74	85	159	
	46.54%	53.46%	100.00%	
Total	89	91	180	

**Table 4.** Anamnestic data on irregular bleeding in AC and squamous lesions

	Irregular bleeding		Total
	no	yes	
AIS AC	9	12	21
	42.90%	57.10%	100.00%
Others more severe	102	21	123
	82.90%	17.10%	100.00%
Total	111	33	144

Sampling and histological diagnostics of adeno-changes can also be inadequate and problematic. Endocervical curettage of adeno-changes shows low sensitivity and may produce false-negative results. The problem is that a curette cannot harvest endocervical crypts in the cervical stroma. For these very reasons, the only diagnostic and therapeutic method in cytological and colposcopic examinations that appear suspicious for cervical adeno-changes is Loop Electrosurgical Excision or traditional conization.

Apart from etiopathogenesis and diagnostics, adenocarcinomas also differ from squamous lesions

in risk factors and socio-epidemiological parameters. The number of sexual partners and smoking are significantly associated with squamous cell carcinoma, but not with adenocarcinoma. Adenocarcinoma risk factors are similar to endometrial carcinoma risk factors, such as the use of hormone therapy, obesity, and genetic factors. Table 5 shows that in case of AC, family history reveals genetic factor more commonly. A family history of gynecologic malignancy was recorded in 71.4% of patients with AC diagnosis.

**Table 5.** Anamnestic data on family history of malignancy in adenocarcinoma and other squamous lesions

PH finding	Family history of malignancy			p
	no	yes	Total	
AC	6	15	21	< 0.001
	28.60%	71.40%	100.00%	
Others	114	45	159	
	71.70%	28.30%	100.00%	
Total	120	60	180	

## Conclusion

According to all aforementioned facts, it can be said that endocervical adenocarcinomas present a heterogeneous group of tumors, having different etiopathogenesis, risk factors and clinical sympto-

matology from squamous cell carcinomas. Despite the development of science and technology, these tumours are still an enigma that requires individual approach, clinical experience and further investigations of novel diagnostic procedures, immunohistochemical and genetic markers.

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## SPECIFIČNOSTI ENDOCERVICALNOG ADENOKARCINOMA U ODNOSU NA SKVAMOZNE INTRAEPITELNE LEZIJE I SKVAMOZNI KARCINOM GRLIĆA MATERICE

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Adenokarcinom cerviksa (AC) predstavlja ogroman dijagnostički i terapijski problem zbog svoje endocervikalne lokalizacije i specifične etiopatogeneze. Ciljevi ovog rada bili su: utvrditi karakteristike cervikalnog adenokarcinoma u odnosu na tip skvamoznih ćelija, proceniti valjanost postojećih dijagnostičkih metoda u dijagnostici cervikalnog adenokarcinoma i utvrditi najbolju kombinaciju dijagnostičkih postupaka, koji smanjuju udeo lažno negativnih rezultata i pokazuju visoku osetljivost u dijagnostici adenokarcinoma cerviksa. Ovo je bilo prospektivno, istraživanje koje je obuhvatilo 180 bolesnica operisanih zbog cervikalne intraepitelne neoplazije II i III, adenokarcinoma *in situ* i karcinoma grlića maternice, na Klinici za ginekologiju i akušerstvo Univerzitetskog kliničkog centra Niš. Cilj istraživanja bio je posebno usmeren na bolesnice sa histološkom dijagnozom adenokarcinoma. Sve bolesnice popunjavale su upitnik pre operacije i od svih su prikupljeni uzorci za testiranje na humani papiloma virus (HPV). Tipizacija HPV-a izvedena je u Institutu za javno zdravlje Niš, PCR metodom za otkrivanje onkogenog tipa i genotipa HPV-a. Rezultat HPV-a statistički je značajno češće povezan sa adenokarcinomom. NILM nalaz statistički je značajno češće povezan s patohistološkim nalazom adenokarcinoma, dok su ostali citološki nalazi statistički značajno češće povezani s ostalim rezultatima PH. Normalni kolposkopski nalaz češće je povezan s patohistološkim nalazom AC. Iregularna krvarenja u odnosu na definisane grupe patoloških nalaza statistički su značajna i pokazuju srednju jačinu značajnosti. Iregularna krvarenja statistički su značajno češća u adenokarcinomu u poređenju sa teškim oblicima skvamoznih promena.

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**Ključne reči:** endocervikalni adenokarcinom, dijagnostika, HPV testiranje, kolposkopski nalaz, iregularno krvarenje



## POSSIBILITY OF USING BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN EVALUATING ADNEXAL MASSES

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Current diagnostic approach to adnexal masses (medical history, clinical examination, transvaginal sonography, tumour markers) does not provide an accurate prediction for potential malignancy. There is a possibility of using hematological and biochemical parameters (platelets count, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, platelet distribution width, level of C-reactive protein) in predicting ovarian malignancy. A retrospective study was conducted. Analysis of aforementioned parameters was performed in patients with histopathologically verified benign/malignant ovarian tumours. CRP levels, total count of granulocytes, and total count of platelets were statistically significantly higher in patients with malignant changes ( $p < 0.001$ ,  $p = 0.001$ , or  $p = 0.023$ ). Total lymphocytes count was statistically significantly lower in patients with malignant changes ( $p < 0.001$ ). Platelet count was statistically significantly higher in patients with stage III ( $p = 0.011$ ). Pl/Ly ratio was statistically significantly higher in patients with stage III ( $p = 0.043$ ). CRP was statistically significantly higher in stage III ( $p < 0.001$ ). Lymphocyte count was statistically significantly lower in stage III ( $p < 0.001$ ), and granulocyte count was statistically significantly higher in stage III ( $p = 0.001$ ). Platelet count was statistically significantly higher in stage III ( $p = 0.001$ ). MPV was statistically significantly lower in stage III ( $p = 0.031$ ). Pl/Ly ratio was statistically significantly higher in patients with stage III ( $p = 0.044$ ). Analyzed biochemical and hematological parameters are of limited utility in differentiating benign from malignant ovarian masses. Elevated levels of C-reactive protein, neutrophils and platelets suggest potentially malignant ovarian masses. Analyzed biochemical parameter (high levels of C-reactive protein, reduced lymphocyte count, increased granulocyte count, increased platelet count, increased PLR, as well as lower MPV values) may suggest advanced malignancy.

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**Key words:** ovarian tumours, biochemical, hematological parameter

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### Introduction

Adnexal masses represent different changes of gynaecological (functional cysts, inflammatory changes, benign and malignant neoplasma) and nongynaecological origin. It is not diagnosed easily and diagnostic errors and problems are possible (1-4). Evaluation of adnexal masses involves an accurate anamnesis, careful clinical examination, transvaginal sonography, and determination of tumour markers. Ultrasound diagnostics is non-invasive, cheap

and available for adnexal masses evaluation. Transvaginal sonography is the preferred technique (no need for special equipment, area of interest is close to the probe). Ultrasound diagnostics identifies adnexal masses. Masses that are bilateral, large, complex, irregular, and accompanied by ascites are considered suspicious (5). By using ultrasound as a diagnostic tool, patients can be grouped into those in whom surveillance using serial ultrasonography can be applied, and those who require surgery (6). The Gynecologic Imaging Reporting and Data System (GI-RADS) is a reporting system that was created for reporting the findings in adnexal masses based on transvaginal ultrasonography (7). Still, there is a possibility that GI-RADS classification gives a great number of false-positive results, so it is recommended to additionally use tumour markers. Additional measurements to GI-RADS system do not alter clinical approach (6-9).

The success of surgical treatment depends on appropriate preoperative plan. There is a need to define steps in treating indeterminate adnexal changes (10). Monitoring the morphology of tu-

mours, the presence of ascites, and Doppler parameters facilitate approaches in making preoperative plans and the choice of medical center for patients to be treated. Patients with diagnosed adnexal masses indicative of malignancy should be referred to a gynecological oncologist for optimal patient treatment (11). Surgeries performed by a well-trained surgeon, in a well-equipped center, provide optimal surgical treatment (12-15).

Current diagnostic approach to adnexal masses (history taking, clinical examination, transvaginal sonography, tumour markers) does not provide accurate prediction of potential malignancy, thus potentially affecting the choice and time of treatment, the choice of medical center, specialist doctor and surgical approach (laparotomy/laparoscopy). There is a need to define additional parameters for a more accurate preoperative prediction of potential malignancy. There is a possibility to utilize hematological parameters (platelets count, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, platelet distribution width) that have been used in routine preoperative diagnostics and preoperative preparation. Preoperative determination of these parameters is cheap, available, repeatable, and potentially useful in evaluating adnexal masses (16-20).

### Methods of the study

The study is a retrospective one. Patients operated at our Clinic for diagnosed adnexal masses were analyzed. The study enrolled 100 surgically treated patients. They were all operated for ovarian tumours, and all of them were operated at our Clinic according to current oncologic principles (full staging). Standard hematological and biochemical parameters were controlled preoperatively. After the surgery, according to histopathological reports on removed adnexal masses, we formed a group of patients with benign ovarian tumours and a group of patients with malignant ovarian tumours. Patients with detected inflammatory changes (tubo-ovarian abscesses), metastatic ovarian changes, as well as patients with incomplete staging were excluded from the study.

Patients' age, histopathological type of tumour, and stage of the disease were analyzed in patients with malignant ovarian disease. Preoperative hematological parameters, total count of erythrocytes/ml, total count of leukocytes/ml, total count of neutrophils/ml, lymphocyte count/ml, monocyte count/ml, platelet count/ml, neutrophils/platelets count ratio, neutrophils/lymphocyte count ratio, platelet distribution width, platelet volume, and the level of C-reactive protein were analyzed in both groups.

Biochemical and hematological parameters were compared in patients with adnexal masses: a group with histopathologically verified benign ovarian tumours, and a group with verified malignant ovarian neoplasm.

The analyzed parameters were compared in relation to histopathological type of malignant ova-

rian tumour. We also observed the possibility of statistical difference in monitored biochemical parameters between the groups with early and advanced ovarian cancer, as well as between benign ovarian tumours and values of monitored parameters in patients with advanced ovary cancer (stage III).

### Statistical data processing

The data are presented in the form of arithmetic mean  $\pm$  standard deviation. If data distribution was normal, t-test and ANOVA were used. If not, Mann-Whitney test and Kruskal-Wallis test were used. Hypothesis was tested with significance threshold of  $p < 0.05$ : Statistical data analysis was performed by software package SPSS 16.0.

### Results

The study enrolled 100 patients, mean age  $51.40 \pm 15.92$  years (Min 19, Max 83 years). Patients with malignant adnexal changes were statistically significantly older in comparison to patients with benign changes ( $p = 0.037$ ). CRP values, total count of granulocytes and total count of platelets were statistically significantly higher in patients with malignant changes ( $p < 0.001$ ,  $p = 0.001$ , or  $p = 0.023$ ). Total count of lymphocytes was statistically significantly lower in patients with malignant changes ( $p < 0.001$ ). Other analyzed parameters showed no statistically significant difference in total count of erythrocytes and monocytes. Also, there was no statistically significant difference in the parameters related to platelet characteristics (MPV, PCT, PDW), neither regarding neutrophil/lymphocyte count ratio nor platelet/lymphocyte count (Table 1).

In the group with malignant tumours, 25 patients (50.0%) had serous carcinoma, 14 patients (28.0%) mucinous carcinoma, and 11 patients (22.0%) had other types of carcinoma. It has been noted that there was no statistically significant difference in relation to histopathological type of cancer (Table 2).

There were 20 patients (40.0%) in the group with malignant disease stage I, 7 patients (14.0%) in the stage II group and 23 patients (46.0%) in the stage III group. Platelet count was statistically significantly higher in patients with stage III ( $p = 0.011$ ). Pl/LY ratio was statistically significantly higher in patients with stage III ( $p = 0.043$ ) (Table 3).

Patients with stage III disease were statistically significantly older ( $p = 0.003$ ). CRP was statistically significantly higher in stage III ( $p < 0.001$ ). Lymphocyte count was statistically significantly lower in stage III ( $p < 0.001$ ), and granulocyte count was statistically significantly higher in stage III ( $p = 0.001$ ). Platelet count was statistically significantly higher in stage III ( $p = 0.001$ ). MPV was statistically significantly lower in stage III ( $p = 0.031$ ). Pl/Ly ratio was statistically significantly higher in patients with stage III disease ( $p = 0.044$ ) (Table 4).

**Table 1.** Biochemical and hemoatological parameters in analyzed population in comparison to the type of change (benign/malignant)

	Total	Malignant	Benign	p
Age	51.40 ± 15.92	54.44 ± 15.47	48.36 ± 15,94	0.037 <sup>1</sup>
CRP	17.84 ± 35.05	30.46 ± 46.09	5.22 ± 5.78	< 0.001 <sup>2</sup>
ER	4.37 ± 0.50	4.35 ± 0.57	4.38 ± 0.41	0.737 <sup>1</sup>
LE	8.74 ± 3.49	8.46 ± 3.23	9.01 ± 3.75	0.699 <sup>2</sup>
LY	4.42 ± 3.16	3.07 ± 2.43	5.77 ± 3.26	< 0.001 <sup>2</sup>
GR	3.75 ± 3.04	4.65 ± 3.26	2.84 ± 2.50	0.001 <sup>2</sup>
MO	3.51 ± 29.06	6.51 ± 41.07	0.50 ± 0.30	0.252 <sup>2</sup>
TR	287.29 ± 112.30	316.26 ± 138.75	258.32 ± 67.21	0.023 <sup>2</sup>
MPV	10.20 ± 12.11	9.78 ± 11.56	10.62 ± 12.74	0.062 <sup>2</sup>
PCT	0.26 ± 1.18	0.38 ± 1.67	0.15 ± 0.14	0.887 <sup>2</sup>
PDW	16.33 ± 5.17	16.37 ± 5.87	16.30 ± 4.46	0.468 <sup>2</sup>
Ne/Ly	3.54 ± 3.12	4.11 ± 4.05	2.96 ± 1.59	0.339 <sup>2</sup>
Tr/Ly	155.05 ± 125.69	178.30 ± 157.95	131.80 ± 76.58	0.394 <sup>2</sup>

1- t test, 2- Mann-Whitney test

**Table 2.** Biochemical parameters in relation to histopathological type of malignant tumours

	Serous	Mucinous	Other	p <sup>1</sup>
Age	58.36 ± 14.61	52.57 ± 14.72	47.91 ± 16.99	0.138
CRP	30.32 ± 40.34	41.81 ± 65.10	16.33 ± 24.33	0.449 <sup>2</sup>
ER	4.33 ± 0.60	4.27 ± 0.53	4.50 ± 0.57	0.588
LE	8.44 ± 3.35	9.18 ± 2.43	7.61 ± 3.89	0.238 <sup>2</sup>
LY	2.89 ± 2.25	3.31 ± 2.22	3.17 ± 3.20	0.802 <sup>2</sup>
GR	4.98 ± 3.17	5.03 ± 2.96	3.43 ± 3.86	0.081 <sup>2</sup>
MO	0.55 ± 0.29	1.19 ± 2.43	26.84 ± 87.61	0.551 <sup>2</sup>
TR	357.36 ± 163.05	289.42 ± 84.72	256.99 ± 109.98	0.235 <sup>2</sup>
MPV	8.30 ± 1.29	7.99 ± 1.34	15.40 ± 24.58	0.186 <sup>2</sup>
PCT	0.16 ± 0.17	0.14 ± 0.22	1.20 ± 3.55	0.715 <sup>2</sup>
PDW	16.15 ± 4.53	14.23 ± 6.31	19.89 ± 7.00	0.215 <sup>2</sup>
Ne/Ly	3.81 ± 3.98	4.06 ± 2.52	4.88 ± 5.76	0.697 <sup>2</sup>
Pl/Ly	221.08 ± 193.50	131.57 ± 114.34	140.54 ± 78.70	0.189 <sup>2</sup>

1- ANOVA, 2- Kruskal-Wallis test

**Table 3.** Biochemical parameters in relation to the stage of the disease

	Stage I+II	Stage III	p <sup>1</sup>
Age	5048 ± 16.60	59.09 ± 12.87	0.071
CRP	28.08 ± 50.91	33.25 ± 40.66	0.102
ER	4.34 ± 0.52	4.37 ± 0.64	0.953
LE	7.77 ± 2.92	9.28 ± 3.45	0.104
LY	2.93 ± 2.24	3.24 ± 2.69	0.97
GR	4.13 ± 3.11	5.27 ± 3.42	0.185
MO	11.54 ± 55.88	0.62 ± 0.31	0.066
TR	266.59 ± 97.50	374.56 ± 158.30	0.011
MPV	11.12 ± 15.70	8.20 ± 1.40	0.325
PCT	0.56 ± 2.27	0.18 ± 0.17	0.115
PDW	16.20 ± 7.10	16.57 ± 4.12	0.942
Ne/Ly	4.23 ± 4.06	3.97 ± 4.13	0.899
Pl/Ly	141.59 ± 129.62	221.39 ± 179.22	0.043

1-Mann-Whitney test

**Table 4.** Comparison of biochemical parameters between patients with ovarian carcinoma stage III and patients with benign ovarian tumours

	Ovarian carcinoma stage III	Benign tumours	P
Age	59.09 ± 12.87	48.36 ± 15.94	0.003 <sup>1</sup>
CRP	33.25 ± 40.66	5.22 ± 5.78	< 0.001 <sup>2</sup>
ER	4.37 ± 0.64	4.38 ± 0.41	0.906 <sup>1</sup>
LE	9.28 ± 3.45	9.01 ± 3.75	0.510 <sup>2</sup>
LY	3.24 ± 2.69	5.77 ± 3.26	< 0.001 <sup>2</sup>
GR	5.26 ± 3.42	2.84 ± 2.50	0.001 <sup>2</sup>
MO	0.62 ± 0.30	0.50 ± 0.30	0.064 <sup>2</sup>
TR	374.56 ± 158.30	258.32 ± 67.21	0.001 <sup>2</sup>
MPV	8.20 ± 1.40	10.62 ± 12.74	0.031 <sup>2</sup>
PCT	0.18 ± 0.17	0.15 ± 0.14	0.242 <sup>2</sup>
PDW	16.57 ± 4.12	16.30 ± 4.46	0.582 <sup>2</sup>
Ne/Ly	3.97 ± 4.13	2.96 ± 1.59	0.288 <sup>2</sup>
Tr/ly	221.39 ± 179.22	131.80 ± 76.58	0.044 <sup>2</sup>

1- t test, 2- Mann-Whitney test

## Discussion

Inflammation and inflammatory response play a pivotal role in the development and progression of malignant diseases. Biochemical and hematological inflammatory markers may be incorporated into prognostic score for various carcinoma types. Patients with malignant ovarian tumours had statistically significantly higher values of C-reactive protein and higher levels of total granulocytes, as observed in our study. High levels of C-reactive protein and leukocytosis are markers of systemic inflammatory response. Many studies suggest that high neutrophil-to-leukocyte ratio (N/LR) is associated with poor survival rate in patients with malignant diseases (20). It seems that an increase of NLR is greater in patients with metastatic disease and longer inflammatory response (21). We have not observed statistically significant difference in NLR between the group with malignant and the group with benign ovarian changes. We also have not registered statistically significant difference in this parameter by comparing groups of patients with an early and advanced disease stage III (21). Our study showed that patients with advanced disease had significantly higher count of granulocytes in comparison to those with benign ovarian changes. Neutrophils have a role in immune response, they inhibit the immune system by suppressing cytolytic activity of lymphocytes (22). The presence of tumour-infiltrating lymphocytes is suggestive of better response to treatment (23). Patients in our study have statistically significantly lower total lymphocyte count. A change in neutrophil to lymphocyte ratio may be more useful in tailoring therapy for patients with advanced malignant disease, rather than being an early disease marker (21). There is evidence that neutrophil-to-leukocyte ratio is an available marker of systemic inflammation, but, it has also been reported that this ratio may be useful in predicting operable

disease and in forming pretreatment and pre-operative strategy (24).

Research has shown that inflammation plays a key role in cancer pathogenesis. It affects tumour microenvironment, cell proliferation signaling, factors that impact programmed cell death, proangiogenesis, capability for invasion and metastasis (25). Inflammation may be evident in some cases at early stages of neoplastic progression. It is believed that inflammatory cells can release agents that may accelerate genetic changes (25).

Besides aforementioned parameters, a complete blood count also includes platelet count and platelet indices. We followed platelet count (Tr, Plt), mean platelet volume (MPV), plateletcrit (Pct) and platelet distribution width (PDW). PDW - Platelet distribution width represents a variation of platelet size distribution index (distribution curves for platelet distribution width). This platelet index is calculated from the distribution curve of the platelet volume and represents the width of the curve. Platelet distribution width (PDW) is a regular parameter in blood routine examination which reflects variation of platelet size distribution with a range from 8.3% to 56.6% (26). Morphological changes of platelets are always present when they are activated during inflammatory response. Plateletcrit (Pct) is a platelet index representing platelet volume as a percentage, or the relation between a total volume of full blood sample and the total number of platelets. Diagnostic value of this index remains to be seen (26).

Our study showed that patients with malignant ovarian tumours had statistically significantly higher platelet levels, but there was no statistically significant difference in PLR in comparison to the patients with benign changes. Some authors report that, despite limited sensitivity and specificity, PLR and NLR may be used as predictive factors for survival (19, 27). We have not found a statistically significant difference in platelet count and platelet parameters in benign/malignant changes (20).

Expressed inflammation, thrombocytosis, and platelet hyperreactivity are often associated with malignancies. In our patients with advanced stages, thrombocytosis, higher PLR and low MPV were registered. Some of these parameters are associated with advanced stages of the disease, inoperability and poor outcome, so these parameters are more useful in predicting advanced stage disease than as early disease markers (18).

### **Conclusion**

The analyzed biochemical parameters are of

limited value in differentiating benign from malignant ovarian masses. Elevated levels of C-reactive protein, neutrophils and platelets are indicative of potentially malignant ovarian mass.

The analyzed biochemical parameters (high levels of C-reactive protein, low lymphocyte count, higher granulocyte count, higher platelet count, elevated PLR and lower levels of MPV) may be suggestive of advanced malignant disease, thus potentially changing therapeutic approach.

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## MOGUĆNOST KORIŠĆENJA BIOHEMIJSKIH I HEMATOLOŠKIH PARAMETARA U PROCENI ADNEKSALNIH MASA

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Sadašnji dijagnostički pristup adneksalnim masama (anamneza, klinički pregled, transvaginalna sonografija, tumorski markeri) ne omogućava preciznu predikciju mogućeg maligniteta. Postoji mogućnost korišćenja hematoloških i biohemijskih parametara (broj trombocita, odnos neutrofila i limfocita, odnos trombocita i neutrofila, trombocitna širina, nivo C reaktivnog proteina) u predikciji ovarijalnog maligniteta.

Sprovedeno je retrospektivno istraživanje. Analiza pomenutih parametara kod bolesnica sa histopatološki verifikovanim benignim/malignim ovarijalnim tumorima.

Vrednosti CRP-a, ukupan broj granulocita i ukupan broj trombocita statistički su značajno veće kod bolesnica sa malignim promenama ( $p < 0,001$ ,  $p = 0,001$ ; odnosno  $p = 0,023$ ). Ukupan broj limfocita statistički je značajno manji kod bolesnica sa malignim promenama ( $p < 0,001$ ). Broj trombocita statistički je značajno veći kod bolesnica sa stadijumom III tumora ( $p = 0,011$ ). Odnos Tr/LY statistički je značajno veći kod bolesnica sa stadijumom III tumora ( $p = 0,043$ ). CRP je statistički značajno viši kod bolesnica III stadijuma tumora ( $p < 0,001$ ). Broj limfocita statistički je značajno niži kod bolesnica III stadijuma tumora ( $p < 0,001$ ), a broj granulocita je statistički značajno veći kod bolesnica sa III stadijumom tumora ( $p = 0,001$ ). Broj trombocita statistički je značajno veći kod bolesnica sa III stadijumom tumora ( $p = 0,001$ ). MPV je statistički značajno manji kod bolesnica sa III stadijumom tumora ( $p = 0,031$ ). Tr/Ly odnos statistički je značajno veći kod bolesnica na III stadijumom tumora ( $p = 0,044$ ).

Ispitivani biohemijski i hematološki parametri su od ograničene vrednosti u diferencijaciji benignih od malignih ovarijalnih masa. Povišeni nivoi C reaktivnog proteina, neutrofila i trombocita ukazuju na, verovatno, malignu ovarijalnu masu. Ispitivani biohemijski parametri (visoki nivoi C reaktivnog proteina, nizak broj limfocita, viši broj granulocita, viši nivoi trombocita, povišeni odnos PLR-a, kao i niži nivoi MPV-a) mogu ukazati na uznapređovalu malignu bolest.

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**Ključne reči:** karcinom jajnika, biohemijski, hematoloski parametri

## ASSESSMENT OF THE SOCIAL BURDEN ON PATIENTS WITH PRIMARY OPEN-ANGLE GLAUCOMA

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Glaucoma is a chronic ophthalmic disease characterized by progressive, irreversible loss of visual acuity, long-term progression and lifelong treatment, declining work ability and self-sufficiency, which can generate social burden in patients. The study is designed to understand the social, clinical and pharmacological aspects of assessing the social burden of primary open-angle glaucoma. It is a cross-sectional study with a quantitative analytical approach, which includes 182 male and female patients with primary open-angle glaucoma, aged 20-67, with preserved visual acuity > 6/18 (0.33), according to the ICD-10 classification, conducted in the period August-November 2020 on the territory of North Macedonia. Fifty-three point three percent of the respondents were female and 46.7% male, of whom 79.12% were treated with prescribed medications, 8.8% with laser and 12.08% underwent a surgical procedure. Fifty-seven point sixty-nine percent of the respondents received treatment regularly, which in 43.96% had a negative outcome, partial success in 30.22%, and in 25.82%, the treatment prevented further vision loss. Fourteen point twenty-eight percent of the respondents experienced a social family burden and 34.07% discomfort, depression, anxiety, hopelessness and other psychosocial disorders. Primary open-angle glaucoma generates a significant socio-economic burden as a result of irreversible visual impairment, reduced work ability and productivity, and high treatment costs of patients. The degree of the social burden depends on the involvement and clinical stage of the disease, the percentage of preserved vision, availability, manner and regularity of treatment and socio-demographic parameters such as gender, age, occupation, genetic predisposition, comorbid conditions, family history, etc. which in glaucoma play the role of predisposing risk factors.

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**Key words:** *primary open-angle glaucoma, social burden, socio-demographic parameters, treatment*

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### Introduction

The eye disorder caused by glaucoma is one of the most common pathological conditions in ophthalmology. It affects all ages, both genders, all occupations, people of different social categories and existential status and depending on the clinical

stage, the severity of the clinical picture and the degree of the preserved acuity, it generates more or less pronounced individual and family social burden to the diseased people (1-3).

Glaucoma is an optic neuropathy which is characterized by damage to the papilla of the optic nerve, specific defects in the visual field and progressive irreversible loss of visual function (4, 5).

Glaucoma has a prevalence of 1-2% in the world's population and it is the second most common cause of irreversible vision loss after cataracts, accounting for 13% of all cases of global blindness (6-8). Primary open-angle glaucoma accounts for 75% of all glaucoma cases, 55% of which are female (9).

Clinically, they start with non-specific symptoms, which is why they are usually diagnosed in the later clinical stages, after 40 years of age. Due to the long asymptomatic period, in addition to the diagnosed ones, there are more than 60% of still undiagnosed cases of the disease (10, 11).

Primary open-angle glaucoma generates individual and family social burden on patients and has



a huge socio-economic impact on the population and society (12-14).

The decreased visual acuity impedes mobility and leads to difficulties in movement, spatial orientation, recognition of objects and people in the surroundings, performing daily obligations and taking care of oneself without any assistance of others (15, 16).

The opportunities for education, training, employment, socialization, interaction are limited, and as a consequence they live stigmatized on the margins of society, often experiencing hopelessness and loss of independence (17).

The long-term, usually lifelong, daily use of antiglaucoma therapy causes discomfort, adverse local reactions accompanied by pain and recurrent infections in patients (18-20). Topical administration of beta-blockers containing preservatives may cause erosions and inflammation of the cornea, secondary cataracts and systemic adverse reactions in the form of bronchial asthma (21, 22).

Progressive loss of visual acuity, uncertainty of the outcome and the possibility of blindness can cause discomfort, anxiety, depression and other mental disorders in patients (23, 24).

The Health-Related Quality of Life (HRQL) as one of the main determinants (predictors) which define the subjective perception of well-being, social status, and thus the social burden which is reflected on the diseased, according to Guantiamo and Floriani, are inversely related to the degree of lost visual acuity (25, 26).

### Purpose

The study is designed to determine the social, clinical and pharmacological aspects when assessing the social burden on patients with primary open-angle glaucoma.

### Materials and methods

It is a cross sectional study with a quantitative analytical approach conducted in the period between August and November of 2020 on the territory of North Macedonia, which included 182

male and female employed respondents with primary open-angle glaucoma, aged 20-67.

The research was carried out using clinical history and a custom designed survey, whereas the patients' diagnoses were confirmed by a clinical ophthalmological examination and the accompanying medical history. The clinical research and surveys were performed within the periodic health examinations, while the degree of impaired vision was determined according to the ICD 10 classification, which ranged from 0.7 to 0.3 of affected people. All the respondents were diagnosed with primary open-angle glaucoma at different clinical stages with progression over a period from 2 to 20 years.

The socio-demographic parameters of the respondents included in the study were assessed according to gender, age, education, occupation, family burden, individual consequential predictors (discomfort, depression, anxiety), whereas the clinical-pharmacological assessment included method of treatment, duration of treatment, regularity in taking prescribed medication and outcome of treatment.

The statistical data processing was performed using descriptive and comparative statistical procedures, utilising statistical programmes such as statistics for Windows 7.0 and SPSS 17.0.

Pearson Chi-square homogeneity test was used to determine the differences in the attributive values of dichotomous features (variables) between male and female respondents, while a non-parametric Mann-Whitney Test was used to determine the significance of the differences in the independent features (variables). To determine the statistical significance, a significance level is determined,  $p < 0.05$ . The obtained results are presented numerically and in tables.

### Results

The scientific study included 182 people aged 20-67, patients with primary open-angle glaucoma, of whom 85 (46.70%) were male and 97 (53.30%) were female and were organised into 3 age groups, 20-50, 51-60 and 61-67 (Table 1).

**Table 1.** Structure of the respondents by age and gender

Age		Gender		Total
		Male	Female	
Age 20-50	Number	15	20	35
	%	8.24	10.99	19.23
Age 51-60	Number	38	40	78
	%	20.88	21.98	42.86
Age 61-67	Number	32	37	69
	%	17.58	20.33	37.91
Total	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 4.02721, df = 2, p = 0.083576

Eighty-five (46.70%) of the respondents included in the research were male and 97 (53.30%) were female. Thirty-five (19.23%) of whom were up to 50 years of age, 78 (42.86%) were aged 51-60, and 69 (37.91%) between 61 and 67, i.e. most of the respondents, male or female, were aged 51-60. The statistical analysis showed that there was no significant age difference between the two genders (Pearson Chi-square = 4.02721,  $df = 2$ ,  $p = 0.083576$ ). The youngest respondent in the research was 44, and the oldest 67.

A descriptive analysis was also made on the individual age of all respondents involved in the study (Table 2).

The average age of the male respondents included in the study was 57.85, SD 6.343, median 59, minimum age 44 and maximum age 67, and the average age of the female respondents was 57.40, SD 6.858, median 59, minimum age 44 and maximum age 67.

Statistical analysis showed that no significant difference was observed between the two genders in terms of age (Mann-Whitney U Test  $Z = 0.373668$ ,  $p = 0.804904$ ).

The respondents involved in the scientific study had different education and occupation. The employees with primary education were of different occupations, and most of them were construction and agricultural workers, workers in the cardboard industry, telephone operators, porters, craftsmen, etc., while in the rest of the patients, those with secondary and higher education, different occupations predominated (Table 3).

Sixty-two (34.07%) of the respondents included in the study had a career with primary education, 77 (42.31%) with secondary education, and 43 (23.62%) with higher education. Statistical analysis showed that there is no significant difference in terms of these parameters with male and female respondents (Pearson Chi-square = 2.96,  $df = 2$ ,  $p = 0.1138$ ).

**Table 2.** Descriptive analysis of the age of the respondents

Gender	Number of people	Average value (Means)	Standard deviation (Std.Dev.)	Standard Error (Std.Err.)	(Median)	(Min)	(Max)
Male	85	57.85	6.343	1.255504	59	44	67
Female	97	57.40	6.858	1.418532	59	44	67
<b>Total</b>	182	57.63	6.601	0.944411	59	44	67

Mann-Whitney U Test  $Z = 0.373668$ ,  $p = 0.804904$

**Table 3.** Structure of the respondents by occupation/education

Occupation/education		Gender		Total
		Male	Female	
Occupation with primary education	Number	29	33	62
	%	15.93	18.13	34.07
Occupation with secondary education	Number	36	41	77
	%	19.78	22.53	42.31
Occupation with higher education	Number	20	23	43
	%	10.99	12.64	23.62
<b>Total</b>	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 2.96,  $df = 2$ ,  $p = 0.1138$

From the socio-demographic characteristics, the parameter place of residence, city/village (urban/rural environment) was also analysed among the surveyed respondents (Table 4).

Out of the total of 182 people, 139 (76.37%) lived in urban areas and 43 (23.63%) in rural areas. 65 (35.71%) of the male respondents lived in the city and 20 (10.99%) in the countryside, and the largest number 74 (40.66%) of the female respondents also lived in the city, 23 (12.64%) in the

countryside. There is no statistically significant difference in terms of place of residence between male and female respondents (Pearson Chi-square = 2.713,  $df = 1$ ,  $p = 0.132$ ).

All patients included in the study were treated with pharmacotherapy, while in the cases of unsuccessful treatment and progression of visual impairment some of them received laser therapy or surgery (Table 5).

**Table 4.** Place of residence of respondents

Place of residence		Gender		Total
		Male	Female	
City	Number	65	74	139
	%	35.71	40.66	76.37
Village	Number	20	23	43
	%	10.99	12.64	23.63
Total	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 2.713, df = 1, p = 0.132 \* significance for p < 0.05

**Table 5.** Treatment method of respondents

Treatment method		Gender		Total
		Male	Female	
Medication	Number	68	76	144
	%	37.36	41.76	79.12
Laser Therapy	Number	7	9	16
	%	3.85	4.95	8.80
Surgical Therapy	Number	10	12	22
	%	5.49	6.59	12.08
Total	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 8.35064, df = 1, p = 0.012465

One hundred forty-four (79.12%) male and female respondents were treated with medication, 22 (12.08%) with surgery, and 16 (8.80%) with laser therapy. The analysis of the results indicates a statistical significance in favour of medication, as the most common type of treatment for the subjects, in comparison with the other types of therapy (Pearson Chi-square = 8.5064, df = 1, p = 0.012465).

Depending on the clinical stage in which they were diagnosed with glaucoma, the subjects used anti-glaucomatous treatment for a period of 1-20

years and the analysis of this parameter is presented in Table 6.

Fourteen (7.69%) of the male respondents received treatment for 1-5 years, 32 (17.58%) for 6-10 years, 29 (15.94%) for 11-15 years and 10 (5.49%) for a period of > 15 years, while 21 (11.54%) of the female respondents received treatment for a period of 1-5 years, 34 (18.68%) for a period of 6-10 years, 31 (17.03%) for a period of 11-15 years and 11 (6.05%) for a period of > 15 years.

**Table 6.** Descriptive analysis of the duration of antiglaucoma treatment

Duration of treatment		Gender		Total
		Male	Female	
1-5 years	Number	14	21	35
	%	7.69	11.54	19.23
6-10 years	Number	32	34	66
	%	17.58	18.68	36.26
11-15 years	Number	29	31	60
	%	15.94	17.03	32.97
> 15 years	Number	10	11	21
	%	5.49	6.05	11.54
Total	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 4.37340, df = 2, p = 0.223871

No statistically significant difference was observed between male and female respondents in relation to the duration of prescribed therapy (Pearson Chi-square = 4.37340, df = 2, p = 0.223871).

The outcome from the prescribed glaucoma treatment depend on the type of treatment, the manner of administration and adherence to the directions for its use, and the outcome of these examinations is presented in Table 7.

Most of the patients, i.e. 105 of them (57.69%) adhered to the directions for administration of the treatment, 43 (23.63%) adhered partially and 34 (18.68%) did not adhere. There is no statistically significant difference in this parameter between male and female patients (Pearson Chi-square = 2.047, df = 3, p = 0.563).

Primary open-angle glaucoma is a chronic ophthalmic disease which requires long-term or lifelong treatment with an uncertain prognosis. The analysis of the outcome of the treatment prescribed to the respondents is shown in Table 8.

Thirty-nine (21.43%) of the male respondents had negative outcome of the treatment, 25 (13.73%) had partial and 21 (11.54%) had positive outcome, whereas 41 (22.53%) of the female respondents had negative, 30 (16.48%) partial and 26

(14.29%) positive outcome of the treatment. Regarding this parameter, there is no statistically significant difference between male and female respondents (Pearson Chi-square = 4.982, df = 2, p = 0.083).

All the glaucoma cases, due to progressive loss of visual acuity, are accompanied by a social burden which prevents patients from carrying out normal daily functions, movement, self-care and imposes assistance from others, while, at the same time, the unsuccessful outcome of the treatment can cause onset of discomfort, depression and anxiety from complete visual impairment to patients. The analysis of the clinical and pharmacological aspects of the social burden to the respondents is presented in Table 9.

Fourteen (7.69%) female and 12 (6.59%) male respondents have a family social burden. Simultaneously, 33 (18.13%) female and 29 (15.93%) male respondents developed depression, anxiety and discomfort in a more severe form. Regarding these two social parameters, there is no significant difference between female and male respondents (Pearson Chi-square = 2.048, df = 2, p = 0.359).

**Table 7.** Regularity of prescribing treatment

Adherence to directions for treatment		Gender		Total
		Male	Female	
Yes	Number	49	56	105
	%	26.92	30.77	57.69
Partially	Number	20	23	43
	%	10.99	12.64	23.63
No	Number	16	18	34
	%	8.79	9.89	18.68
Total	Number	85	97	182
	%	46.70	53.30	100

Pearson Chi-square = 2.047, df = 3, p = 0.563

\*significance for p < 0.05

**Table 8.** Outcome of treatment of respondents

Outcome of treatment		Gender		Total
		Male	Female	
Successful treatment	Number	21	26	47
	%	11,54	14,29	25,82
Partial success of treatment	Number	25	30	55
	%	13,73	16,48	30,22
Negative outcome of treatment	Number	39	41	80
	%	21,43	22,53	43,96
Total	Number	85	97	182
	%	46,70	53,30	100

Pearson Chi-square = 4.982, df = 2, p = 0.083

\* significance for p < 0.05

**Table 9.** Social burden on the respondents

Social burden		Gender		Total
		Male	Female	
Family burden	Number	12	14	26
	%	6.59	7.69	14.28
Discomfort/depression/anxiety	Number	29	33	62
	%	15.93	18.13	34.07
Total	Number	41	47	88
	%	22.52	25.82	48.35

Pearson Chi-square = 2.048, df = 2, p = 0.359

\* significance for p &lt; 0.05

## Discussion

The current findings indicate the existence of complex exact relations and connection of the social burden with the impairment of the visual acuity, the clinical stage and treatment of the disease and the socio-demographic parameters in the patients (27-29).

Our research demonstrates that in primary glaucoma, female patients are more frequent (53.30%) compared to male (46.70%), whereby from the total number, 19.23% were aged 20-50, 42.86% were 51-60 and 37.91% were 61-67 years of age.

Gender has no pathognomonic significance in the occurrence of glaucoma, but it has been proven that of all the glaucoma cases 50-55% are female, while in primary closed-angle glaucoma cases females dominate with 70% of the total number (6, 9, 30).

With reference to age, according to the WHO program "Right to Sight 2020", the number of visually impaired people progressively increases with age, 31% of the people with severe forms of visual impairment and blindness were aged 45-59, and 58% were over 60 years of age (31, 32).

Primary open-angle glaucoma is treated with medication, laser and surgery and it is administered at home, on an outpatient basis or in an inpatient setting (33, 34).

Most of the respondents, i.e. 79.12% were treated with medication, 8.80% with laser therapy and 12.08% with surgical treatment.

The medical treatment includes the use of mono and combined antiglaucoma medication, beta blockers, carbonic anhydrase inhibitors, alpha 2 antagonists, prostaglandin analogues, miotics, neuroprotective and multivitamin and other medication (35-37).

If the application of antiglaucoma medication does not normalize the intraocular pressure, argon, diogen or similar types of laser therapy are indicated (38-40) or the application of various surgical treatment techniques (41-43).

Despite the regular treatment, which was adhered to by 57.69% of the respondents, glaucoma is an optical progressive neuropathy with an uncertain outcome of treatment, and in our study it was determined that in 43.96% of the cases the

treatment had a negative outcome with further loss of visual acuity, partial success of treatment in 30.22% cases and in 25.82% of the respondents the treatment was successful and prevented further vision loss.

The reason for not adhering to the medical treatment is usually the insufficient patient education about the essence of the disease (14, 44, 45) or the high costs of prescription medicine and the economic burden they cause (46, 47).

A cross-sectional study which examined the social burden of outpatient care on glaucoma patients in Cairo found that 88% of 68 patients did not attend outpatient appointments, 40 of whom did not do so due to lack of education and 28 for economic reasons (48).

The disproportion between the increased treatment costs and the reduced income due to productivity loss, in 14.28% of the respondents was reflected with the occurrence of social family burden, and in 34.07% of the respondents the progressive loss of visual acuity caused discomfort, depression, anxiety, hopelessness and other psychosocial disorders that have adversely affected their health-related quality of life (HRQL). A retrospective study conducted in the United States to assess social burden with DALY states that there is a trend of progression of social burden on people with lower social status and older age, while it also observed that there is higher social burden on women rather than men in all age groups. The largest increase in the social burden was registered among people with reproductive ability at the age of 60 and in patients older than 75 (49). Early diagnosis in the initial stage of the disease, availability of medication and proper administration of treatment with regular ophthalmological examinations, in 70-80% of cases can lead to a positive outcome, prevention of progression of the disease and occurrence of blindness (44, 45, 50, 51).

## Conclusion

Primary open-angle glaucoma generates a significant socio-economic burden as a result of irreversible visual impairment, reduced work ability and productivity, and high treatment costs of patients.

The impact of the social burden depends on the degree of visual impairment, clinical stage of the

disease, adequacy, method of administration, regularity and treatment costs and socio-demographic parameters such as gender, age, genetic predis-

position, race, comorbid conditions, familial predisposition, etc. which in glaucoma play the role of predisposing risk factors.

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## PROCENA SOCIJALNOG OPTEREĆENJA BOLESNIKA SA PRIMARNIM GLAUKOMOM OTVORENOG UGLA

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Glaukom je hronična oftalmološka bolest koju karakteriše progresivan, nepovratan gubitak vidne oštine, dugotrajna progresija i doživotno lečenje, opadanje radne sposobnosti i samodovoljnosti, što može da generiše socijalno opterećenje kod bolesnika. Studija je dizajnirana tako da obuhvati socijalne, kliničke i farmakološke aspekte procene društvenog opterećenja bolesnika primarnim glaukomom otvorenog ugla. Reč je o studiji preseka sa kvantitativnim analitičkim pristupom, koja obuhvata 182 bolesnika muškog i ženskog pola, sa primarnim glaukomom otvorenog ugla, starosti od 20 do 67 godina, sa očuvanom oštrinom vida > 6/18 (0,33), prema ICD-10 klasifikaciji, sprovedena u periodu od avgusta do novembra 2020. godine na teritoriji Severne Makedonije. 53,3% ispitanika činile su žene i 46,7% činili su muškarci, od kojih je 79,12% lečeno propisanim lekovima, 8,8% laserom i 12,08% podvrgnuto je hirurškom zahvatu. Redovno se lečilo 57,69% ispitanika, 43,96% imalo je negativan ishod, delimičan uspeh u 30,22%, a kod 25,82% ispitanika lečenje je sprečilo dalji gubitak vida. 14,28% ispitanika iskusilo je socijalno i porodično opterećenje, a 34,07% nelagodnost, depresiju, anksioznost, beznađe i druge psihosocijalne poremećaje. Primarni glaukom otvorenog ugla stvara značajan socio-ekonomski teret, kao rezultat nepovratnog oštećenja vida, smanjene radne sposobnosti i produktivnosti i visokih troškova lečenja bolesnika. Stepenn socijalnog opterećenja zavisi od razvoja i kliničkog stadijuma bolesti, procenta očuvanog vida, dostupnosti, načina i redovnosti lečenja i socio-demografskih parametara, kao što su pol, starost, zanimanje, genetska predispozicija, komorbidna stanja, porodična anamneza i dr., koji kod glaukoma igraju ulogu predisponirajućih faktora rizika.

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**Ključne reči:** primarni glaukom otvorenog ugla, socijalno opterećenje, socio-demografski parametri, terapija



## CATALASE ACTIVITY AND MALONDIALDEHYDE CONCENTRATION IN THE BRAIN TISSUE OF RATS TREATED WITH CARBON TETRACHLORIDE

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Carbon tetrachloride (CCl<sub>4</sub>) is a potent oxidative agent, used in animal models for the induction of liver and neuronal damage. In this study, we tracked the changes in the concentration of malondialdehyde (MDA) and the activity of catalase (CAT) in the brain tissue of Wistar rats exposed to CCl<sub>4</sub>. The animals were divided into two groups of six rats each. The control group was treated with vehicle olive oil (10 ml/kg) and the experimental group included CCl<sub>4</sub>-treated animals (1 ml/kg). The level of oxidative stress was determined in a 10% homogenate of whole encephalic mass (WEM). The levels of MDA in the experimental group were significantly increased ( $p = 0.0009$ ), while CAT activity was significantly decreased ( $p = 0.0143$ ) in the CCl<sub>4</sub>-treated group compared to the control group. The results confirmed the theory about the CCl<sub>4</sub>-induced oxidative damage on the brain tissue in rats and may be a basis for further research related to potentially protective substances in this animal model.

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**Key words:** carbon tetrachloride, brain, oxidative stress, malondialdehyde, catalase

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### Introduction

The oxygen is essential for regular brain activity, hence the human brain uses around 20% of its total basal consumption (1-3). However, the harmful effects of oxygen are also described, throughout the term of oxidative stress (4). Brain, and especially neurons, appear to be particularly vulnerable to the effects of oxidative and nitrosative stress. Free radicals are components of the processes of neuroinflammation and neurodegeneration, hence they are involved in the pathogenesis of Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease and multiple sclerosis (4). Under physiological conditions, there is a balance between oxygen products generated by mito-

chondria – free radicals and their elimination, which is the function of the antioxidant system (5, 6). Under pathological conditions, there is a decrease in adenosine triphosphate (ATP) synthesis and antioxidants and increasing of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS), which results in oxidative stress (7). Oxidative stress is a consequence of the alteration in the balance between oxygen products called pro-oxidants (ROS and RNS) and cellular antioxidant substances, in favor of the former, which can lead to potential damage (5, 6). Antioxidant capacities can be endogenous or exogenous. Endogenous antioxidant system is determined by enzymatic and non-enzymatic defenses, precursors and cofactors of antioxidants (8). Enzymatic antioxidant system includes following enzymes: superoxide dismutase (SOD), catalase, (5, 6), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), NADH-dehydrogenase and NADPH-dehydrogenase (6) and peroxiredoxins (8).

One of the potential inductors of oxidative stress is exogenous substance carbon tetrachloride (CCl<sub>4</sub>), which is a noninflammable, colorless liquid with an extreme destructive capacity of damaging cells. (9). This industrial solvent is a potent hepatotoxic agent, mostly used to induce hepatotoxic effects in animals, for the purposes of experiment (6), but it also has systemic effects, including brain damage (10), as well as kidneys, lungs, muscles, testis damage (11-14), through the action of free radicals (10). The toxicity of CCl<sub>4</sub> is mostly related to

its lipophilicity, which mostly leads to distribution and deposition in the liver and brain (15, 16). Toxic effects of  $\text{CCl}_4$  involves lipid peroxidation, mediated by the free radicals that are generated during its metabolism by cytochrome P450 (16). The most important products of  $\text{CCl}_4$  are trichloromethyl ( $\text{CCl}_3$ ) and trichloromethyl peroxy ( $\text{OCCl}_3$ ) radicals (17). Acute toxic doses of  $\text{CCl}_4$  are responsible for hepatocellular necrosis and degeneration, but hepatic failure is not so frequent when the liver capacities for regeneration are preserved (18). Nevertheless,  $\text{CCl}_4$ -induced oxidative damage is currently an approved experimental animal model for central nervous system damage, because  $\text{CCl}_4$ -induced hepatic dysfunction may lead to neurotoxicity as well, mainly via oxidative stress (10). When it comes to the brain, this tissue is more sensitive to the harmful effects of  $\text{CCl}_4$ , because of the following facts: being rich in polyunsaturated fatty acids (PUFA), more oxygen utilization and low amount of antioxidants combined with high levels of non-hem iron (6).

While there are numerous known facts about hepatotoxic effects of  $\text{CCl}_4$ , research about its influence on the brain is incomplete.

### **The aim**

Our study aimed to determine the extent of changes in levels of malondialdehyde (MDA) and activity of catalase (CAT) in the brain tissue of rats, after administration of  $\text{CCl}_4$ .

### **Materials and methods**

#### *Chemicals*

Chemicals were of analytical grade and were purchased from Sigma-Aldrich, Sr. Louis, USA. Drug solutions were made on the day of the experiment.

#### *Animals and housing*

Experiments were performed using male Wistar rats, weighing 250-300 g. They were maintained in plastic cages in groups of 6, at  $22 \pm 2$  °C, relative humidity 60%, with 12/12 h light/dark cycle and had free access to food and water. Animals were obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia. Experimental procedures were conducted following the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EU Directive of 2010; 2010/63/ EU) and with the consent of the National Ethics Committee. All efforts were made to reduce the number of laboratory animals used and to minimize the pain or distress encountered by animals in this experiment.

#### *Experimental design*

All the animals were separated into two equal groups, each consisting of 6 rats. The control group comprised animals that were administered only with

vehicle (olive oil) in a dose of 10 ml/kg, while the experimental group included  $\text{CCl}_4$ -treated animals. Dose of 1 ml/kg  $\text{CCl}_4$  was given to rats via intraperitoneal injection, 24 h before sacrificing. During the experiment, the animals were sacrificed by an overdose of ketamine, and the whole encephalitic mass - WEM (composed of the removed brain tissue without the cerebellum and brainstem) and cerebellar tissue were dissected, washed in PBS, stored at -80 °C until 10% homogenates were prepared for later biochemical analysis.

#### *Determination of malondialdehyde (MDA) concentrations*

Malondialdehyde (MDA) is a prooxidant marker and one of lipid peroxidation end products (20). Concentrations of MDA were measured to determine the extent of lipid peroxidation in WEM, by using a thiobarbituric acid reactive substance (TBARS) and measuring the absorbance spectrophotometrically (21). This method is based on the reaction between thiobarbituric acid and MDA under increased heat and under low pH. The absorbance of the reaction mixture was recorded at 532 nm and values were expressed in nmol/mg proteins.

#### *Determination of catalase (CAT) activity*

Activity of antioxidant marker - catalase (20) was determined by the method which Goth with co-workers previously described (22). This method includes the formation of a yellow complex between ammonium molybdate and remaining  $\text{H}_2\text{O}_2$ . Results were obtained by spectrophotometric measuring and the absorbance was determined at 405 nm and the enzyme activity was expressed as U/g of WEM tissue proteins.

#### *Protein estimation*

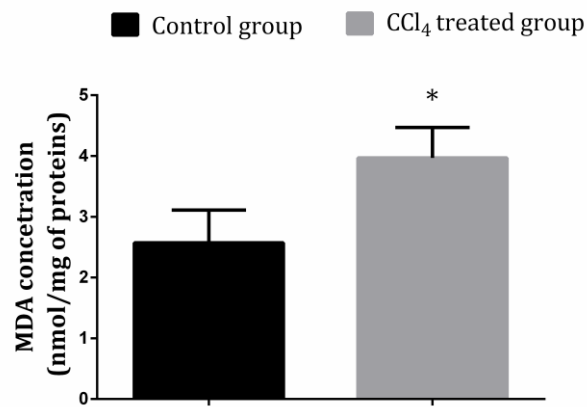
Protein content was measured and determined by the Lowry's method, using bovine serum albumin standard curve (19).

### **Statistical analysis**

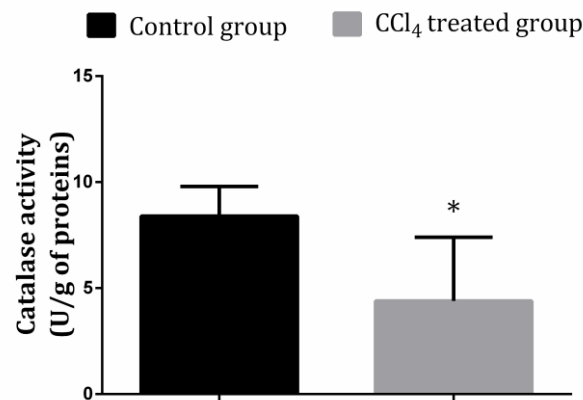
Calculated results are presented as mean values  $\pm$  SD. Data comparison was done by applying Students t-test and the difference was considered significant when  $p < 0.05$ . Statistical calculations were conducted using the Statistical Package for Social Sciences (SPSS) version 13.0.

### **Results**

In our study, we examined values of  $\text{CCl}_4$ -induced oxidative stress, by measuring the values of its markers MDA, as the end product of lipid peroxidation and CAT, as the antioxidant enzyme. Levels of MDA ( $p = 0.0009$ ) were significantly increased, as well as the activity of CAT ( $p = 0.0143$ ) (Figures 1, 2).



**Figure 1.** MDA concentration in rats' WEM, after exposure to CCl<sub>4</sub>, in the experimental group, compared with the control group. Values are expressed in terms of nmol/mg protein, as mean  $\pm$  SD (n = 6). The significance of differences among groups is determined by Student's t-test. \* p = 0.0009 compared to the control group of rats.



**Figure 2.** Catalase activity in rats' WEM, after exposure to CCl<sub>4</sub>, in the experimental group, compared with the control group. Values are expressed in terms of U/g protein, as mean  $\pm$  SD (n = 6). The significance of differences among groups is determined by Student's t-test. \* p = 0.0143 compared to the control group of rats.

## Discussion

Our research has shown that neurotoxicity induced by CCl<sub>4</sub> is a result of combined two effects: oxidative stress generated by free radicals of CCl<sub>4</sub> and decreased activity of antioxidant defenses (6). In general, the most important free radicals are ROS and RNS and the antioxidant system includes superoxide dismutase (SOD), CAT, (5, 6) reduced glutathione (GSH) and glutathione peroxidase (GPx) (6). Reactive oxygen species are important in normal metabolism, although, in excess, they are known to induce apoptosis, cellular injury or necrosis (2, 7).

Free radical CCl<sub>3</sub> is the leading factor of lipid peroxidation in different cells/organelles membranes. Hence, measuring markers of lipid peroxidation (MDA in this case) point at the consequences of the

harmful effects of CCl<sub>4</sub> radicals (6). That is the reason for being a marker of lipid peroxidation in tissues and the signal for the existing oxidative stress. It is also responsible for cellular changes, such as dehydration, deformity or even cell death (9). One study on mice also showed elevated levels of MDA in the brain (23). Increased level of oxidative stress in the hippocampus of mice treated with CCl<sub>4</sub> can be explained by activation of one type of cytochrome P450 – CYP2E1 (18).

Catalase is an antioxidant enzyme located in peroxisomes, but minor concentration is found in the rest of the cell, precisely in cytoplasm and mitochondria. This enzyme performs the conversion of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into oxygen and water and cofactor of this process may be iron or manganese. Its role is most important when the levels of H<sub>2</sub>O<sub>2</sub>

are higher, while when the levels are lower, their removal is mainly related to the activity of peroxidoreductases (PRX) (8). A couple of previous research articles showed the same, statistically significant reduction in CAT activity and an increase of MDA level in the brain tissue of rats exposed to CCl<sub>4</sub> (14, 24-26). The increased MDA level in the brain suggests enhanced peroxidation leading to tissue damage and failure of the catalase, as an antioxidant enzyme, to prevent the excessive production of free radicals.

Despite the fact that more studies describe significant hepatotoxicity of CCl<sub>4</sub>, some researches, such as the one by Ritesh and co-workers (6) showed that neurotoxicity, induced by CCl<sub>4</sub> is even more manifested, than hepatotoxicity caused by the same single dose. The reason for this may lie in richer lipid content of the brain tissue which represents a better surrounding for lipid peroxidation. This especially applies to white matter, because it contains lower PUFA in its myelin sheath, than is the case with grey matter (6). Additionally, there have been some explanations about decreased activities of antioxidants that suggest that the enzymes are inactivated by lipid peroxides, which leads to a deficiency of antioxidant defenses and further tissue damage. However, this process is also happening in reverse, because decreased activities of antioxidant

enzymes, such as CAT and SOD may lead to superoxides' overproduction within the cell, which then causes even more lipid peroxidation. The high sensitivity of the brain can also be described by a higher level of metabolism and a naturally lower level of antioxidants' concentrations. This specifically is referred to CAT activity and GSH concentrations (9).

### **Conclusion**

Our results showed a significant elevation of oxidant forces and a decrease in antioxidant defenses which confirms the suggested theory related to the toxic effects of CCl<sub>4</sub> and a generation of free oxygen radicals that could cause damage to the brain tissue in rats. This knowledge may be a basis for some future research about potentially protective effect of different molecules, not only against the action of CCl<sub>4</sub> but also towards some other harmful factors with similar consequences on brain tissue.

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doi:10.5633/amm.2021.0406**AKTIVNOST KATALAZE I KONCENTRACIJA MALONDIALDEHIDA U MOŽDANOM TKIVU PACOVA TRETIRANIH UGLJEN-TETRAHLORIDOM***Ivana Mihajlović<sup>1,2</sup>, Aleksandra Maslovarić<sup>1</sup>, Aleksandra Mladenović<sup>1</sup>, Tamara Miljković<sup>1</sup>, Nikola Miljković<sup>1</sup>, Dušan Sokolović<sup>1,3</sup>*<sup>1</sup>Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija<sup>2</sup>Vojna bolnica Novi Sad, Novi Sad, Srbija<sup>3</sup>Univerzitet u Nišu, Medicinski fakultet, Katedra za biohemiju, Niš, Srbija

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Ugljen-tetrahlorid (CCl<sub>4</sub>) je snažno hepatotoksično oksidaciono sredstvo, koje se koristi u animalnim modelima indukcije oštećenja jetre i nervnog tkiva. U ovom istraživanju, pratili smo promene koncentracije malondialdehida (MDA) i aktivnost katalaze (CAT) u moždanom tkivu Wistar pacova, koji su bili izloženi dejstvu CCl<sub>4</sub>. Životinje su bile podeljene u dve grupe od po šest pacova. Pacovi iz kontrolne grupe tretirani su maslinovim uljem (10 ml/kg), dok je pacovima iz eksperimentalne grupe apliciran CCl<sub>4</sub> (1 ml/kg). Nivo oksidativnog stresa određen je u desetoprocentnom homogenatu celokupne encefalične mase. Nivoi MDA kod pacova eksperimentalne grupe bili su statistički značajno povećani ( $p = 0,0009$ ), dok je aktivnost CAT signifikantno smanjena ( $p = 0,0143$ ) kod pacova iz eksperimentalne grupe u poređenju sa pacovima iz kontrolne grupe. Rezultati su potvrdili teoriju o oksidativnom oštećenju moždanog tkiva ugljen-tetrahloridom kod pacova i mogu biti osnova za dodatna istraživanja potencijalno protektivnih supstanci u ovom animalnom modelu.

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**Ključne reči:** ugljen-tetrahlorid, mozak, oksidativni stres, malondialdehid, katalaza

## REVERSE OBLIQUITY FRACTURE OF THE PROXIMAL PART OF THE FEMUR (AO/OTA 31-A3) TREATED WITH INTRAMEDULLARY NAIL

Igor M. Kostić

Intertrochanteric fractures of the femur with reverse fracture line are unstable fractures of unique anatomical and biomechanical characteristics, which are surgically treated with extramedullary or intramedullary fixation methods.

The purpose of this study was to evaluate intertrochanteric femoral fractures with intramedullary nail treatment in regard to surgical procedure, complications, and clinical outcomes.

We retrospectively analyzed outcomes of thirty-two elderly patients with AO/OTA 31-A3 intertrochanteric fractures of the femur treated by proximal cephalomedullary nails available at our institution, Clinic of Orthopaedic and Traumatology Niš, Serbia, during the period from 2012 to 2020 years. Postoperative follow-up ranged from 12-22 (12.36) months.

Surgical procedures were performed on average 4.45 days after the injury. The average duration of the surgical intervention was 64 minutes. Closed reduction of fracture and internal fixation were achieved in 26 cases. Acceptable anatomical reposition was achieved in 17 cases (53.12%), and anatomical reposition in 15 cases (46.88%). The mean value of the Harris hip score was 74.66 (65-96), and the mean value of Barthel's activity score was 15.71 (12-20). Fracture healing after intramedullary fixation was achieved in 29 cases, while in 2 cases complications in the form of failure of internal fracture fixation and non-union of fractures (6.25%) were noted.

Intramedullary fixation of reverse transtrochanteric fractures (AO/OTA 31-A3) with short or long cephalo medullary nails provides adequate biomechanical conditions for fracture healing in the optimal time period with the possibility of performing a minimally invasive surgical procedure.

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**Key words:** reverse transtrochanteric fracture, intramedullary fixation, minimally invasive surgery

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Trauma Association (AO/OTA) classification as A3, and they account for 2-23% of all trochanteric fractures. This type of fracture may also be divided into three subgroups:

- A3.1 are oblique fractures,
- A3.2 have a transverse fracture patterns, and
- A3.3 are more comminuted with the fracture line involving the lesser trochanter, and are considered the most unstable fractures in this category (2) (Figure 1).

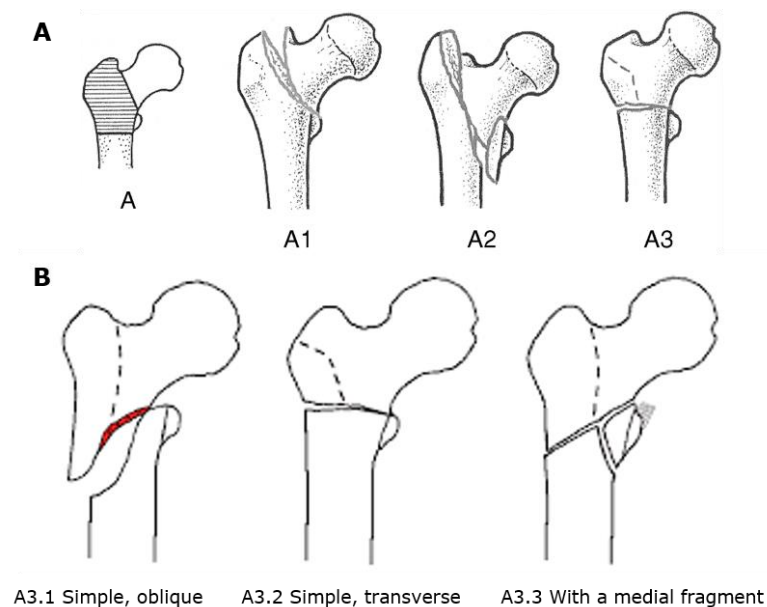
Because of their opposite fracture configuration, which is characterized by the fracture line running obliquely from the proximal greater trochanter to the distal lesser trochanter, reverse obliquity intertrochanteric fractures are unstable with unique anatomical and mechanical characteristics and present challenging fracture to treat whatever implant is used. There are two main types of implant available for the treatment of these fractures, namely, extramedullary and intramedullary implants (3, 4). Although the most widely used extramedullary implant is the dynamic hip screw, which consists of a sliding hip screw connected to a plate in the lateral

### Introduction

Intertrochanteric fractures continue to be one of the most common causes of high geriatric morbidity and mortality (1). Reverse oblique intertrochanteric fractures are classified by the Orthopaedic

femoral cortex, most authors have reported that this device is not suitable for AO/OTA A3 reverse oblique or transverse fractures due to high incidence of fixation failures (5, 6). Intramedullary hip nailing for these fractures reportedly has less potential for cut-out of the lag screw because of their loadbearing capacity when compared with extramedullary implants. Cephalomedullary nailing was introduced in

the late 1980s for the treatment of trochanteric femoral fractures (7-9). However, clinical reports regarding intramedullary hip nailing for reverse obliquity intertrochanteric fractures are few in number (9-14). Despite improved techniques and various implant modifications, implant failure remains a challenging problem for these unstable fractures.



**Figure 1.** Diagrams of AO/OTA 31-A fractures (A) and of three subgroups of A3 fractures (B).

The A1 (perthrochanteric) fractures are always two-part fractures with an intact posteromedial buttress.

The A2 (perthrochanteric multifragmentary) fractures always have a posteromedial fragment involving the lesser trochanter and a varying amount of adjacent posteromedial cortex.

In both the A1 and A2 fractures, the primary fracture line runs from proximal-lateral to distal-medial.

The A3 fractures are intertrochanteric, with the fracture line exiting the lateral femoral cortex distal to the vastus ridge.

In the reverse oblique form of A3 fractures, the primary fracture line runs from proximal-medial to distal-lateral.

(Reprinted from: Orthopaedic Trauma Association Committee for Coding and Classification.

Fracture and dislocation compendium. *J Orthop Trauma*. 1996;10(Suppl 1):31-2, 35.)<sup>2</sup>

In this study, we present the results of intramedullary nailing surgery performed for reverse obliquity intertrochanteric of thirty two patients with AO/OTA 31-A3 fractures using standard or long Proximal Femoral Nail Antirotation (PFNA; Synthes, Oberdorf, Switzerland) and Gamma nail (Stryker, Mahwah, New Jersey, USA) available in our trauma centre during the period of 2012-2020 (15, 16). Proximal nailing surgery was performed for reverse obliquity intertrochanteric fractures in treatment of a non-pathologic fracture, using one lag screw proximally with one or two distal locking screws, and we evaluated the quality of the reduction, operative time, complications and functional status of the patients.

### The aim

The aim of this study is to evaluate the outcome of the treatment of unstable reverse obliquity intertrochanteric fractures (with fracture line that runs distally in a medial to lateral direction) using the proximal cephalomedullary nails.

### Materials and methods

Between 2012 and 2020, we treated 32 reverse oblique intertrochanteric fractures (type 31-A3 of the AO/OTA classification).



We excluded patients with pathological fractures, fractures associated with polytrauma, a pre-existing femoral deformity preventing hip screw osteosynthesis or intramedullary nailing, previous surgery on the ipsilateral hip or femur, and fractures extending 5 cm distally to the inferior border of the lesser trochanter.

We retrospectively recorded age, gender and distribution of the fractures subgroups in our series of patients. Patients were evaluated with perioperative complications, operation time, fluoroscopy time and duration of hospital stay. During a mean of 12.36 months (range 12-22), the results, as well as the intraoperative and postoperative complications, were noted. We recorded their ability to walk within their place of residence, their ability to walk outside, and their ability to go shopping independently or with external support. Operative and postoperative data included specific information on the intramedullary fixation device used, initial reduction of fracture and position of implant, as a radiographs of the affected hip at each follow-up visit, and any changes in the position of the implant and the extent of fracture union were noted. Radiological consolidation was defined when there were visible bone trabeculae between the fragments in the frontal and lateral radiographs. Delayed union was defined as the absence of radiological and clinical union four months after surgery and non-union after nine months. Malunion was defined by more than 10 degrees of varus or valgus deformity and more than 10 mm of shortening compared with the unaffected hip. Nonunion was defined as radiographic lucency around the implants, persistent fracture line that failed to show progressive healing after 9 months, loss of fixation, and pain associated with radiographic findings described above during walking at latest follow-up. Loss of fixation was defined as cut-out or penetration of the blade into the joint or nail breakage.

### Treatment protocol

All operations were performed with the patient in a supine position on an orthopedic fracture table. The operation was done using a C-arm fluoroscopy with an image intensifier in order to verify fracture reduction and fixation. All patients were given one dose (1.5 g) of prophylactic intravenous antibiotics (Primaceph, PharmaSwiss d.o.o.) and all patients were treated with low-molecular-weight heparin (Fragmin 5000 IU SC, Pfizer Manufacturing Belgium NV) beginning on the day of surgery for prophylactic anticoagulation continuing three weeks postoperatively, unless there was a medical contraindication to such treatments.

We used a minimally invasive technique through the lateral border of the tip of the greater trochanter, using guided fluoroscopy to achieve alignment by a closed reduction technique when possible. Fracture reduction was graded as anatomical, acceptable nonanatomical and poor. All the pre- and post-operative radiographs were reviewed (AP and lateral of the hip and femur), and the fracture

reduction was considered to be "anatomical" when there was less than 4 mm of displacement between the major fragments and when the normal neck-shaft angle had been re-established with less than 5 degrees of varus, valgus, anteversion or retroversion in the post reduction images seen on the Image intensifier. It was considered acceptable nonanatomical if the deviation was between 5-10 degrees and poor if it exceeded 10 degrees. The ideal recommended screw tip position was in the inferior half of the femoral head as seen in the anteroposterior radiograph and in the central part of the head in the lateral radiograph, with the sum of the distances from the screw tip to the apex of the femoral head as measured on anteroposterior and on lateral views had to be less than 25 mm.

### Follow-up protocol

Postoperatively, all patients were encouraged to walk fully weight bearing assisted by external support as soon as possible after surgery. The patients were allowed to bear as much weight as they could tolerate. The patients were routinely examined at our outpatient clinic by an orthopedic doctor at 3 and 6 weeks, and at 3, 6, 12 months, and two years after the surgery, until fracture union was evident, as shown by the patient's anamnesis, physical examination and X-ray. The fracture was considered to have healed when the fracture was filled with callus and hip movement and walking was painless.

### Statistical analysis

Statistical analysis was performed using standard data processing programs - MS EXCEL and software package R. Tests were performed with Chi-square, Fisher's exact test and t-test for independent samples. A value of  $p < 0.05$  was considered as significant. Continuous variables were presented as mean  $\pm$  stdev or as median (range); categorical data were presented as number (%). Student's t-test was used for determining any differences in age, operative and fluoroscopy times, and follow-up times between groups. Clinical and functional outcomes were assessed according to Harris hip score and Barthel activity score, respectively. We performed statistical analyses using SPSS1 Version 16.0 (SPSS Inc, Chicago, IL, USA). Details collected included pre- and postoperative mobility and patient residence, operative details, perioperative medical and orthopaedic complications, re-operations, follow-up details and radiographic findings. The primary outcome measure was fixation failure needing re-operation.

### Results

The mean patient age was 78 (range: 47-95) years, with 18 female and 14 male patients, and the left side was affected in 20 cases. According to the AO-OTA classification, 11 fractures were of type 31-A3.1, three were type 31-A3.2 and 18 type 31-A3.3.

The distribution of the fractures in our series is included in Table 1.

The mean follow-up was 12.36 months (range 12-22), and all operations were performed within 4.45 days of injury. Closed reduction was achieved in 26 patients whilst six required open reduction and four of these required cerclage wire fixation. Fracture reduction was considered satisfactory acceptable nonanatomic in seventeen (53.12%) patients and anatomical in fifteen (46.88%) patients, while the position of the hip screw was

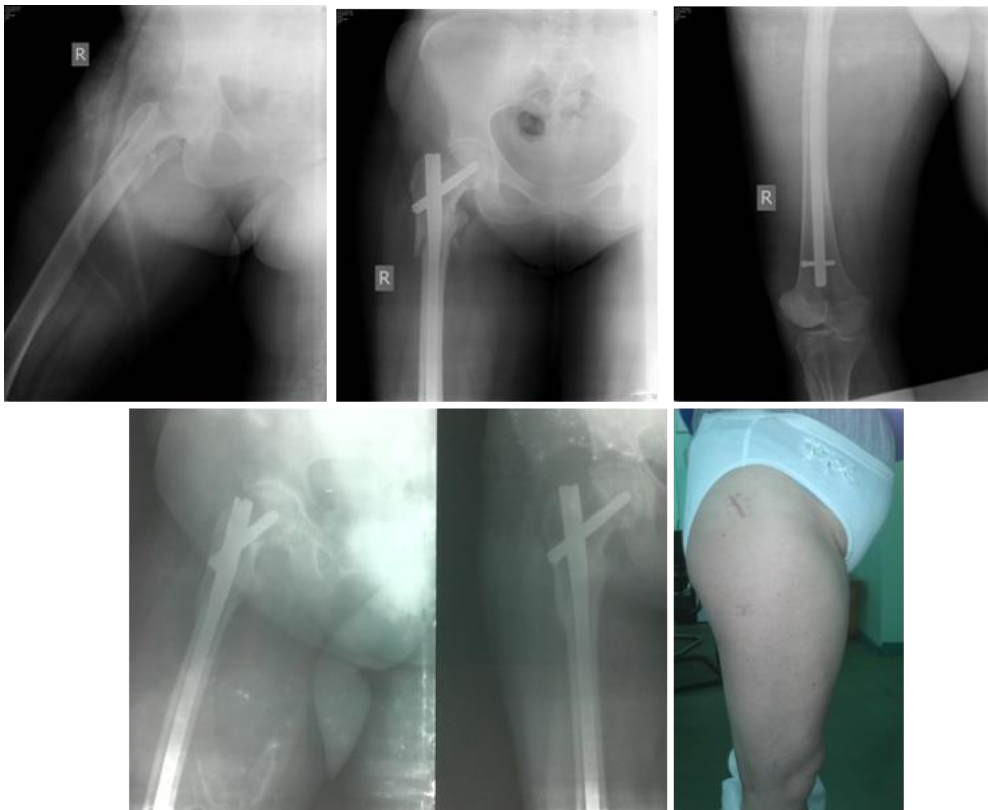
correct in 28 (85%). In our study, the amount of subtrochanteric extension of fracture line and femoral bowing was determined and decision regarding the usage of the standard or long proximal femoral nail. The long nail was taken to prevent the nail impinging against the anterior femoral cortex in four cases and in nine cases for subtrochanteric extension of fracture. In 19 patients we used the standard proximal femoral locking nail (Figure 2), and in 13 the long nail also with distal locking (Figure 3).

**Table 1.** Distribution of the three subgroups of 31A-A3 fractures according to gender

	Male	Female
A3.1 Simple, oblique	2 (6.25%)	2 (6.25%)
A3.2 Simple, transverse	5 (15.62%)	7 (21.87%)
A3.3 With a medial fragment	7 (21.87%)	9 (28.12%)
$\Sigma$	14 (43.75%)	18 (56.25%)
Total	32	



**Figure 2.** Images illustrate the case of a 68-year-old male patient with reverse oblique trochanteric fracture subtype 31A3-A3, treated with a standard (short) PFNA nail, using the closed method



**Figure 3.** Images illustrate the case of a 47-year-old woman with reverse oblique trochanteric fracture subtype 31A3-A3, treated with a long gamma nail (GN), using the closed method

Comparison of demographic and perioperative data for reverse oblique fractures treated with standard (short) or long cephalomedullary nail in our series of patients revealed that values were comparable (Table 2).

There were no perioperative complications, but 14 patients developed one or more major com-

plications during their hospital stay. These included heart failure, respiratory insufficiency and pulmonary or urinary infection. The average period of hospital stay was 5.2 (range: 2-11) days.

There were three major "orthopaedic" complications: late implant breakage, cutting-out and non-union (Table 3).

**Table 2.** Demographic and perioperative data of patients with reverse oblique fractures of the proximal femur treated with standard or long proximal femoral nails

Variable	Standard nail (n = 19)	Long nail (n= 13)	p Value
Demographic			
Age (years) *	78 (67-88)	81(47-96)	0.255
Sex (number of males/females)	4/11	4/14	0.767
Side (number of right/left)	7/8	6/12	0.493
Follow up (months) *	14 (12-18)	15 (12-22)	0.153
Operation time (minutes) *	52.6 (34-65)	71.8 (57-94)	< 0.001
Fluoroscopy time (seconds) *	58.6 (45-79)	75.3 (56-103)	< 0.001
Hospital stay (days) *	5.4 (2-11)	4.9 (2-9)	0.51

\* Values are expressed as mean, with range in parentheses

**Table 3.** Summary of orthopaedic complications

Major complications	Malrotation	2
	Nonunion	2
	Late implant breakage	1
Minor complications	Locking bolts breaking	2
	Distal locking bolts backing out	2
	Breach of anterior femoral cortex	1

The mean Harris hip score was 74.66 (range 65–96) and the mean Barthel activity score was 15.71 (range 12–20). Nineteen patients had excellent results, nine had good results and four had poor results according to Harris hip score; three had low range, five patients had a median range and twenty-four patients had a high range of mobility according to the Barthel activity score. The mean duration of surgery was 64 minutes. The fractures healed in 29 patients; the average consolidation time was 8.6 weeks (range 7–13). In 19 patients (59%) there was no pain after fracture consolidation, in 5 (16%) there was slight pain that was controlled with analgesics and in three there was moderate pain. In one patient this was associated with radiographic hypertrophy of the anterior femoral cortex at the distal tip of a long cephalomedullary nail. The pain disappeared after nail removal.

Four patients (12%) were unable to walk, of whom three had Alzheimer's disease. Nineteen pa-

tients (59%) walked independently or with a single walking aid, five (16%) needed two crutches and four (12%) used a four-point walker. Fourteen patients (43%) needed some assistance with daily activities, eight patients (26%) needed continuous assistance and were living in residences for the elderly, and ten (31%) were completely independent.

### Discussion

There is a permanent confusion regarding the best treatment for fractures of the trochanteric region, especially in unstable reverse oblique fracture patterns of proximal femur. The results of extramedullary and intramedullary fixation of reverse oblique fractures in different studies are variable. The literature suggests that there is a huge range of failure rates for the same type of intramedullary nail used for the reverse oblique fracture (Table 4).

**Table 4.** Summary of clinical studies on reverse oblique fractures of the proximal femur

Author	Year	Number of patients	Implant Type	Failure rate (%)
Hernandez-Vaquero <sup>12</sup>	2005	47	Gamma nail	6.4
Min et al. <sup>14</sup>	2007	11	Gamma nail	27
Sadowski et al. <sup>9</sup>	2002	20	Proximal Femoral Nail	5
Honkonen et al. <sup>11</sup>	2003	36	Proximal Femoral Nail	14
Honkonen et al. <sup>15</sup>	2003	36	Gamma nail	3
Hernandez-Vaquero <sup>12</sup>	2005	47	Gamma nail	6.4
Min et al. <sup>14</sup>	2007	11	Gamma nail	27
Sadowski et al. <sup>9</sup>	2002	20	Proximal Femoral Nail	5
Honkonen et al. <sup>11</sup>	2003	36	Proximal Femoral Nail	14
Min et al. <sup>13</sup>	2007	11	Proximal Femoral Nail	9
Park et al. <sup>13</sup>	2008	21	Proximal Femoral Nail	29
Ozkan et al. <sup>17</sup>	2010	15	Proximal Femoral Nail	0
Park et al. <sup>13</sup>	2008	19	Intertrochanteric subtrochanteric nail	0
Wang et al. <sup>25</sup>	2009	30	Proximal Femoral Nail Antirotation	3
Chou et al. <sup>22</sup>	2012	63	Intramedullary hip screw	7.9
Warschawski et al. <sup>18</sup>	2021	40	Gamma nail	20

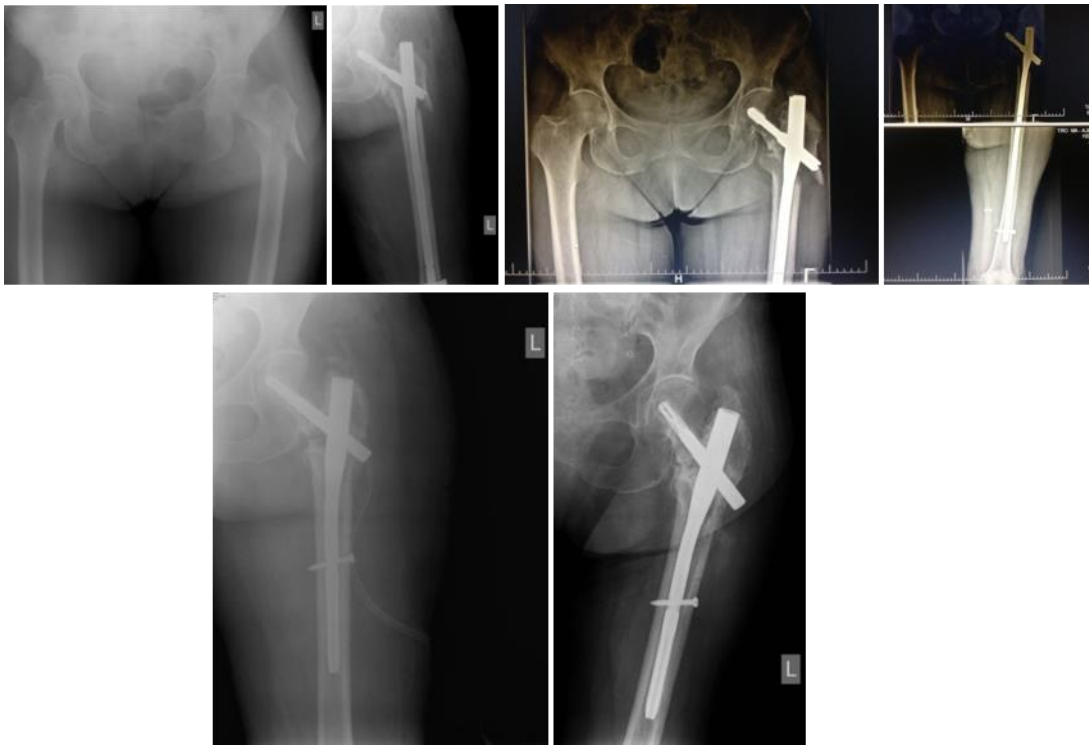
Most of the comparative studies have low numbers of patients and therefore drawing conclusions about relative superiority of one implant over another is inappropriate. Often the failure is due to inadequate fracture reduction, poorly placed femoral head screw or malpositioning of the implant. In our series, two fractures were poorly reduced and subsequently required revision surgery. Overall, in our series, four fractures were poorly reduced; therefore, the remaining two reduced in varus malalignment did, in fact, go to union. The total percentage of fixation failure in our series of patients was 6.25% (two patients). The relatively low number of patients in our series prevent the drawing of any firm conclusions with regard to the importance of fracture reduction. A review by Haidukewych et al. (17) of reverse oblique fractures demonstrated that poor implant placement and fracture malreduction had strong negative effect on the outcome. Almost every hip IMN has standard and long versions (varying from 30 to 42 cm) for fixing the proximal part of femoral fractures. However, the indications for choosing either a standard or long IMN are somewhat unclear and usually subjective, and it is unclear whether long nails reduce the rates of reoperation and nonunion (18). It is well known that, when the nails were statically locked with two distal screws, the weak points were protected and the mechanical stresses were shifted towards the diaphyseal area, overloading the part of the nail close to the nonunion (19). Nevertheless, cephalomedullary nailing systems combine the biomechanical advantages of a sliding hip screw with those of intramedullary nailing. The sliding hip screw provides a controlled impaction of the fracture, leading to increased fracture stability, less collapse and decreased bone healing time. The intramedullary nail is located closer to the central weight bearing axis of the femur, reducing the bending stresses up to 30 % due to smaller moment of inertia of the lag screw (20). Thus, in intertrochanteric fractures, the compression of both main fragments occurs along the femoral shaft axis, i.e., along the axis of the nail. With regard to anatomy of the proximal fragment, the lag screw passes almost through the lateral cortex of the proximal fragment. Therefore without distal locking, most of these fractures are rotationally and longitudinally unstable (21, 22). We used two screws distally in eleven patients (34.37%) and one screw in twenty-one patients (65.63%).

Secondary displacement of the system was considered when the screw changed its position inside the femoral head, and "cutting-out" of the screw was defined when the hip screw had penetrated into the acetabulum. Cutout, Z-effect or

reversed Z-effect of the lag screws were scored as technical failures (23). Despite correct technical application, complications such as varus angulation of the proximal fragment and medialisation of the distal fragment during nail insertion, which may necessitate open reduction, may occur. Excessive loading of the femur may also occur due to distraction between the proximal and distal fragments that may cause stress fracture of the nail. This can be prevented with dynamic stabilisation or distal stabilisation of the nail after releasing the traction (24).

The use of cephalomedullary nailing systems has been complicated with implant breakage in only rare occasions. Breakages occurred mainly in complex unstable fracture patterns. In the literature, the mean time to implant breakage was 9 months (range 3-24 months), and all cases occurred after a period of several months of full weight bearing (25). In our study cases, we have one late implant breakage, 6 months postoperatively, due to nonunion at the fracture site. Possible biomechanical and/or biological causes of nail breakage could be attributable to (1) the initial inadequate primary reduction demonstrating varus axis deviation of the proximal fragment as the main cause for nail breakage, (2) use of the short standard nail to manage unstable intertrochanteric fractures with subtrochanteric extension, (3) the open reduction inducing devascularisation of the fragments or disturbance of the osteoinductive fracture hematoma, (4) the use of cerclage wiring which might compromise the periosteal vascularity, (5) the incorrect insertion point of the nail (the selection of the correct insertion point prevents the occurrence of varus axis deviation as well as the occurrence of shear forces) (26, 27). If those cannot be neutralized, secondary dislocation is inevitable.

In our study hardware or fixation failure was not related to the type of nail, implant material or subtype of AO/OTA 31-A3 fractures, but a neck-shaft angle of  $< 125^\circ$  led to a significant increase in fixation failure. Thus, reverse trochanteric fractures require cautious follow-up studies to identify delayed or missing bone healing. In these cases of delayed or nonunion, additional treatment may include mobilization with partial weight bearing, dynamization of implant or even consequent operative revision using exchange nailing. In one case of late implant breakage in our series of patients, eight months postoperative, we did the revision intervention using exchange nail surgical technique, and nonunion site healed inevitably 6 months after post revision surgery (Figure 4).



**Figure 4.** Images illustrate the case of a failure of the fixation, implant breakage 8 months postoperative in 81-year old female patient, treated primary with long gamma nail. X rays after revision surgery using exchange nailing surgical technique with standard PFN nail and bone grafting of the nonunion site

The limitations of this study must be recognised. Limitations of our study are the lack of control group and relatively small patient population, but these are uncommon fractures accounting for 5-23% of all trochanteric fractures even in the centralised trauma centres. It is a retrospective study carried out over a long period of time, potentially giving rise to inconsistencies in classification and treatment. Some of the CMN implants discussed in this study have now been upgraded and may not be in common use today, therefore making it difficult to make direct comparisons with the extramedullary implants. Further limitations include lack of rando-

misation in selecting the implant type and length and a relatively small number of patients.

### Conclusion

In spite of the limitations imposed by the retrospective study, we conclude, that the cephalomedullary devices are a good option for the treatment of reverse oblique intertrochanteric femoral fractures. We therefore recommend that surgeons aim to achieve good fracture reduction and use a long cephalomedullary implant device that they are familiar with to achieve ideal implant placement.

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## PROKSIMALNI PRELOMI FEMURA SA OBRNUTOM KOSINOM (AO/OTA 31-A3) LEČENI METODOM INTRAMEDULARNE FIKSACIJE

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Intertrohanterni prelomi femura sa obrnutom kosinom su nestabilni prelomi jedinstvenih anatomskih i biomehaničkih karakteristika, koji se hirurški zbrinjavaju ekstramedularnim ili intramedularnim metodama fiksacije.

Cilj ove studije je procena efikasnosti primene metode proksimalne cefalomedularne fiksacije transtrohanternih preloma sa inverznom kosinom u odnosu na samu hiruršku proceduru, komplikacije i ishod lečenja.

Retrospektivno su analizirane primene proksimalne intramedularne fiksacije u hirurškom zbrinjavanju 32 bolesnika sa kosim, inverznim, intertrohanternim prelomima, koje su bile izvršene u periodu od 2012. do 2020. godine na Klinici za ortopediju i traumatologiju, UKC Niš. Postoperativno praćenje bolesnika kretalo se u rasponu od 12 meseci do 22 meseca (12,36 meseci).

Operativni zahvati izvedeni su u proseku 4,45 dana od povrede. Prosečno vreme trajanje hirurške intervencije iznosilo je 64 minuta. Zatvorena repozicija preloma i unutrašnja fiksacija postignute su u 26 slučajeva. Prihvatljiva anatomska repozicija postignuta je u 17 slučajeva (53,12%), a anatomska repozicija u 15 slučajeva (46,88%). Srednja vrednost Harris hip skora bila je 74,66 (65 – 96), a srednja vrednost Barthelovog skora aktivnosti iznosila je 15,71 (12 – 20). Zarastanje preloma nakon intramedularne fiksacije postignuto je u 29 slučajeva, dok su u 2 slučaja zabeležene komplikacije u vidu neuspeha unutrašnje fiksacije preloma i nezarastanja preloma (6,25%).

Intramedularna fiksacija inverznih transtrohanternih preloma (AO/OTA 31 A3) kratkim ili dugim cefalomedularnim klinom obezbeđuje adekvatne biomehaničke uslove za zarastanje preloma u optimalnom vremenskom periodu, uz mogućnost izvođenja minimalno invazivne hirurške procedure.

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**Ključne reči:** inverzni transtrohanterni prelom, intramedularna fiksacija, minimalno invazivna hirurgija



## HONEY, MORE THAN JUST FOOD: THE POSSIBILITY OF APPLICATION IN REGENERATIVE MEDICINE

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Honey has long been recognized as a special natural product, not only because of its nutritional value, but also because of its healing properties. Although it is mostly composed of carbohydrates, it also contains numerous enzymes, amino acids, organic acids, polyphenols, minerals, vitamins and other substances, whose presence varies depending on the type of honey and its botanical and geographical origin. Thanks to its antimicrobial properties, as well as its beneficial effect on wound healing, tissue repair and regeneration, honey could have a potential role in the fields of tissue engineering and regenerative medicine.

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**Key words:** honey, wound healing, tissue repair, tissue regeneration, tissue engineering

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### Introduction

Honey is produced by honey bees (genus *Apis*) through processing nectar and honeydew (1) using enzymes of their hypopharyngeal glands (2). Nectar honey can be monofloral (from the nectar of one plant type) or polyfloral (obtained from various nectar types). Honeydew honey is obtained by processing plants secretions or excretions of plant-sucking insects. Strictly speaking, pure monofloral honey cannot be obtained, because many other honey plants bloom at the same time as the main plant. Monofloral honey is considered to be one that is obtained mainly from a certain plant and has a characteristic composition and properties (3, 4).

Since ancient times, honey has been in human use, primarily as a food. With the development of human civilization, the possibilities of using this natural product expanded, so it began to be used, for example, as a medicine and preservative (5). Such use is based on its antioxidant, antibacterial,

antifungal, antiviral, immunomodulatory, wound healing and many other properties (6, 7), for which there is growing evidence in the scientific literature.

Injury of any tissue is followed by repair. Tissue repair means that tissue architecture and function are restored after injury through regeneration and replacement processes. Regeneration completely restores damaged tissue to its normal form. Repair of severe tissue injuries or those which are not prone to regeneration ends by filling the injury site with connective tissue and is accompanied by scarring. It is replacement process. Most tissue repair involves both of these processes. Along with tissue repair, it is important to prevent wound infection, which can prolong the healing process and also lead to additional damage not only to injured parts of tissue, but also to healthy surrounding tissue (8). Honey antimicrobial and pro-healing activity, as well as the possibility of preventing scars, makes it a good candidate for use in regenerative medicine (9).

### Chemical composition of honey - the main features

Honey has complex chemical composition and contains a large number of biologically active substances. Richness of honey content is due to its plant origin, but also to the components that bees add to nectar. In honey are found different carbohydrates, enzymes, amino acids, organic acids, polyphenols, minerals, vitamins and many other substances.

The most common compounds in honey are carbohydrates, over 90% of its dry weight, and they

are mostly in the form of monosaccharides fructose and glucose. Less common are disaccharides and trisaccharides such as: sucrose, isomaltose, panose, maltotriose, melezitose, erlose, isomaltotriose, isopanose, maltopentaose and others (10, 11). The high content of carbohydrates in honey is considered to be the main reason for its high osmolarity (12).

Right after carbohydrates, water is the next most represented honey component ranging from 17 to 20% (11).

Proteins are also constituents of honey, but are present in much smaller quantities, such about 0.5%. They are mainly in the form of enzymes (such as  $\alpha$ -amylase,  $\beta$ -amylase, glucose oxidase, invertase, catalase) and amino acids, whose most common representative is proline, which makes up to 85% of the total amino acid content. Proteins originate from pollen (primary source) and secretions of bee glands (13-15). Bee antimicrobial peptide, defensin-1, is also honey constituent (16, 17). Likewise, another antimicrobial bee-derived peptide named hymenoptaecin can be also found in some honey types (17).

Polyphenols are products of secondary plant metabolism. They can be classified into two groups: flavonoids and non-flavonoids (phenolic acids) (15). The content of these compounds varies among different honeys, so their qualitative content can be used to determine the floral origin of honey (18). Their presence is associated with the honey anti-oxidant and antimicrobial properties (19).

The main organic acid in honey is gluconic acid. Citric acid, acetic acid, formic acid and many others are represented as well. Organic acids originate from nectar, or are formed from sugars by the action of bee enzymes during honey production (20). Presence of organic acids, primarily gluconic acid, in honey is the main cause of its low pH.

Vitamins and minerals are present in small amounts. Among vitamins, those from B complex and C vitamin are the most common, while among minerals it is primarily potassium, but a whole range of other minerals is represented too. Minerals are also a very stable component of this natural product (14).

The wide range of substances contained in honey gives it special properties that increase its nutritional value, and at the same time are important for human health. The share of these substances in honey is generally not constant, and largely depends on the type of honey and its botanical and geographical origin. Likewise, some compounds contained in honey are subject to changes due to chemical reactions during long-term storage (14).

### **Antimicrobial effects of honey**

Having in mind the richness and diversity of compounds found in honey, its antimicrobial properties cannot be attributed only to one of them, but to the whole constellation of molecules that give it special antimicrobial characteristics.

Foremost, the pH value of honey is in between 3.2 and 4.5, so it represents an acidic

environment unfavorable for growth of microorganisms. In addition, high osmolarity of honey, due to its high sugar content, is also one of the factors responsible for its antimicrobial properties (5, 21), given that osmotic stress leads to changes that are reflected in depolarization and increased permeability of bacterial cell membrane, as well as reduction in cell size (22). However, a study which compared different natural honeys with culinary processed commercial honey has shown that in the case of the latter there was no antibacterial effect, indicating that this effect is not only conditioned by the honey sugar content (23). In fact, the result of this comparison suggests that not only high osmolarity and low pH are participating in its antimicrobial effect, but that some other honey compounds have such function.

Hydrogen peroxide is considered to be one of the main honey's constituent that has antimicrobial properties. It arises from glucose as a product of reaction catalyzed with bee-derived enzyme, glucose oxidase (24). In the previously mentioned study, specifically, several different types of honey from various Danish floral sources were examined and their antibacterial activity was compared with culinary processed commercial honey and raw and medical grade Manuka honey (MH). Not only was it found that all honey types had an antibacterial effect, except the commercial one, but also that some of the examined honeys had an even stronger antibacterial effect than MH. Antibacterial action of tested honeys, especially those that had a stronger antibacterial effect, was attributed primarily to the content and activity of hydrogen peroxide (23). Natural role of hydrogen peroxide is in preventing the development of microbes in unripe honey, and after its maturation this role is taken over by a high concentration of sugars, while glucose oxidase is inactivated. This enzyme is reactivated upon honey dilution (25, 26). Thus, it is found that binding of enzyme to glucose is much easier in diluted honey, and production of hydrogen peroxide is more continuous, so honey antimicrobial effect is increased (27). The amount of hydrogen peroxide in different honeys varies, and this depends on the type of honey and its dilution (26, 28). Although there is, to some extent, a positive correlation between the degree of honey dilution and hydrogen peroxide concentration, hydrogen peroxide activity is highest at 30-50% honey dilution (26). In fact, the presence of free water molecules in honey is important for maintaining the native conformation and enzymatic activity of the glucose oxidase, as well as the activity of the enzyme catalase. Catalase is an enzyme of pollen origin that participates in destruction of hydrogen peroxide. So, the total amount of hydrogen peroxide in honey also depends on the relationship between actions of these two enzymes (29). There is also synergy between hydrogen peroxide and gluconic acid, which some authors underline as crucial for honey antibacterial activity. It has been shown that these two components lead to bacterial membrane depolarization and destruction of bacterial cell wall in dose-dependent manner (22).

Additionally, polyphenols as the constituents of honey are involved in its antimicrobial action as

well. These compounds can also contribute in hydrogen peroxide production, as was reported with honeydew honey (30). Polyphenols content varies between honey types. Forest honey (honeydew) is rich in polyphenols. It has more than ten times higher total phenolics and flavonoids contents than acacia honey, as it has been shown in an experiment in which forest honey, polyfloral and monofloral acacia honey from the Šumadija District (Central Serbia) were compared. This investigation has shown that all honey types have antibacterial activity against different bacterial strains, and this activity is most pronounced in forest honey. But forest honey has also shown antifungal activity against some fungal species such as *Trichoderma longibrachiatum*, *Trichoderma harzianum*, *Penicillium canescens* and *Penicillium cyclopium* (31). Phenolic fractions isolated from 33 Iranian honey samples have shown antimicrobial activity against four bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* and this property was attributed not only to honey's individual phenolic compounds but to their joint action (32).

Defensin-1 is a bee-derived antibacterial peptide, which has been found in royal jelly and honey (17, 28). The presence and amount of defensin-1 varies strongly and in some types of honey it is completely lacking (33). Mechanism of defensin-1 action is based on bacterial cell membrane permeabilization and then RNA, DNA and protein synthesis inhibition (34). Another antimicrobial peptide, hymenoptaecin, is variable honey component and it is assumed that its presence depends on the microbial pressures in honeybee colonies (17). This antimicrobial peptide forms pores in bacterial envelopes (35).

The antimicrobial activity of MH is based primarily on the methylglyoxal presence. Methylglyoxal is formed by conversion of dihydroxyacetone, highly present substance in nectar of manuka tree (*Leptospermum scoparium*) flowers, during honey storage (33). Investigation of the effects of different methylglyoxal dilutions on four bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) has shown that methylglyoxal negatively affects bacterial fimbriae and flagella, damages bacterial cell membrane and causes changes in bacterial cell shape (36).

### **The application of honey in tissue repair and regeneration**

The use of honey for medicinal purposes has been known since ancient times. Inter alia, many studies suggest that honey has a positive effect on wound healing, tissue repair and regeneration. Acacia honey was used in treatment of excision, incision, burn and dead-space wound models in Swiss Wistar rats of either sex (37). Honey was applied topically and, for that purpose, 5 or 10% concentration of honey was added to 2% w/w gel sodium alginate gel. The results of this study confirmed that honey treatment aids wound healing by increasing wound epithelization and wound contraction in the excision and burn wound model,

breaking strength of the wound in the incision wound model, as well as breaking strength, dry tissue weight, and hydroxyproline content in the dead-space wound model.

Application of honey to the wound site, can serve as a protective coating against infections, mimicking the action of antibiotics. Also, the high sugar content in honey is a good nutritional source for cells at the site of injury (9). Likewise, the high sugar content creates an osmotic pressure that triggers and draws lymph fluid from tissues at the site of application in wound treatment. This supports the process of removing debris and necrotic tissue, which is important at the beginning of tissue repair (16). Mphande and coworkers have investigated whether there was a difference between honey and sugar applied as dressing in wound management of 40 patients. Results of this investigation have shown that honey has greater antibacterial activity than sugar, and is more successful in promoting wound healing (38). Honey can reduce the pH at the wound site, while stimulating the activity of fibroblasts. The addition of acacia honey to culture media has encouraged migration and differentiation of cultured rabbit corneal fibroblasts. This effect was attributed to the high glucose content in honey, which served as an important source of energy for cells, subsequently to the hydrogen peroxide releasing and lowering the pH of the medium, which are also factors that can positively affect fibroblast proliferation (39).

Gradual release of hydrogen peroxide from honey applied to the site of injury can be initiated by its dilution with wound exudates. Thus honey, with its antimicrobial action, can prevent wound infection, and at the same time the concentration of hydrogen peroxide is such that it is not toxic to the tissue cells. Also, hydrogen peroxide within physiological range stimulates angiogenesis, a process which is considered as critical for tissue repair (26, 40). Namely, blood vessels' network development is an important part of any tissue repair process, since it allows nutrients, cytokines, growth factors, oxygen and many other molecules to reach damaged tissue (41-44). Martinotti and colleagues have identified hydrogen peroxide as main honey compound involved in regenerative process. Namely, using *in vitro* model of immortalized human keratinocyte cell line, they have demonstrated that extracellularly released hydrogen peroxide from honey enters cells through specific aquaporin channels. When found in cytoplasm, hydrogen peroxide facilitates extracellular  $\text{Ca}^{2+}$  influx by activating  $\text{Ca}^{2+}$ -permeable ion channels. An increase in intracellular  $\text{Ca}^{2+}$  levels facilitates wound healing, as  $\text{Ca}^{2+}$  is essential for activating various cellular-level processes involved in tissue regeneration (45). In order to activate hydrogen peroxide activity, researchers recommend diluting honey before using it as an antiseptic dressing on wounds to an extent appropriate to the presumed amount of fluid that will be released from the wound during treatment (26).

The activity of macrophages, cells whose role in tissue repair is already known, can also be modulated by honey constituents (46). Actually, tissue repair begins with an inflammatory reaction.

Inflammatory phase lasts up to two weeks, after which it needs to be silenced. Then, through anti-inflammatory mediators, synthesis of extracellular matrix and angiogenic process are promoted, which ultimately leads to tissue repair. Main actors of the inflammatory phase are macrophages which, depending on wound conditions, secrete a number of different molecules (47-49). Inflammatory process can be modulated under the influence of honey or its components, through inhibition or stimulation of the synthesis of various cytokines by macrophages and their monocyte precursors (46). Human monocytes exposed to MH *in vitro* were stimulated to produce pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (50). Some authors attribute the stimulatory effect of honey on the inflammatory process to the presence of endotoxins as well (46). On the other hand, the anti-inflammatory effect of honey has also been described and it is attributed primarily to its flavonoid content. Thus, Candiracci and coworkers investigated the effect of a honey flavonoid extract on pro-inflammatory cytokine production by N13 microglia cells pretreated with lipopolysaccharide. Honey flavonoid extract has significantly reduced TNF- $\alpha$  and IL-1 $\beta$  production (51). Given the both pro-inflammatory and anti-inflammatory effect, it can be altogether said that honey has immunomodulatory properties. Their mechanisms are very complex and conditioned by different honey components, which vary considerably by their presence and quantity, depending on the honey type. Further extensive research is needed in this field, but the assumption is that whether applied honey will have pro-inflammatory or anti-inflammatory effect depends on the wound environment (52).

Defensin-1, in addition to having antimicrobial properties, has also been shown to participate in wound healing process. This peptide in a culture of human keratinocytes promoted secretion of matrix metalloproteinase 9 (MMP-9), cell migration, as well as improved wound closure *in vivo* (53).

Since the use of honey in superficial wounds treatment has been shown as highly effective, the possibilities of its application in the repair of hard tissues are expanding. Here, the principle of honey action is essentially the same as that on soft tissues, and is largely a result of the action of its numerous components. Honey has been shown to be effective in treating small mandibular bone defects in rats (54). In this study, healing of mandibular defects, filled with sterile honey or untreated, was monitored. According to the presented results, defects' healing process in the experimental group was more significant than in the control group, indicating that honey may support bone healing. Often, in large bone defects' treatment, it is necessary to aid tissue repair with biomaterials, which are frequently on hydroxyapatite base (41-44, 47-49, 55-57). Combining hydroxyapatite with honey, in order to repair the rats' radius bone defects, turned out to be a better solution compared to the independent use of honey or hydroxyapatite (58). Another application in oral surgery was conducted within one small clinical trial, when honey was placed in the socket, immediately after surgical removal of impacted third

molars (59). In the honey-treated group, less pain, lower frequency of postoperative complications and less swelling was observed in comparison with the untreated group (control). The effects of honey on cartilage defects repair were also examined. In that purpose, MH, gellan gum (GG) and mesoporous silica-MS composite hydrogels were prepared (60). First, *in vitro* analysis has shown that these composites had significant changes in peak elastic and dynamic moduli during 45 days of *in vitro* incubation period with human mesenchymal stem cells. In addition, MS-composite hydrogels ensured good *in vitro* cytocompatibility and antibacterial activity in a co-culture model, so they were chosen for application in a subcutaneous implantation mice model. *In vivo*, there was no severe immune response, which means that GG-MH-MS composites represent a very promising tool for cartilage tissue engineering. Honey has the ability to modulate inflammatory cells activity which can actually increase its application as a scaffold additive in the cases of bone infection (61). For example, MH decreases inflammation and promotes collagen deposition and migration of fibroblast. Therefore, this type of honey can probably promote tissue-material integration/regeneration and accelerate healing of site that surrounds the wound (62-64). Cryogels are biomaterials that have favorable porosity, elasticity and ability to retain three-dimensional architecture, which makes them interesting for application in bone tissue engineering. To overcome the problem of bacterial infection, the application of honey for incorporation into cryogels has attracted a lot of attention recently (65). In the research conducted by Hixon and his team (61), different amounts of MH were incorporated into cryogel scaffolds made of either gelatin or silk fibroin. Likewise, MH properties were utilized in a sustained release fashion to help in the bone healing process, while cryogel structure was used as a tissue template. All types of cryogels were examined regarding the effects of MH on porosity, swelling potential, mechanical durability and cell compatibility. In order to mimic native bone, these scaffolds were mineralized. It was found that a 5% MH silk fibroin cryogel had the potential to inhibit bacterial growth while still maintaining adequate porosity, mechanical properties, and cell infiltration. These features are favorable for the application in bone tissue regeneration.

## Conclusion

There are many characteristics of honey, which classify it as a natural product suitable for various medical purposes. In addition to the fact that its price is affordable and that it has an unlimited shelf life, honey has no side effects and is easy to apply to the site of interest. The antimicrobial and wound healing abilities of honey are based on the presence of numerous compounds that give it special characteristics. Application of honey to the site of injury creates a physical barrier between wound and its environment, prevents contamination, and accelerates healing process. Thanks to the antimicrobial properties of honey, its use provides the

possibility of reducing the utilization of antibiotics during wound healing treatment. In addition to the already proven positive effect on the healing of superficial wounds, its use in the healing of hard tissue defects is being investigated, after independent application or in combination with biomaterials. However, although for now the results of various researches are promising, there are not many scientific papers on the influence of honey in hard tissue repair, and more clinical studies are missing. Despite all mentioned positive effects of honey, some dilemmas remain. One of the issues is

the stability of its chemical components during prolonged storage. Likewise, not all mechanisms by which honey influences tissue repair and regeneration through its components have been fully investigated. Also, it is known that there are large differences in the representation of different compounds among honeys, which depend on their type, botanical and geographical origin. Therefore, in order to implement honey in the frameworks of regenerative medicine, it is necessary to standardize its individual components and their effective doses, especially in relation to different tissue types.

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**Pregledni rad**

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## **MED, VIŠE OD HRANE: MOGUĆNOST PRIMENE U REGENERATIVNOJ MEDICINI**

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Med je od davnina prepoznat kao dragoceni prirodni produkt, ne samo zbog svoje nutritivne vrednosti, već i zbog svojih lekovitih svojstava. Iako je u najvećoj meri sačinjen od ugljenih hidrata, on sadrži i brojne enzime, aminokiseline, organske kiseline, polifenole, minerale, vitamine i druge supstance, čija zastupljenost varira u zavisnosti od vrste meda i njegovog botaničkog i geografskog porekla. Zahvaljujući svojim antimikrobnim svojstvima, kao i blagotvornom efektu na zarastanje rana, reparaciju i regeneraciju tkiva, med može imati potencijalnu ulogu u oblastima tkivnog inženjerstva i regenerativne medicine.

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**Ključne reči:** med, zarastanje rana, reparacija tkiva, regeneracija tkiva, tkivno inženjerstvo



## THE INFLUENCE OF THERAPEUTIC MODALITIES ON THE FUNCTIONAL ABILITY OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease. The progressiveness of the disease and damage to the joints caused by RA can make a significant impact on the functioning ability. The gold standard for assessing functional status in RA is the HAQ questionnaire.

The aim of the study was to assess the impact of sociodemographic characteristics, laboratory parameters of interest and therapeutic modalities on the functional ability of patients with RA.

A retrospective cross-sectional study involved 109 patients, 29 male and 83 female, with a diagnosis of RA. Data on the demographic and clinical characteristics of the patients, laboratory findings and applied therapeutic modalities were collected. Microsoft Office Excel and PASW were used for statistical analysis. An estimation error level of less than 5% (0.05) was used as the statistical significance threshold.

The results of the study indicate that more than a third of the participants have a HAQ-DI greater than 1, which indicates a significant decrease in functional ability. The largest number of subjects with a high HAQ-DI index (41.2%) was observed in the group of subjects who used glucocorticoids in combination with several synthetic disease-modifying drugs (GC + multiDMARD). The values of HAQ-DI differ statistically significantly in relation to the applied therapeutic modalities ( $p = 0.004$ ). HAQ-DI significantly correlated with the values of sedimentation rate ( $p < 0.05$ ), CRP ( $p < 0.01$ ) and anti-CCP ( $p < 0.05$ ). PLUM regression analysis showed that functional ability was impaired in the group of subjects without and with a smaller number of comorbidities. In addition, the results of regression analysis indicate that the therapeutic modalities GC + methotrexate ( $p = 0.023$ ) and GC + multiDMARD ( $p = 0.002$ ) can significantly contribute to better functional ability in patients with RA.

Methotrexate, alone or in combination with other drugs, can significantly contribute to better functional ability and is effective in the treatment of most patients with RA.

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**Key words:** rheumatoid arthritis, methotrexate, HAQ-DI

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### Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that affects an average of 0.5-1% of the world's population. The disease is

more common in women and can occur at any age, and the highest incidence is between 50-60 years of age (1). Today, it is known that a large number of factors can influence the development of diseases, both genetic and environmental factors. Expressed in a percentage, the chance of inheriting RA from relatives is from 53% to 65%, which is 1.6 times higher compared to people who do not have a genetic basis (1, 2). Disease progression, chronic inflammation of the joints and consequent joint damage make RA significantly affect functional ability (2). Decreased functional capacity can contribute to: higher unemployment rates, loss of productivity at work, increased health costs and impaired quality of life of patients with arthritis (RA). The Health Assessment Questionnaire (HAQ-DI) (2) is used as the gold standard for assessing the functional status of a patient with RA (2).

Individual approach to each patient, early diagnosis and timely application of anti-inflammatory and immunomodulatory drugs are the

imperative of modern treatment of RA. There is a wide range of drugs that can be used in the treatment of RA, with the aim of alleviating or absolutely reducing the symptoms of the disease, its progression and preserving the functional status (3). The traditional approach in the treatment of RA is based on the use of conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), glucocorticoids (GC) and non-steroidal anti-inflammatory drugs (NSAIDs) (4). The most commonly used csDMARDs are: methotrexate, sulfasalazine, hydroxychloroquine and leflunomide. Additionally, biological disease-modifying anti-rheumatic drugs (bDMARDs) may be used in the treatment of RA, which include: TNF- $\alpha$  inhibitors, anti-B cell therapy, T-cell costimulatory blockers, IL-6 and IL-1 inhibitors and protein kinase inhibitors (5).

### The aim

The aim of the research was to assess the impact of sociodemographic characteristics, laboratory parameters of interest and therapeutic modalities on the functional ability of patients with RA.

### Patients and methods

The research in the form of a retrospective cross-sectional study was conducted at the Clinic of Rheumatology of the Military Medical Academy in Belgrade. The study included 109 patients diagnosed with rheumatoid arthritis treated during 2019 and 2020. All patients underwent a detailed clinical examination, which included taking anamnestic data, physical examination and standard laboratory diagnostics, which, in addition to classical biochemical analyzes, also included determination of sedimentation rate, reactive C protein (CRP), rheumatoid factor (RF) and anticitrulline antibodies (antiCCP). The functional status of the subjects was assessed based on of the HAQ questionnaire, while the disease activity was assessed based on of the DAS-28 index. The HAQ-DI questionnaire contains questions classified into 8 categories, and they refer to activities related to everyday life (retrieving objects, catching, getting up, getting dressed, feeding, walking, usual daily activities, personal hygiene). By scoring the answers from the questionnaire, the value of the HAQ-DI index is obtained, ranging from 0 (no limit) to 3.0 (complete disability), with values above 1.0 being considered a significant decrease in the patient's functional ability. Subjects were divided into groups according to: gender, age, HAQ values, DAS 28 and therapeutic modalities they applied.

### Data processing and statistical analysis

Standard descriptive and analytical statistical methodology was used in data processing. The following statistical parameters were presented by

descriptive statistical analysis: arithmetic mean, standard deviation, min-max range, absolute frequency (N) and structure index (%). Analytical statistical methodology was used to measure the statistical significance of mutual differences in the average values of certain characteristics of the participants.

The comparison of the average values of the numerical features between the two independent groups of participants was performed by Student's t-test. For categorical variables, the comparison of the frequency of occurrence of individual modalities of attribute features between groups was performed by Pearson  $\chi^2$  test.

The comparison of the average values of the numerical features between the three independent groups of subjects was performed by the ANOVA test. To measure the correlation of certain features, a correlation analysis (Spearman) was performed. PLUM regression analysis was performed to measure the predictor influence of certain features on the functional ability index.

### Statistical analysis

Statistical analysis was performed using Excel programs from Microsoft Office software package and PASW program, version 18.0. An estimation error level of less than 5% (0.05) was used as the statistical significance threshold. The results of the statistical analysis are presented in a table.

### Results

The conducted study included 109 subjects, 26 males (23.85%) and 83 female participants (76.15%). Table 1 shows the demographic characteristics of the participants.

The average age was  $60.46 \pm 10.97$  years. The average age of men was  $60.12 \pm 8.126$  years, and women  $60.57 \pm 11$ , and statistically did not differ significantly, while a statistically significant difference was observed between the age categories of participants ( $p < 0.0001$ ).

Clinical characteristics and laboratory parameters of importance are shown in Table 2. Based on the values of the disease activity index, it can be noticed that 48.62% of the participants had a highly active disease (DAS28 > 5.1). In more than one third of the subjects, the values of the HAQ index were > 1, which indicates a significant decrease in functional ability.

**Table 1.** Demographic characteristics of participants

Demographic characteristics		N (%)	Age	p
Gender	Men	26 (23.85%)	60.12 ± 8.126	> 0.005
	Women	83 (76.15%)	60.57 ± 11.757	
Age	31-50 years	16 (14.68%)	42.06 ± 6.038	< 0.0001
	51-65 years	55 (50.46%)	58.18 ± 4.423	
	> 65 years	38 (34.86%)	71.50 ± 5.336	

**Table 2.** Clinical characteristics and laboratory parameters of importance to the subject

Clinical characteristics		N (%)
DAS 28*	< 2.6	19 (17.43%)
	3.2-5.1	37 (33.94%)
	> 5.1	53 (48.62%)
HAQ-DI#	0-0.5	16 (14.68%)
	0.55-1	57 (52.29%)
	> 1	36 (33.03%)
Number of comorbidities	RA	20 (18.35%)
	RA + 1	35 (32.11%)
	RA + 2	32 (29.36%)
	RA + 3	14 (12.84%)
	RA + 4	8 (7.34%)
Laboratory parameters		N (%)
Sedimentation	↔	18 (16.51%)
	↑	91 (83.47%)
RF	↔	44 (40.37%)
	↑	65 (59.63%)
Anti-CCP	↔	38 (34.86%)
	↑	71 (65.14%)
CRP	↔	44 (40.37%)
	↑	65 (59.63%)
Leukocytes	↔	105 (96.33%)
	↑	4 (3.67%)
Platelets	↔	84 (77.06%)
	↑	8 (7.34%)
	↓	17 (15.6%)

↔ parameter values are within the reference range;

↑ parameter values are above the reference range;

↓ parameter values are below the reference range;

\* DAS28: 0-2.5: disease remission;

2.6-3.1: low disease activity ;

3.2-5.0: moderate disease activity;

&gt; 5.1: high disease activity;

#HAQ DI: 0-3.0 indicates a range of functional abilities without limitation to complete disability.

**Table 3.** Therapeutic modalities in the study group

Therapeutic modalities		N (%)
Therapeutic modality I	GC+MTX*	30 (27.52%)
Therapeutic modality II	GC+other DMARD <sup>#</sup>	27 (24.77%)
Therapeutic modality III	GC+multiDMARD <sup>§</sup>	34 (31.19%)
Therapeutic modality IV	GC+biological therapy	18 (16.52%)
Additional RA therapy		N (%)
Folic acid	Does not apply	37 (33.94%)
	Applies	72 (66.06%)
Vitamin D	Does not apply	17 (15.6%)
	Applies	92 (84.4%)
NSAID	Does not apply	62 (56.88%)
	Applies	47 (43.12%)
Bisphosphonates	Does not apply	95 (87.15%)
	Applies	14 (12.85%)

\* MTX - methotrexate; <sup>#</sup> other disease modifying drug - chloroquine, hydroxychloroquine, sulfasalazine, leflunomide;  
<sup>§</sup> multiDMARD - more than one disease modifying drug

Table 3 shows the therapeutic modalities used in the treatment of the subjects. The largest number of subjects (31.19%) received GC and more than one disease modifying drug (MTX/chloroquine, hydroxychloroquine, sulfasalazine, leflunomide), while the smallest number of subjects received biological therapy (etanercept, tofacitinib, rituximab, tocilizumab, golimumab) (16.52%).

The assessment of the functional ability of the subjects based on the HAQ-DI index, in relation to the applied therapeutic modalities is shown in Table 4.

The analysis of the obtained results showed that the highest number of subjects with a high HAQ-DI index indicating highly impaired functional ability was observed in the group of subjects who used GC in combination with several scDMARD drugs (41.2%). Moderately impaired functional acti-

vity was highest in the group of patients treated with GC + MTX while the largest number of regular HAQ-DI index findings were noted in the group that used biological therapy (44.4%). HAQ value of the index, significant differences compared to the administered therapeutic modality ( $p = 0.004$ ).

Table 5 shows the correlation of the HAQ-DI index with clinical and laboratory parameters. The Spearman index determined the correlation of HAQ index values in relation to gender, age, present comorbidities and values of sedimentation of erythrocytes, CRP and anti-CCP in serum.

The values of HAQ as a parameter for the assessment of functional ability, statistically significantly correlated with the values of erythrocyte sedimentation rate ( $p < 0.05$ ), CRP values ( $p < 0.01$ ) and anti-ACCP ( $p < 0.05$ ).

**Table 4.** Assessment of functional ability in relation to applied therapeutic modalities

	Therapeutic modality I	Therapeutic modality II	Therapeutic modality III	Therapeutic modality IV	$\chi^2$	p
HAQ-DI	N (%)	N (%)	N (%)	N (%)		
Neat finding	1 (3.3%)	5 (18.5%)	2 (5.9%)	8 (44.4%)	18.914	0.004
Moderately impaired functional ability	19 (63.3%)	14 (51.9%)	18 (52.9%)	6 (33.3%)		
Highly impaired functional ability	10 (33.3%)	8 (29.6%)	14 (41.2%)	4 (22.3%)		
Total	30 (100%)	27 (100%)	34 (100%)	18 (100%)		

HAQ-DI: orderly finding (0-0.5);

Moderately impaired functional ability (0.55-1);

Highly impaired functional ability (> 1);

Therapeutic modality I: GC + MTX;

Therapeutic modality II: GC + other DMARDs (chloroquine, hydroxychloroquine, sulfasalazine, leflunomide);

Therapeutic modality group III: GC + multiDMARD (more than one scDMARD);

Therapeutic modality IV: GC + bDMARD

**Table 5.** Correlation of HAQ index with clinical and laboratory parameters

Spearman's correlation	Gender	Age	Comorbidity	AntiCCP	CRP	SE
HAQ	.135	.084	.114	.210*	.267**	.225*
p	.162	.385	.239	.029	.005	.018
N	109	109	109	109	109	109

\*p &lt; 0.05 ; \*\*p &lt; 0.01

PLUM regression was used to assess the predictor effect of individual variables on the degree of functional ability, and the results are shown in Table 6.

The results of the PLUM analysis showed that there was a statistically significantly different increase in the value of HAQ-DI from a normal finding to a moderately impaired functional ability. Up to the highly impaired functional ability, the values of HAQ-

DI in relation to the monitored variables were without statistical significance. The results showed that gender and age were not significant predictors of functional ability, while a lower number of comorbidities was a significant predictor of better functional ability. Therapeutic modalities GC + MTX (p = 0.023) and GC + multiDMARD (p = 0.002) were statistically significant predictor.

**Table 6.** Influence of individual variables on the degree of functional ability of RA patients

-2 Log Likelihood		172.826	p = 0.005	Cox and Snell = 0.237	Nagelkerke = 0.276	Sig.
Parameter Estimates		Estimate	95% Confidence Interval		Sig.	
			Lower Bound	Upper Bound		
Threshold	[HAQ: moderately impaired]	-2.865	-4.795	-.934	.004	.004
	[HAQ: highly impaired]	.225	-1.598	2.047	.809	.809
Location	[Gender: male]	.582	-.352	1.516	.222	.222
	[Gender: female]	0 <sup>a</sup>	.	.	.	.
	[Age: 31-50 years]	-.078	-1.458	1.302	.912	.912
	[Age: 51-65 years]	.212	-.674	1.098	.640	.640
	[Age : > 65 years]	0 <sup>a</sup>	.	.	.	.
	[Comorbidity: without]	-1.984	-3.883	-.085	.041	.041
	[Comorbidity: 1]	-1.565	-3.301	.170	.077	.077
	[Comorbidity: 2]	-1.760	-3.462	-.057	.043	.043
	[Comorbidity: 3]	-.859	-2.717	.999	.365	.365
	[Comorbidity: 4]	0 <sup>a</sup>	.	.	.	.
	[Therapeutic modality I]	1.447	.200	2.694	.023	.023
	[Therapeutic modality II]	1.150	-.110	2.410	.074	.074
[Therapeutic modality III]	1.952	.693	3.211	.002	.002	
[Therapeutic modality IV]	0 <sup>a</sup>	.	.	.	.	

## Discussion

Rheumatoid arthritis is a disease that leads to irreversible damage to the joints, deformities, consequent incapacity for work and reduced quality of life. Therefore, it is necessary to start treatment immediately after the diagnosis. The study involved 109 participants, 26 men and 83 women, with an average age of 60.46 ± 10.97 years (Table 1). The ratio of female and male subjects suffering from RA is approximately 3:1 and is consistent with the

results of other authors (6). Values of laboratory parameters indicating disease activity were elevated in most subjects (RF: 59.63%; anti-CCP: 65.14%; sedimentation: 83.47%; CRP: 59.63%), which is usually associated with aggressive progression illness (7).

The primary goal in the treatment of patients with RA is to establish control of disease activity. In the conducted research, 48.62% of participants had a highly active disease (DAS28 > 5.1) (Table 2). The results of numerous studies indicate a better

functional status in the group of patients in whom treatment with the disease by modifying drugs was started immediately after the diagnosis (8-11). Methotrexate is still the "gold standard" for the treatment of RA and is most commonly used as the drug of first choice of all scDMARDs (12-16). In the conducted research, the largest number of subjects (31.19%) received a therapeutic modality that included GC and more than one scDMARD (MTX + chloroquine/hydroxychloroquine/sulfasalazine/leflunomide), while the smallest number of subjects was on biological therapy (16.52%). (Table 3). The combined use of more than one disease-modifying drug is a widely used pharmacotherapeutic strategy. This type of therapy enables the achievement of better therapeutic goals and is not associated with a significant increase in the rate of adverse events compared with monotherapy (17). In their study, Bergstra et al. showed that continuous low disease activity was better achieved with combination therapy when compared with MTX monotherapy (18). Similar results were obtained by other authors whose studies showed that scDMARDs in monotherapy might be insufficiently effective or that the degree of their effectiveness decreased over time, maintaining the desired therapeutic response in only 5-15% of patients during five-year follow-up (19, 20). Abud-Mendoza and colleagues in their study concluded that the use of therapeutic modalities with higher doses of methotrexate in combination with prednisone or another combination of scDMARD reduced the percentage of patients in need of biological therapy, and the distribution of therapeutic modalities in our study is consistent with their results (17). Concomitant use of GC in combination with scDMARD achieves a higher remission rate, slows down joint destruction and initiates a faster clinical response. In addition to direct therapeutic benefits, clinical studies have suggested that concomitant administration of GK and DMARDs may prolong the effective duration of csDMARD, as well as reduce the occurrence of csDMARD-related side effects (21). Current treatment guidelines and recommendations support the initiation of bDMARD therapy in RA patients who do not achieve their therapeutic goals by receiving csDMARD (22).

The quality of life of patients with RA depends, among other things, on the functional status of the patient, a higher degree of functional incapacity significantly affects everyday life and work ability (23). The results of the research indicate that in more than one third of the participants the values of the HAQ index are higher than 1, which indicates a significant decrease in functional ability (Table 1). Highly impaired functional ability in patients with RA has been reported in other studies (24). The level of the HAQ-DI index is an important instrument for measuring and monitoring the success/failure of treatment and improving the functional ability of patients with RA (25). The values of the HAQ index differ significantly statistically in relation to the applied therapeutic modality ( $p = 0.004$ ). The analysis of the obtained results shows that the correct finding of the HAQ-ID index in the largest percentage was recorded in the group of subjects who applied biological therapy (22%), but it should be

emphasized that there was a small number of subjects (16.52 %) in this group. Given that RA is a progressive disease, it is important to maintain or improve the functional capacity of the RA patient. Accordingly, the results of the conducted research indicate that 63.3% of participants who used the combination of GC + MTX have moderately impaired functional ability (Table 4). Studies suggest that the application of both scDMARD and bDMARDs leads to a statistically significant decrease in HAQ-DI index values (26), while Kopciuch et al. in their study show that lower HAQ-DI index values are achieved when bDMARDs are more likely to achieve remission (27).

In the conducted research, there is no statistically significant difference in the values of the HAQ index in relation to the gender, age and number of comorbidities of the participants. The results of gender influence are incoherent and there are those that confirm that the values of the HAQ index are higher in women than in men (28). When it comes to the influence of age, the results of other studies suggest an inversely proportional effect of age on HAQ-DI values, probably due to the increased number of comorbidities in this group of patients (29). The values of HAQ-DI parameters in the conducted study significantly correlate with the values of erythrocyte sedimentation, CRP and anti-CCP values in serum (Table 5). Given that these laboratory parameters are significant predictors of disease course, their elevated values may be associated with reduced functional capacity of patients with RA (30). A study conducted in Turkey on a small number of subjects did not confirm an association between elevated values of anti-CCP and HAQ-DI index, which is contrary to the results obtained in our study (31). On the other hand, the research conducted on the territory of the Southern Serbia confirms the statistically significant influence of elevated values of sedimentation and CRP on HAQ-DI values, which is in line with the results of our research (30).

In order to identify the variables that most significantly correlate with the functional status of the participants, regression analysis was applied. The results indicate that functional ability is impaired in the group of subjects without comorbidity and in those with a lower number of comorbidities, while in patients with a higher number of comorbidities this connection for functional ability was not shown. In order to explain the obtained results, it is necessary to additionally consider the length of presence and severity of associated diseases. In addition, the results of the regression analysis indicate that the combination of GC + MTX ( $p = 0.023$ ) and GC + multiDMARD ( $p = 0.002$ ) can significantly contribute to better functional ability in patients with RA (Table 6). The obtained results confirm that the use of MTX, alone or in combination with other drugs, still represents an effective and most often prescribed disease modifying drug in patients with RA.

## Conclusion

Based on the conducted research, we can conclude that methotrexate, alone or in combination with other drugs, can significantly contribute to

better functional ability and is an effective therapeutic strategy in most patients with rheumatoid arthritis.

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doi:10.5633/amm.2021.0409**UTICAJ TERAPIJSKIH MODALITETA NA FUNKCIONALNU SPOSOBNOST BOLESNIKA SA REUMATOIDNIM ARTRITISOM***Nikola Krstić<sup>1</sup>, Ivana Kostić<sup>2</sup>, Ana Petronijević<sup>3</sup>, Ivana Damjanović<sup>4</sup>*<sup>1</sup>Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija<sup>2</sup>Dom zdravlja Niš, Niš, Srbija<sup>3</sup>Univerzitetska dečja klinika, Beograd, Srbija<sup>4</sup>Univerzitet u Nišu, Medicinski fakultet, Departman za farmaciju, Niš, Srbija

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Reumatoidni artritis (RA) je hronična, sistemska, inflamatorna bolest. Progresivnost bolesti i oštećenje zglobova izazvano RA može značajno uticati na sposobnost funkcionisanja. Zlatni standard za procenu funkcionalnog statusa u RA je HAQ upitnik.

Cilj rada bio je da se proceni uticaj sociodemografskih karakteristika, laboratorijskih parametara od interesa i terapijskih modaliteta na funkcionalnu sposobnost bolesnika sa RA.

Retrospektivna studija preseka uključivala je 109 bolesnika, 29 muškaraca i 83 žene, sa dijagnozom RA. Prikupljeni su podaci o demografskim i kliničkim karakteristikama bolesnika, laboratorijskim nalazima i primenjenim terapijskim modalitetima. Za statističku analizu korišćeni su *Microsoft Office Excel* i *PASW*. Nivo greške procene manji od 5% (0,05) korišćen je kao prag statističke značajnosti.

Rezultati studije ukazuju da više od trećine učesnika ima HAQ-DI indeks veći od 1, što ukazuje na značajno smanjenje funkcionalne sposobnosti. Najveći broj ispitanika sa visokim HAQ-DI indeksom (41,2%) zabeležen je u grupi ispitanika koji su koristili glukokortikoide u kombinaciji sa nekoliko sintetičkih lekova koji modifikuju bolest (GK + *multiDMARD*). Vrednosti HAQ-DI indeksa statistički se značajno razlikuju u odnosu na primenjene terapijske modalitete ( $p = 0,004$ ). HAQ-DI indeks značajno je korelirao sa vrednostima brzine sedimentacije ( $p < 0,05$ ), CRP ( $p < 0,01$ ) i anti-CCP ( $p < 0,05$ ). PLUM regresiona analiza pokazala je da je funkcionalna sposobnost oštećena u grupama ispitanika bez komorbiditeta i sa manjim brojem komorbiditeta. Pored toga, rezultati regresione analize ukazuju na to da terapijski modaliteti GK + metotreksat ( $p = 0,023$ ) i GK + *multiDMARD* ( $p = 0,002$ ) mogu značajno doprineti boljoj funkcionalnoj sposobnosti bolesnika sa RA.

Metotreksat, sam ili u kombinaciji sa drugim lekovima, može značajno doprineti boljoj funkcionalnoj sposobnosti i efikasan je u lečenju većine bolesnika sa RA.

*Acta Medica Medianae 2021;60(4):63-71.***Ključne reči:** reumatoidni artritis, metotreksat, HAQ-DI

## EPIDEMIOLOGY OF ESOPHAGEAL CANCER, RISK FACTORS AND POSSIBILITIES FOR PREVENTION

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The incidence and mortality of esophageal cancer in developed countries increased in the last four decades, as opposed to the reduced incidence and mortality of other cancers. Esophageal cancer is the eighth most common and the sixth leading cause of cancer death in the world. In the last 3 decades, there has been a shift in the leading histological type of esophageal cancer in developed countries from esophageal squamous cell to esophageal adenocarcinoma. Risk factors lead to the appearance of precancerous conditions, squamous dysplasia as a precursor of esophageal squamous cell carcinoma and Barrett's esophagus as a precursor of esophageal adenocarcinoma. Tobacco, alcohol, hot Mate drink and pickled vegetables are associated with increased risk of esophageal cancer, while increased fruit and vegetable intake could have a preventive effect on esophageal cancer. The evidence about the influence of distal esophageal sphincter relaxants and histamine 2 receptor antagonists is contradictory. There is an inverse association of *Helicobacter pylori* infection and Barrett's esophagus and esophageal adenocarcinoma. We have low certainty evidence about the effect of infection with human papilloma virus and different chemical carcinogens like polycyclic aromatic hydrocarbons, N-nitroso compounds, acetaldehyde, and fumonisins on esophageal cancer. A number of predisposing conditions like gastro-esophageal reflux disease, Barrett's esophagus, obesity, hiatal hernia, achalasia, tylosis, and Plummer Vinson syndrome may increase the risk of esophageal cancer. Primary prevention of esophageal cancer consists of lifestyle changes like smoking cessation, stopping of alcohol consumption and increased intake of fruits and vegetables. Secondary prevention consists of chemoprevention, screening and monitoring program.

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**Key words:** esophageal cancer, risk, prevention

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### History

Although the main evidence for esophageal cancer was collected during the 19<sup>th</sup> and 20<sup>th</sup> century, the first data related to the disease came from Egypt around 3000–2500 BC. In a "Smith sur-

gical papyrus" written by Edwin Smith reparation of the esophagus after perforation is described, but without a clear emphasis on cancer (1). Galen, 125-200 AD, a well-known physician and philosopher of the Roman Empire, published a paper on the causes and symptoms of esophageal cancer, pointing out the possibility that meaty growths partially or completely obstruct the passage of food through the esophagus (1). Avicenna, who is considered the father of modern medicine, in one of the papers entitled "Canon of Medicine" published in 1025, pointed out that dysphagia is the most significant symptom of esophageal tumors (2). Earliest evidence came also from some areas of China which are characterized by a high incidence of esophageal cancer. As the main symptom in patients, "Ye Ge" is mentioned, which means dysphagia and belching. Dysphagia syndrome was reported among the inhabitants of Henan Province 2000 years ago. Additionally, a condition called "ge shi bing" has been described in Linxian Province. Arab clinician Avenzoar (1090-1162) gave some very useful advice regarding the treatment of patients with esophageal cancer (2). He recommended "injecting food into the

stomach through a silver tube as well as using nutrient solutions". One of the leading physicians of the 16<sup>th</sup> century, the Frenchman Jean Francois Fernel (1497-1558) described scirrus and other tumors that block the esophagus causing dysphagia. Surgeon John Casaubon, who died of esophageal cancer in 1691, was the first who correctly described the symptoms of the disease (2).

### Epidemiology

In developed countries, the incidence and mortality of esophageal cancer increased in the last three decades, as opposed to the reduced incidence and mortality of other cancers. Between 1973 and 2020, the incidence rate of the most common cancers including breast, colon, and lung was stable or decreased in the United States. There has been an increase in prostate cancer and melanoma, but this is small compared to the 600% increase in the incidence of esophageal adenocarcinoma (EAC) in the same period. The risk of developing esophageal cancer increases with age and is greatest in the seventh decade of life. The prognosis is generally poor. The 5-year survival rate ranges between 10 and 13%, and only 1 in 5 patients survives 3 years or more (3). Therefore, the esophageal cancer prevention strategy must take into account its changing epidemiology. Esophageal cancer is the eighth most common and the sixth leading cause of cancer death in the world (4, 5). The average incidence in the world is 11.5 cases per 100,000 in men and 4.7 per 100,000 in women (6). There are large geographical variations in the world in terms of incidence and mortality from esophageal cancer. The incidence, as well as mortality, can vary from one part of the world to another up to 500 times (7). It is known that Asian countries, especially China, India, Pakistan, and Japan, have the highest rates of esophageal cancer in the world (8). Asian belt of esophageal cancer (the region with the highest incidence rate of esophageal cancer in the world) originates from Turkey, goes through Iraq, Iran and Kazakhstan and ends in North China. The incidence of esophageal cancer in this zone is 100 cases per 100,000 inhabitants. The incidence is equal in men

and women and is more common in people of Turkish and/or Mongolian descent than those of Indo-European descent. Most frequent histological type in the Asian belt is esophageal squamocellular cancer. The overall incidence rate is twice as high in less developed countries than in developed countries with the highest incidence rates in Asia (9). The highest EC incidence rates in the standard age structure population were recorded in China, South and East Africa, and Japan. More than 90% of esophageal cancers are either squamous cell carcinomas (SCC) or adenocarcinomas (AC). Other cancers, melanomas, leiomyosarcomas, carcinoids and lymphomas occur less frequently in the esophagus.

The epidemiology of the two histological types of esophageal cancer (squamous cell and adenocarcinoma) differs significantly. The most common histological type in the world is esophageal squamous cell carcinoma (ESCC) while in the USA and Europe, esophageal adenocarcinoma (EAC) currently predominates (10).

In the last four decades, there has been a shift in the leading histological type of esophageal cancer in developed countries from esophageal squamous cell to esophageal adenocarcinoma (11, 12). In developed countries, esophageal squamous cell carcinoma occurs three to four times more often in men than in women, while in the Asian belt of esophageal cancer the incidence is the same among men and women (13).

The risk of developing of esophageal cancer increases with age. On average, EAC is formed ten years earlier than ESCC, on average at the age of 53 (11).

### Risk factors

Craver (14) and Watson (15), dealing with the problem of risk factors for the development of esophageal cancer listed excessive use of alcohol and tobacco, low socioeconomic status, poor oral hygiene and consumption of hot beverages as one of the main ones. Craver also quoted an article from Argentina that pointed out that consuming Mate drink, consumed in larger quantities in South America, as an additional risk factor (Table 1) (14).

**Table 1.** Risk factors for esophageal cancer

Habits	tobacco, alcohol, hot drinks, mate drinks, pickles
Nutritional deficiencies	insufficient intake of fruits and vegetables, deficiency of vitamins and minerals
Medicines	relaxing the lower esophageal sphincter, histamine 2 receptor antagonists
Infections	Helicobacter pylori, human papillomavirus
Chemical	polycyclic aromatic hydrocarbons, nitrosamines, acetaldehyde
Physiological or pathological predisposing conditions	gastroesophageal acid reflux, hiatal hernia, achalasia, tylose
Occupational exposure	silicone and asbestos
Low socioeconomic status	

Risk factors lead to the appearance of pre-cancerous conditions, squamous dysplasia as a precursor of esophageal squamous cell carcinoma and Barrett's esophagus as a precursor of esophageal adenocarcinoma.

### **Habits**

#### *Tobacco*

Current and former smokers are at higher risk for developing esophageal cancer (EC). Smoking significantly increases the risk of developing ESCC and less of developing EAC. Active smokers have a 3 to 7 times higher risk of developing ESCC and about 2 times higher risk of developing EAC (16). The risk of developing EC is directly correlated with the number of cigarettes smoked as well as the length of smoking experience (17, 18). The risk persists for 30 years after smoking cessation (19).

#### *Alcohol*

Continuous alcohol intake significantly increases the risk of developing ESCC but not EAC (17, 18) although there are observational studies that suggest a possible link between alcohol and EAC. Exposure to tobacco and alcohol has a synergistic effect on the risk of developing EC. The International Agency for Research on Cancer (IARC) classified alcohol as a known cause of EC (20).

#### *Mate*

The hot Mate drink, which is an extract of the herb *Ilex paraguayensis*, and which is characteristic of the countries of South America, is considered a potential risk factor for the formation of EC. The carcinogenicity of mate beverage is considered to be a consequence of repeated thermal damage and exposure to polyaromatic cyclic hydrocarbons. The first study conducted in Uruguay (21) found a relative risk of 12.2 for those who drank 2.5 l of mate beverage per day, and the risk was even higher, 22.6 for those who consumed alcohol and smoked. Another study (22) found a risk of 2.8 in people who drank more than 1 liter of mate per day, and the risk was even higher (7.1) in those who drank alcohol and smoked at the same time. In 1991, the IARC classified the hot mate beverage as a probable carcinogen for humans (Group 2A) (20).

#### *Pickled vegetables*

Environmental studies have indicated an increased risk of EC in areas where higher amounts of pickles are used in the diet. It is assumed that fungi and yeasts that grow in pickles can release carcinogens (23). Results of epidemiological studies are not consistent. While some of them indicated a significant increase in the incidence of EC in others, the consumption of pickled vegetables did not affect the incidence of EC. The IARC concluded in 1993 that pickles were probably carcinogenic to humans (Group 2B) (24).

### **Nutrition deficits**

#### *Fruits and vegetables*

The World Cancer Research Foundation (WCRF-AICR) announced in 2016 that there was evidence, although mostly from observational studies, that increased fruit and vegetable intake could have a preventive effect on esophageal cancer (25). Combining all the evidence, it was concluded that the risk of developing EC is reduced by about 20% for every 50 g of fruit and vegetables consumed daily (25). The results of recently completed cohort studies support this view (26, 27).

#### *Vitamin and mineral deficiency*

Some areas with a very high incidence of EC, such as Linxian, China, are characterized by a large deficiency of vitamins and minerals in the diet. However, two large randomized controlled clinical trials (28, 29) and a Cochrane systematic review (30) have failed to demonstrate a reduction in the incidence of EC after vitamin and mineral supplementation over 5 to 6 years.

### **Medicines**

#### *Distal esophageal sphincter relaxants (DES)*

Asthma medications ( $\beta$ -adrenergic agonists and theophylline-containing drugs), calcium antagonists, nitroglycerin, and benzodiazepines relax the lower esophageal sphincter and increase acid reflux. Therefore, they can increase the risk of developing BE and EAC. Observational studies, however, have yielded contradictory results (31, 32). Some of them indicate that medications that lead to DES relaxation contribute to the formation of about 10% of all EACs (31, 32).

#### *Histamine 2 receptor antagonists*

H2 blockers can reduce EAC risk by reducing the acidic content of gastroesophageal reflux. However, they may increase EAC risk by neutralizing gastric pH, which allows bacteria to proliferate in the stomach which may result in increased production of carcinogens such as nitrosamines and acetaldehyde (12). One study examined the association of H2 blockers and ESCC, finding no association (33). Another recent study revealed possible increased risk of esophageal cancer after H2 antagonists (25).

### **Infections**

#### *Helicobacter pylori infection*

Results of 3 recently published meta-analyses have indicated that gastric colonization with *Helicobacter pylori* is associated with about a 50% reduction in the risk of developing EAC (34-36). One meta-analysis indicated an inverse association of *H. pylori* and BE (37). There is no association of *Helicobacter pylori* with ESCC.

### *Human papillomavirus*

Oncogenic types of HPV, most commonly HPV 16 and HPV 18, are the causes of cervical cancer, and may play a significant role in the development of epithelial cancers of the vulva, anus, penis, and oropharyngeal cavity. The role of HPV in the development of EC is controversial. Despite the fact that the association of HPV and esophageal tumors has not been established in most studies, there are also studies that found this association in about 75% of cases. In 2006, the IARC concluded that there was insufficient evidence to indicate carcinogenicity of HPV in the esophagus (38).

### **Chemical carcinogens**

#### *Polycyclic aromatic hydrocarbons*

Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete roasting of organic substances. The main sources of PAHs are smoking and air pollution. The causal link between PAHs or substances containing PAHs and cancer has been established for skin, lung and bladder cancers. However, the facts indicating the association of PAHs or substances containing PAHs and EC are based solely on environmental studies (39).

#### *N-Nitroso compounds (NNC)*

NNCs are potent animal carcinogens that can affect the development of cancers of the nasal cavity, esophagus and stomach. Their role in developing cancer in humans has not been established with certainty. A recent umbrella review of 72 meta-analyses examining the association of nitrosamines and EC found that consumption of processed meat, the main source of nitrite and nitrosamines, was associated with a higher risk of developing EC (40).

#### *Acetaldehyde*

Ethanol is converted to acetaldehyde by alcohol dehydrogenase and then to acetate by acetaldehyde dehydrogenase. In 1999, the IARC classified acetaldehyde as carcinogen for animals and a possible carcinogen for humans, mostly because there was insufficient evidence from human studies at the time (41).

#### *Fumonisin*

Fumonisin is a toxin secreted by the fungus *Fusarium verticillioides* (formerly *Fusarium moniliforme*), which grows mainly on maize. Evidence for the carcinogenicity of fumonisin in humans is uncertain and has mainly come from environmental studies. An observational study indicated no link between fumonisin exposure and EC (42).

### **Predisposing conditions**

#### *Gastroesophageal reflux disease (GERD)*

Symptomatic GE reflux is perhaps the strongest risk factor for developing EAC (43). People with recurrent reflux symptoms have 8 times higher risk of developing EAC (43). In contrast to EAC, acid reflux is not considered a risk factor for the development of ESCC (43).

#### *Barrett's esophagus*

Barrett's esophagus (BE) is considered to be one of the most significant risk factors for the development of EAC. Patients with BE have 40 times higher risk of developing EAC (44). There is no association of BE and ESCC (44). The overall risk of developing cancer in a patient with BE is about 0.5% per year. However, most patients with BE will not develop EAC.

#### *Obesity*

Previous epidemiological studies have indicated that a higher body mass index increases the risk of developing EAC. A recently published meta-analysis of 22 case-control and cohort studies found that the risk of EC was significantly higher in obese patients (45). Obesity also increases the risk of developing gastroesophageal reflux disease (46), and Barrett's esophagus (47) that are primary risk factors for EAC. The trend is the opposite for ESCC. Progressively higher BMI is associated with progressively lower risk for ESCC (48).

#### *Hiatal hernia*

Hiatal hernia may increase the risk for developing EAC due to increased GE acid reflux. Numerous studies have found an increased risk, with relative risk ranging from 2 to 6 times (49).

In contrast to EAC, the risk of developing ESCC is not increased in the presence of a hiatal hernia.

#### *Achalasia*

EC develop in 3% to 7% of patients with achalasia, which is significantly higher than in the healthy population. Recent results indicate a 10 times higher risk of developing ESCC and EAC in patients with achalasia. Corrosive constrictions increase the risk of developing ESCC by about 1000 times (50).

#### *Tylosis*

Genetic defect in the 17q25 region, characterized by hyperkeratosis of the palms and soles, is associated with a high risk of developing ESCC after 65 years of age (51).

### Plummer Vinson syndrome

PVS is also associated with increased risk of developing esophageal cancer (52).

### Exposure to harmful substances

Various studies have indicated that exposure to asbestos may increase the risk of developing EC 2 to 16 times. The IARC has classified asbestos and silicon in the group of human carcinogens (Group 1 carcinogens), mostly because of their effect on lung cancer and mesothelioma (53).

### Low socio-economic status

A large number of epidemiological studies have confirmed that the risk of developing ESCC is

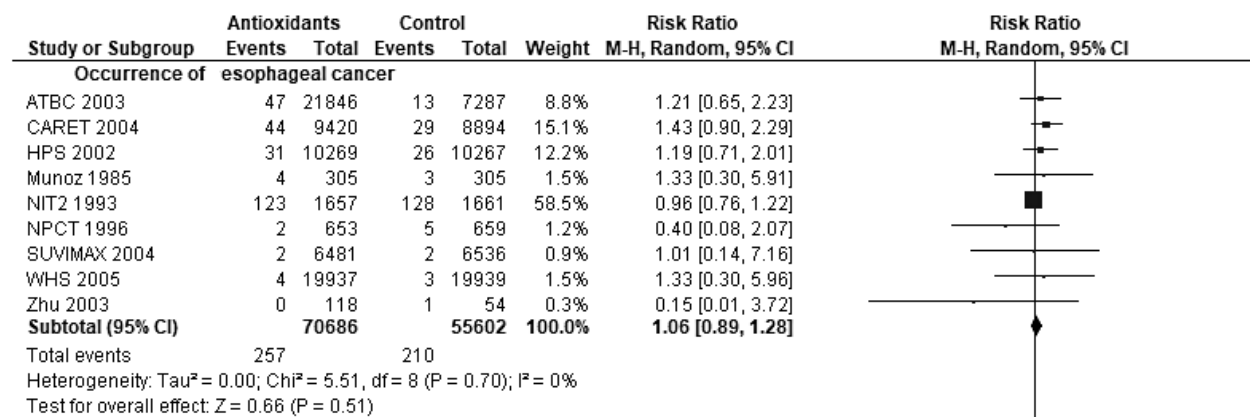
higher in populations with lower socioeconomic status (SES) (13). Higher tobacco and alcohol use or poor nutrition among people with lower SES are responsible for increased risk. Less data is available for EAC (54). Recent studies have indicated that SES is inversely associated with EAC as well, but one study found no association (55).

Population attributable risk (PAR) is defined as the proportion of disease in a population that can be attributed to a particular risk factor.

About 78.7% (95% CI 66.5%-87.3%) EAC compared to 89.4% ESCC cases can be attributed to one or more well-defined risk factors, of which smoking, alcohol, body mass index and GERD contribute the most (Table 2) (56).

**Table 2.** Population attributable risk for esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC)

Risk factor	PAR-EAC
Smoking	39.7%
BMI	41.1%
GERD	29.7%
Low fruit and vegetable intake	15.3%
Combined PAR	78.7%
Risk factor	PAR-ESCC
Smoking	56.9%
Alcohol	72.4%
Low fruit and vegetable intake	28.7%
Combined PAR	89.4%



**Figure 1.** Occurrence of esophageal cancer after supplementation with antioxidants

## Prevention

Prevention of esophageal cancer can be divided into primary and secondary.

### Primary

Primary prevention consists of lifestyle changes that reduce the prevalence of known risk factors like smoking cessation, stopping of alcohol consumption and increased intake of fruits and vegetables (57).

### Secondary

Secondary prevention consists of chemoprevention (use of specific natural or synthetic chemical agents to reverse, suppress, or prevent the progression of precancerous conditions to invasive cancer), screening and monitoring program (58).

## Pharmacological chemoprevention

### Antioxidant supplements

Antioxidants protect against oxidative stress involved in the pathogenesis of many diseases including esophageal cancer. Antioxidant supplements containing beta-carotene, vitamins A, C, E, and selenium have no effect on the incidence of EC (Figure 1) (30).

### NSAIDs, aspirin

A systematic review and meta-analysis of 9 studies concluded that aspirin and other NSAIDs

reduce the risk of EC in a dose-response manner (59). Phase III RCT (AspECT trial: Aspirin Esomeprazole Chemoprevention Trial) evaluated the beneficial and harmful effects of the combination of aspirin and esomeprazole for EC prevention (60). High-dose PPI and aspirin significantly improved outcomes in patients with Barrett's esophagus (60). One RCT in which high doses of IPP were tested in order to suppress acid secretion and reduce BE showed a small but statistically significant regression of BE after elimination of reflux symptoms, which was confirmed by pH monitoring and recording of subjective symptoms.

## Screening - EAC

Screening of patients with BE is still controversial given the lack of evidence that screening has the effect on reducing mortality from EAC (61). The annual rate of neoplastic transformation of BE is 0.5% (61). Standard endoscopy with biopsy is the most reliable in diagnosing BE. There are several limitations of screening like low negative predictive significance, risk, discomfort and cost (Figure 2).

## Supervision - EAC

Endoscopic supervision is recommended due to the association of BE with EAC, slow progression of BE through dysplasia to EAC, increasing incidence of EAC, poor prognosis of EAC when detected in the symptomatic phase, and better prognosis after treatment of dysplasia/neoplasia detected by monitoring (Figure 3) (61).



**Figure 2.** Barrett's esophagus



**Figure 3.** Esophageal cancer

## Conclusion

Esophageal cancer is a very aggressive and common cancer in the world. Although ESCC and EAC differ in histology and geographic distribution, many of their risk factors and carcinogenetic mechanisms are identical. The most common risk factor for

developing EAC is Barrett's esophagus. The most important risk factors for developing ESCC are tobacco, alcohol, and a diet poor in fruits and vegetables. Elimination of risk factors responsible for the development of esophageal cancer can result in reduced incidence and mortality rates.

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## Pregledni rad

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doi:10.5633/amm.2021.0410**EPIDEMIOLOGIJA RAKA JEDNJAKA, FAKTORI RIZIKA I  
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Incidencija raka jednjaka i mortalitet izazvan rakom jednjaka u razvijenim zemljama porasli su u poslednje četiri decenije, za razliku od smanjene incidencije i mortaliteta kod drugih karcinoma. Rak jednjaka je osmi najčešći karcinom i šesti vodeći uzrok smrti od raka u svetu. U poslednje 3 decenije došlo je do promene vodećeg histološkog tipa karcinoma jednjaka, u razvijenim zemljama, sa skvamoznih ćelija jednjaka na adenokarcinom jednjaka. Faktori rizika dovode do pojave prekanceroznih stanja, skvamozne displazije, kao prekursora skvamoznog karcinoma jednjaka i Baretovog jednjaka, kao prekursora adenokarcinoma jednjaka. Duvan, alkohol, topli *Mate* napitak i kiselo povrće povezuju se sa povećanim rizikom od raka jednjaka, dok bi povećan unos voća i povrća mogao preventivno da utiče na rak jednjaka. Dokazi o uticaju relaksansa distalnog ezofagealnog sfinktera i antagonista histamin 2 receptora su kontradiktorni. Postoji inverzna povezanost infekcije *Helicobacter pilori* i Baretovog jednjaka i adenokarcinoma jednjaka. Imamo nisku sigurnost dokaza o uticaju infekcije humanim papiloma virusom i različitim hemijskim kancerogenima, kao što su policiklični aromatični ugljovodonici, N-nitrozo jedinjenja, acetaldehid i fumonizini na rak jednjaka. Brojni predisponirajući uslovi, kao što su gastroezofagealna refluksna bolest, Baretov jednjak, gojaznost, hijatalna hernija, ahalazija, tiloza i *Plummer Vinsonov* sindrom mogu povećati rizik od raka jednjaka. Primarna prevencija raka jednjaka sastoji se od promene načina života, koje obuhvataju prestanak pušenja, prestanak konzumiranja alkohola i povećan unos voća i povrća. Sekundarna prevencija sastoji se od hemoprevencije, skrininga i programa praćenja.

*Acta Medica Medianae 2021;60(4):72-81.*

**Ključne reči:** rak jednjaka, rizik, prevencija

## INVASIVE CANDIDIASIS IN PRETERM NEWBORN: A CASE REPORT

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*Candida* is the main etiologic factor in the development of invasive fungal infections in children in the hospital environment. We reported our autopsy findings from clinically undiagnosed neonatal invasive candidiasis. Male preterm newborn 27 gestational weeks old died after 24 days after delivery with clinical diagnosis of intracranial and intrapulmonary hemorrhage, threatened with surfactant, antibiotics and invasive mechanical ventilation. Histologically, multiple colonies of fungi were found in lung blood vessels, kidney, suprarenal gland, brain, endocardial and myocardial tissue. In the liver, centrilobular necrosis was found.

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**Key words:** *Candidiasis, invasive, preterm newborn*

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### Introduction

The majority of candidemia cases are noted among one year olds (particularly in the neonatal period) (1). Invasive candidiasis ended in mortality in 14-40% of the cases and with significant neurological and developmental abnormalities in 30-70% of surviving children (2).

We reported our autopsy findings from clinically undiagnosed neonatal invasive candidiasis in a preterm newborn.

### Case report

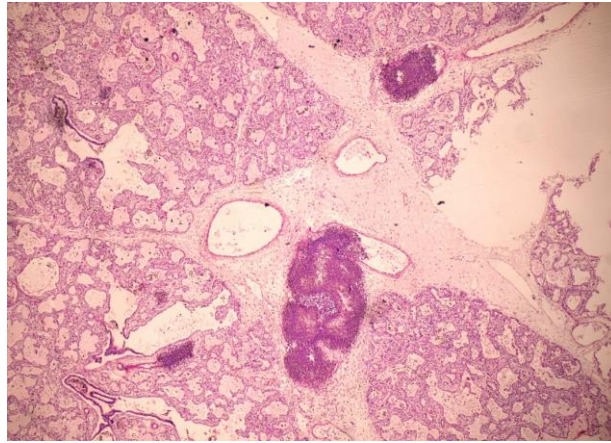
Male preterm newborn 27 gestational weeks old (body weight 1040 g, crown-heel length 34 cm,

head circumference 25 cm) was treated with surfactant, antibiotics (ampicillin, gentamicin, meropenem and piperacillin/tazobactam) and invasive mechanical ventilation in the Intensive Care Unit, at the Pediatric Clinic, Clinical Center Niš Serbia. In the culture of aspirated fluid, *Pseudomonas aeruginosa*, *Klebsiella* spp. (at the second postnatal week), and *Stenotrophomonas maltophilia* (at the third postnatal week) were found.

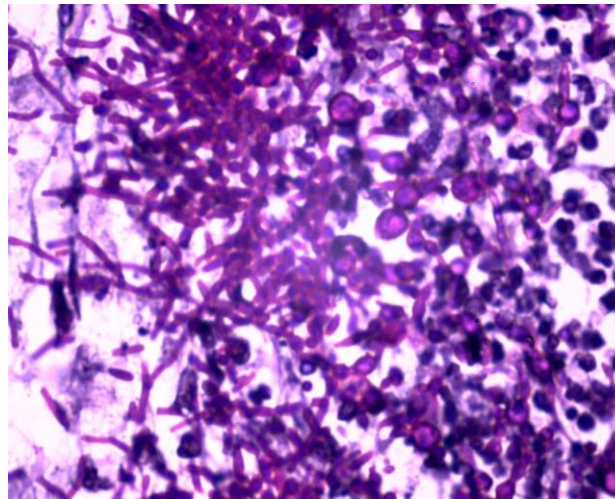
A newborn died 24 days after the delivery with clinical diagnosis of intracranial and intrapulmonary hemorrhage.

At autopsy, diffuse petechial skin hemorrhages were found on male preterm newborn (body weight 1460 g, body length 40 cm, toe-heel length 52 mm, biparietal diameter 7 cm, crown-rump length 21 cm). In the left cerebral ventricle hemorrhage was found. Lung and liver parenchyma were heavily congested.

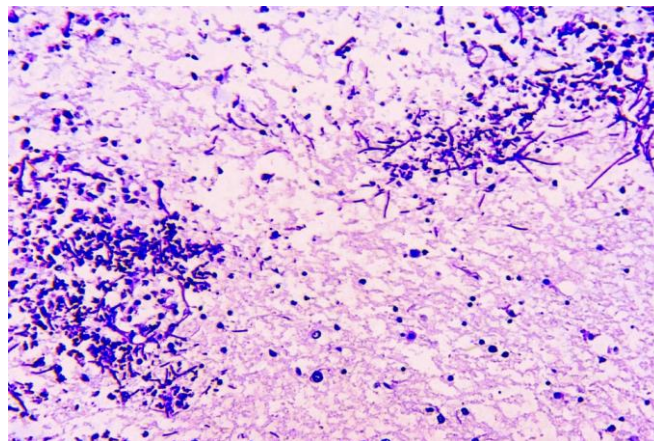
Histologically, using alcian blue-periodic acid-Schiff (AB-PAS) stain at pH 2.5, multiple colonies of fungi were found in lung blood vessels (Figure 1), composed of spherical or ovoid blastospores or yeast cells, about 5 µm in diameter, elongated pseudo-hyphae with constriction at septations (Figure 2), and true hyphae. Similar colonies of fungi were found in the kidney, suprarenal gland, brain (Figure 3), and endocardial and myocardial tissue (Figure 4). In the liver, centrilobular necrosis was found. Involution was found in the thymus. Fungi were not found in the gut lumen.



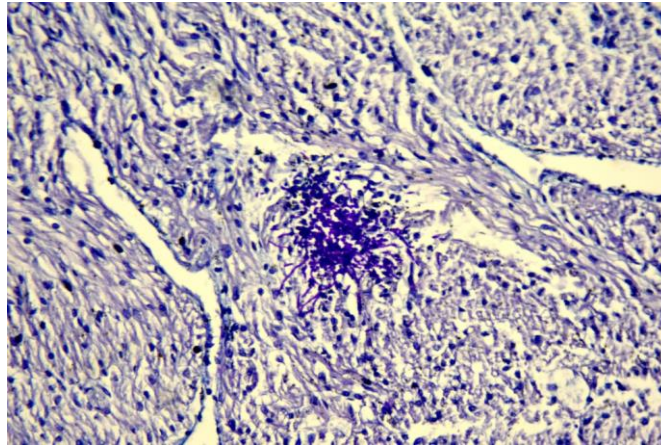
**Figure 1.** Colonies of fungi in lung blood vessels. AB-PAS, x40



**Figure 2.** Spherical blastospores, about 5  $\mu\text{m}$  in diameter, elongated pseudohyphae with constriction at septations, and true hyphae. AB-PAS, x600



**Figure 3.** Colonies of fungi in brain tissue. AB-PAS. x100



**Figure 4.** Fungi in myocardial tissue. AB-PAS, x200

### Discussion

*Candida* species are present in humans as commensal organisms, and candidiasis in intrahospital conditions is usually endogenous and caused by those very types. Invasion and spreading occurs through the damaged mucosae and has also been linked with the overuse of antibiotics. Exogenous origin of invasive candidiasis has also been found (caused by types acquired from other patients, medical staff and apparatus, as well as local hospital environment (3).

Invasive candidiasis encompasses candidemia (presence of fungal elements in the blood) with/or without the infection of deep tissues and internal organs (4). This fungal species is the most common and frequent cause of invasive fungal disease in preterm children (5). Studies identified risk factors that could be linked with candidiasis such as: intubation, insertion of venous catheter, and administration of parenteral therapy in the form of intravenous lipid emulsion (6). Our patient was intubated for two weeks.

Although *C. albicans* is the most common type (45-55% of *Candida* species) responsible for invasive candidiasis among infants, recent studies have shown an increased incidence of non-*albicans Candida* (NAC) species, which led to higher mortality and poorer antifungal therapy response. *Candida*

*parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei* together make up about half of all *Candida* types found in blood specimen cultures. Causes of this change in incidence could be explained by an increased usage of caspofungin and azoles as a part of antifungal treatment protocol (2, 5, 7, 8). In our case, in aspirate culture Gram-negative bacteria were found. Culture of fungi was not done and diagnosis was made after autopsy.

*C. albicans* and non-*albicans Candida* species vary in micromorphology and severity of harmfulness towards the organism they infect. *Candida* species can be found in form of spherical or oval shaped blastospores or yeast cells that can produce chains of elongated blastospores or pseudohyphae. *C. albicans* can also be found in the form of elongated filamentous cells (true hyphae) (9). In our case true hyphae were found. *C. Albicans* infection is often accompanied by severe inflammatory response while non-*albicans Candida* organisms are far more indolent and less noticeable pathohistologically (5).

### Conclusion

Our findings show the association of prolonged mechanical ventilation and antibiotic therapy in preterm newborn with invasive candididasis.

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**Prikaz bolesnika**

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## **INVAZIVNA KANDIDIJAZA KOD PRETERMINSKOG NOVOROĐENČETA: PRIKAZ SLUČAJA**

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Kandida je vodeći uzrok invazivnih gljivičnih infekcija kod hospitalizovane dece. Prikazujemo autopsijski slučaj klinički neotkrivene neonatalne invazivne kandidijaze. Muško pretermensko novorođenče gestacione starosti 27 nedelja umrlo je 24 dana nakon porođaja sa kliničkom dijagnozom intrakranijalne i plućne hemoragije, lečeno surfaktantom, antibioticima i invazivnom mehaničkom ventilacijom. Histološki, mnogobrojne kolonije gljivica otkrivene su u krvnim sudovima, bubrezima, nadbubrežnim žlezdama, mozgu, tkivu endokarda i miokarda. U jetri je uočena centrolobularna nekroza.

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***Ključne reči:*** kandidijaza, invazivnost, pretermensko novorođenče



Проф. др Иван Јовановић (1970 – 2021), *In memoriam*

Проф. др Иван Јовановић рођен је 21. 12. 1970. године у Нишу, где је завршио основну школу „Ратко Вукићевић“ а затим и средњу школу Гимназију „Светозар Марковић“. Након одслуженог војног рока, 1992. године уписао је студије медицине на Медицинском факултету Универзитета у Нишу. Дипломирао је на Медицинском факултету 1999. године са просечном оценом 9,37. Стручни испит положио је 2001. године.

Проф. др Иван Јовановић преминуо је 18. маја 2021. године у својој 51. години живота.

Последипломске студије уписао је 1999. године, најпре, из области нефрологије, али након заснивања радног односа на Катедри за анатомију Медицинског факултета у Нишу у звању асистента приправника маја 2000. године, пребацио се на смер анатомија. Након три године рада на Катедри за анатомију (јула 2003. године) поштовани професор одбранио је магистарску тезу под насловом: „Морфолошке и морфометријске карактеристике конкремената у структурама централног нервног система у току старења човека“. Докторску тезу под називом „Имунохистохемијске и морфометријске карактеристике псамоматозних телашаца хороидног плексуса у току старења човека“ одбранио је децембра 2006. године. Ментор магистарске тезе и докторске дисертације поштованом професору била је проф. др Светлана Антић. Након одбрањене докторске дистертације започео је волонтерску специјализацију из патологије.

Свој радни век проф. др Иван Јовановић провео је на Катедри за анатомију. Радни однос на Медицинском факултету у Нишу засновао је маја 2000. године, најпре у звању асистента приправника, док је 2004. године изабран у звање асистента. У прво наставничко звање, звање доцента, на предмету анатомија, проф. др Иван Јовановић изабран је 2008. године, у звање ванредног професора 2013. године, док је у звање редовног професора изабран 2018. године. На Катедри за анатомију Медицинског факултета провео је 21 годину. Оставио дубоки траг у многим апсектима рада Катедре за анатомију и самог Медицинског факултета, који се огледао у изузетно професионалном, али и пријатељском односу према студентима и колегама. Дугогодишњим ангажовањем учествовао је у припреми материјала за практичан рад са студентима, као и у припреми и издавању значајних уџбеника и практикумима на нашем предмету. Значајно је унапредио научноистраживачки рад на Катедри за анатомију кроз бројне студентске радове, као и учешћем у изради бројних научних радова и докторских дистертација. Посебно је битно његово залагање у оснивању и опремању Лабораторије за морфометријску анализу на Медицинском факултету у Нишу, као и успешно руковођење два предмета на докторским академским студијама. Верујем да би се сви који су познавали професора Ивана Јовановића сложили са мном у мишљењу да је био изузетно интелигентан, посвећен и вредан

наставник, поуздан колега и пријатељ и изнад свега праведан и скроман човек. Покушаћу да, као колегица и пријатељ са којом је провео свој радни век, представим професионални рад и допринос нашег професора Ивана Јовановића.

#### Рад са студентима и допринос развоју наставе

Континуиран успешан и предан рад са студентима свих академских и струковних студија Медицинског факултета, на почетку кроз практичну, а затим и теоријску наставу, уз коришћење савремених начина презентације комплексног градива из анатомије човека, обележио је радни век цењеног професора. За почетак бих истакла његово континуирано ангажовање у припреми коштаних и влажних кадаверичних препарата за практичну наставу из анатомије, као и само извођење практичне наставе, у чему је истрајао од звања асистента приправника до своје преране смрти. Да су студенти поштовали рад професора Јовановића доказују и високе оцене на анкетама студената за вежбе и предавања (од 4,57 до 5,00). Био је један од омиљених професора. Дугогодишње ангажовање професора Јовановића у извођењу и унапређењу дисекције кадавера, а затим и ангажовање приликом фотографисања истих, значајно је допринело издавању практикума „Водич кроз практични испит из анатомије“ за студенте медицине и стоматологије (прво издање 2012. године, последње двојезично издање 2021. године). Професор је био спреман да у сваком тренутку помогне млађим колегама у дисекционој сали, говорио је да одговарајући на питања и дискутујући са колегама још боље разјасни себи теме о којима се говорило и научи још више и боље. Неки од његових препарата део су збирке анатомских препарата у остеолошкој сали Института за анатомију. Поред поменутог практикума, професор је током своје каријере био један од аутора 8 уџбеника из анатомије за студенте интегрисаних академских студија медицине, као и за студенте струковних студија Медицинског факултета у Нишу. Навешћу последња два издања.

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- Угреновић С, **Јовановић И**, Стојановић В, Павловић М, Кундалић Б. Анатомија главе и врата за основне струковне студије (струковни зубни протетичар). Ниш: Медицински факултет Универзитета у Нишу; **2020**.

Проф Иван Јовановић је своју љубав према науци и истраживању преносио студентима кроз менторства и коменторства у студентским радовима (9 студентска рада), од којих су неки и награђивани као најбољи на конгресима. Са појединим студентима, професор је наставио сарадњу и након њиховог дипломирања, кроз наставу на докторским студијама. Иначе, професор је био ментор 6 дипломских радова. Две школске године, професор је био ангажован као наставник по уговору на предмету Анатомија на Факултету за спорт и физичку културу у Нишу. Пет пута је учествовао, као предавач, у склопу састанака континуиране медицинске едукације (у периоду од 2010. до 2012. године).

Поред рада са студентима академских и струковних студија проф. др Иван Јовановић радио је и са студентима докторских студија, као и са колегама, на специјалистичким студијама. Као доцент, а касније ванредни и редовни професор, учествовао у организовању и извођењу практичне и теоријске наставе на предмету Методе у молекуларној медицини – део морфометрија. Од 2017. године професор је осмислио план и програм и преузео дужност

одговорног наставника на изборном предмету II године докторских академских студија „Морфолошке и морфометријске методе у биомедицинским истраживањима“ на Медицинском факултету, Универзитета у Нишу. У циљу унапређења квалитета наставе за студенте докторских студија, професор је покренуо иницијативу и уз подршку Управе Медицинског факултета учествовао у формирању нове Лабораторије за морфометријску анализу на Институту за анатомију, која је почела са радом школске 2017/2018.године.

#### Научни и стручни рад

Професор др Иван Јовановић био је аутор и коаутор у око 167 радова, од којих 3 представљају поглавља у монографијама домаћег или међународног значаја, 95 је штампано у међународним или домаћим научним часописима у целини, док је 70 штампано у зборницима међународних и домаћих научних скупова (нумерички подаци су приближни и добијени из мени доступних материјала и портала). Укупна научна компетентност др Ивана Јовановића је 2018. године износила 363,07 поена. У октобру 2021. године укупан број хетероцитата на ISI/Web of Science и у SCOPUS индексној бази података, видљив на КОБСОН порталу за радове др Ивана Јовановића износио је 520.

Ауторски радови професора Јовановића објављени у страним и домаћим часописима проистекли из његове магистарске и докторске тезе, припадали су области неуроанатомије, неуроимунологије, као и неуроимуноендокринологије. Имунохистохемијска, морфометријска и статистичка анализа испитиваних структура нервног система чиниле су срж његових публикација, а већину метода научно-истраживачког рада савладавао је самостално, користећи страну литературу и бројне *online* курсеве. Први је савладао и успешно користио програм за анализу и дигиталну обраду слике Image-J а и своје знање и искуство пренео најпре нама, колегама са Института за анатомију, а затим и студентима основних и докторских студија.

Професор Јовановић објавио је укупно 7 ауторских радова у часописима међународног значаја и 4 рада у домаћим часописима. Један од његових најцитиранијих ауторских радова је следећи:

- **Jovanović I, Ugrenović S, Vasović L, Petrović D, Cekić S.** Psammoma bodies -friends or foes of the aging choroid plexus. *Med Hypotheses*. 2010; 74 (6):1017-20. **(ИФ 2010 = 1,296)**

Током своје каријере, професор је био учесник бројних конгреса и симпозијума, како у земљи, тако и у иностранству. Одабрала сам само неколико његових излагања.

- **Jovanović I, Ugrenović S, Trandafilović M, Stojanović V, Kundalić B.** Morphometric characteristics of choroid plexus epithelium in cases with significantly different presence of psammoma bodies. *Book of abstracts. 7th International Symposium of Clinical and Applied Anatomy (ISCAA)*. September 17 – 20, 2015; Bratislava, Slovakia; p. 72.
- **Jovanović I, Ugrenović S, Čukuranović R, Stojanović V, Pavlović M, Kundalić B, Čukuranović Kokoris J, Trandafilović M, Antić M.** Folliculo-stellate cells– potential mediators of the inflammaging- induced hyperactivity of the hypothalamic-pituitary-adrenal axis in healthy elderly individuals *Zbornik sažetaka. V Kongres srpskog anatomskog društva Srbije sa međunarodnim učešćem*, 8 -10. septembar 2016; Novi Sad, Srbija; 2016.p.13.
- **Ivan Jovanović, Slađana Ugrenović, Aleksandra Jovanović.** Application of the Image Processing and Analysis in the Quantification of the Parahippocampal Region Synaptophysin Immunoreactivity. *Book of Proceedings. LII INTERNATIONAL SCIENTIFIC CONFERENCE ON*

INFORMATION, COMMUNICATION AND ENERGY SYSTEMS AND TECHNOLOGIES. Serbia, Niš, June 28 - 30, 2017. Faculty of Electronic Engineering, Niš, Serbia, Faculty of Communications and Communication Technologies, Sofia, Bulgaria, Faculty of Technical Sciences, Bitola, Macedonia. Niš, Serbia, 2017: 305 – 308.

Нарочито желим да истакнем велики број коауторских радова професора Јовановића, који није само обичан троцифрени број. Сведок сам тога да је у већини публикованих радова професор учествовао као идејни творац, креирао план истраживања, бирао методу хистохемијских и имунохистохемијских бојења, планирао стереолошке и астереолошке студије на различитим материјалима, учествовао у формирању базе података, статистичке анализе и доношењу закључака из добијених резултата. Један је од ретких истраживача који је своја истраживања реализовао самостално, од идеје до публикације у часописима, а да при томе своје знање и вештине није скривао, већ је као прави интелектуалац делио са колегама. Жеља да константно учи и ради на себи никада га није напустила, иако је 14 година боловао од дијабетеса и био срчани болесник. Често сам се шалила говорећи да сам ја његов вишегодишњи вредни ђак. Све моје радове преводио је на енглески језик и у свим мојим радовима учествовао у статистичкој анализи и графичким приказима, заједно смо бирали часописе, заједно смо их слали, радовали се сваком прихваћеном раду и бодрили једно друго након сваког неуспешног покушаја. Хвала ти пријатељу.

Највећи број коауторских радова професор Јовановић публиковао је заједно са колегама са Катедре за анатомију Медицинског факултета у Нишу, у часописима од међународног значаја 34 радова, као и у часописима од националног значаја око 25 радова. Заједно са колегама Катедре за физиологију учествовао је у изради и публиковању 7 радова, са колегама Катедре за патологију 2 рада, док је са колегама Катедре за патофизиологију учествовао у изради и публиковању једног рада. Из сарадње са колегама клиничких предмета публиковано је 5 радова из области офталмологије, 2 рада из области радиологије, као и један рад из области кардиологије. Заједно са колегама са Катедре за судску медицину учествовао је у изради и публиковању једног рада. Као део тима научног пројекта Министарства просвете, науке и технолошког развоја (Етиологија, дијагностика, превенција и терапија ендемске нефропатије и са њом повезаних тумора уротела – значај истраживања генома и протеома) учествовао је у изради и публиковању 5 радова. Последњих година, сарађивао је и са колегама Клинике за стоматологију и тако учествовао у изради и публиковању приближно 5 радова. Заједно са колегама са Факултета спорта и физичке културе у Нишу учествовао је у изради и публиковању једног рада. Заједно са колегама Медицинског факултета у Београду учествовао је у изради и публиковању једног рада.

У току процеса педагошког и научног сазревања професора Ивана Јовановића проистекле су идеје за бројне докторске тезе, које је идеолошки и методолошки осмислио и учествовао, заједно са докторандима, у њиховој реализацији. Био је ментор у изради следеће три докторске дисертације које су одбрањене у периоду од 2013. до 2015. године :

- Мирјана Бакић, Морфометријске и имунохистохемијске карактеристике паракхипокампадне коре великог мозга човека током процеса старења, област: анатомија; 2013. године; Медицински факултет у Нишу;
- Миљана Павловић, Морфометријска и имунохистохемијска анализа структура аденохипофизе укључених у систем хипоталамо-хипофизно-адреналне осовине током старења човека, област: анатомија; 2014. године.; Медицински факултет у Нишу;

- Владимир Антић, Морфометријска и имунохистохемијска анализа соматотропних ћелија аденохипофизе и њихова повезаност са присуством саркопеније током старења човека, област: анатомија; 2015. године; Медицински факултет у Нишу.

Осим менторства у докторским дисертацијама, професор Иван је, као члан комисије значајно допринео изради и одбрани 8 докторских дисертација својих колега са Катедре за анатомију Медицинског факултета у Нишу и Медицинског факултета у Београду, као и Катедре за физиологију Медицинског факултета у Нишу.

Професор Иван Јовановић још је као асистент препознат као вредан, способан, организован сарадник спреман да стално учи и шири спектар својих вештина, па је као истраживач био део тима на следећим научним пројектима:

- Етиопатогенетски аспекти Балканске ендемске нефропатије и тумора уротракта; руководилац: проф. др Раде Чукурановић; Министарство просвете, науке и технолошког развоја, број: 1609; 2001.-2005.г.;
- Истраживање узорка, механизма настанка, превенције и лечења ендемске нефропатије и тумора уротелијума; руководилац: академик проф. др Владисав Стефановић; Министарство просвете, науке и технолошког развоја; број: 145004Б, 2006.-2010.г.;
- Етиологија, дијагностика, превенција и терапија ендемске нефропатије и са њом повезаних тумора уротела – значај истраживања генома и протеома; руководилац: Љубинка Јанковић Величковић; Министарство просвете, науке и технолошког развоја; број: 175092; 2011.-2017.г.

Поред ангажовања у извођењу наставе из анатомије на свим студијским програмима и на докторским и специјалистичким студијама, професор Јовановић дао је значајан допринос раду Медицинског факултета као члан Одбора за научно-истраживачки рад, као члан Савета Медицинског факултета, члан уређивачког одбора часописа *Acta medica Medianae* и, такође, као члан уређивачког одбора универзитетског часописа *Facta Universitatis (Series Biology and Medicine)*.

Био је члан Српског анатомског друштва Србије и самим тим и Европске федерације за експерименталну морфологију (ЕФЕМ) и Интернационалне федерације асоцијација анатома (ИФАА), затим Српског лекарског друштва и Лекарске коморе Србије.

Значајно је поменути то да је професор Јовановић дао свој допринос као рецензент уџбеника, као и научних радова у домаћим часописима од националног значаја (*Acta medica Medianae, Acta facultatis medicae Naissensis, Facta Universitatis*), интернационалног значаја (Војносанитетски преглед), као и иностраним часописима (*Histology and Histopathology, Computers in Biology and Medicine, Case Reports in Radiology*).

У покушају да на најбољи начин представим професионални рад професора Јовановића, његову племениту душу и радознао ум, извињавам се уколико неки од наведених бројева није прецизан (јер се свакодневно мењају) и уколико нисам навела или истакла неке од бројних референци нашег драгог професора Јовановића. Његов рад остаје академској заједници, као и његовој породици, супрузи Александри, Милитину и Даници, родитељима.

Као сведок дугогодишње сарадње професора Јовановића и доктора Николе Стојановића, почев од консултација везаних за анатомију на првој години студија медицине, преко израде студентских радова до плодне научне сарадње крунисане публикованим

радовима у значајним часописима, замолила сам др Николу да опише рад са професором из угла студента и колеге асистента са Катедре за физиологију.

„Први реалан сусрет са медицином и са анатомијом стекао сам током конатка са проф. Јовановићем још 2009. године, када сам уз његову несебичну помоћ савладавао градиво. Већ наредне године сам са професором направио прве кораке у области морфометрије, а резултате нашег заједничког труда и рада презентовао сам на интернационалном конгресу 2011. године у Новом Саду, где је овај рад и награђен. Током своје истраживачке каријере, заједничким залагањем са професором објавили смо неколико радова из различитих области биомедицине. Оно што је њега чинило правим професором, колегом и истраживачем је невероватна енергија и спремност да помогне уколико би му се неко обратио. У појединим случајевима његова истрајност и спремност да се нешто уради била је огромна, а све у циљу проналажења решења за неки проблем. Рад са професором Јовановићем се током десет година претворио не само у диван колегијалан однос пун узајамног поштовања и разумевања, већ и у право пријатељство. Његов одлазак представља велики губитак за мене лично, јер сам поред професора, ментора, колеге изгубио и пријатеља.”

За мог пријатеља и његову дивну породицу  
Проф. др Слађана Угреновић

## JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa – International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

### Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obeležavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legendi moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarском broju **Index Medicusa**. Ova lista se takođe može naći na adresi [www.nlm.nih.gov](http://www.nlm.nih.gov)

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

### Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pisanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

#### Članci u časopisima

##### 1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. *Ann Intern Med* 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood-leukaemia in Europe after Chernobyl: 5 year follow-up. *Br J Cancer* 1996;73:1006-12.

##### 2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. *Med J Aust* 1996;164:282-4.

##### 3. Članak bez poznatih autora

Cancer in South Africa (editorial). *S Afr Med J* 1994;84:15.

##### 4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. *Environ Health Perspect* 1994; 102 Suppl 1:275-82.

##### 5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. *Semin Oncol* 1996;23(1 Suppl 2):89-97.

##### 6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. *Ann Clin Biochem* 1995;32(Pt 3):303-6.

##### 7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. *N Z Med J* 1994;107(986 Pt 1):377-8.

##### 8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. *Clin Orthop* 1995;(320):110-4.

##### 9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. *Curr Opin Gen Surg* 1993;325-33.

#### 10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) *Lancet* 1996;347:1337.

Clement J, De Bock R. Hematological complications of hantavirus nephropathy (HVN) (abstract). *Kidney Int* 1992; 42:1285.

#### Udžbenici i monografije

##### 11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

##### 12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

##### 13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

##### 14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

##### 15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

##### 16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

#### 17. Istraživački ili tehnički izveštaji

##### Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

##### Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

#### 18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

#### Druge vrste publikovanog materijala

##### Neobjavljeni materijal

##### 19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. *N Engl J Med*. In press 1996.

##### Elektronski zapisi

##### 20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

##### 21. Monografija u elektronskom formatu

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

##### 22. Kompjuterski podaci

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



## PROPOZICIJE ZA PISANJE RADOVA U ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) je tematski časopis iz oblasti medicinskih nauka. Časopis objavljuje originalne radove koji nisu prethodno publikovani.

U AMM se objavljuju: uvodnici, naučni i stručni članci, prethodna ili kratka saopštenja, revijski radovi tipa opšteg pregleda, aktuelne teme, meta-analize, prikazi slučajeva, prikazi knjiga i drugi prilozi. Radovi se štampaju na srpskom ili engleskom jeziku sa apstrak-tom na srpskom i engleskom jeziku. Radovi na engles-kom jeziku se prezentuju u elektronskom formatu na sajtu Medicinskog fakulteta u Nišu, kao i na među-narodnim sajtovima iz oblasti medicinskih nauka. Acta Medica Medianae izlazi četiri puta godišnje, od 1962 godine.

Svi radovi koji se objavljuju u AMM podležu anonimnoj recenziji, a Uredivački odbor određuje redosled njihovog štampanja. Primedbe i sugestije urednika i recenzenata dostavljaju se autoru radi konačnog oblikovanja. Radovi se predaju u pisanom ili elektronskom obliku na srpskom i engleskom jeziku. Rukopisi radova prihvaćenih za štampu ne vraćaju se autoru.

Rukopis treba predati sa jednostrukim proredom, formata A4, sa levom marginom od 3 cm.

**Prva strana** rada treba da sadrži: a) naslov rada b) puna imena i prezimena autora c) puni nazivi ustanova i organizacijskih jedinica u kojima je rad realizovan i mesta u kojima se ustanove nalaze, d) znacima \*, \*\*, \*\*\*, #, ##, ###,...označavaju se redom autori i njihove institucije e) puna adresa, broj telefona i e-mail osobe zadužene za korespondenciju u vezi predatog rukopisa.

**Druga strana** treba da sadrži samo naslov rada, rezime i ključne reči, bez imena autora i institucija. Veličina rezimea za naučne i stručne članke, revijske radove tipa opšteg pregleda i meta-analize može da bude do 250 reči. Ispod rezimea sa podnaslovom "Ključne reči" navesti 3-5 ključnih reči ili izraza. Poželjno je da autori za ključne reči koriste odgovarajuće deskriptore, tj. definisane termine iz *Medical Subject Heading* (MeSH) liste *Index Medicus-a*. Prva i druga strana se predaju na srpskom i engleskom jeziku i ne obeležavaju se brojevima.

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