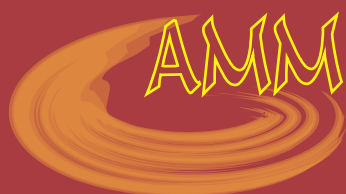
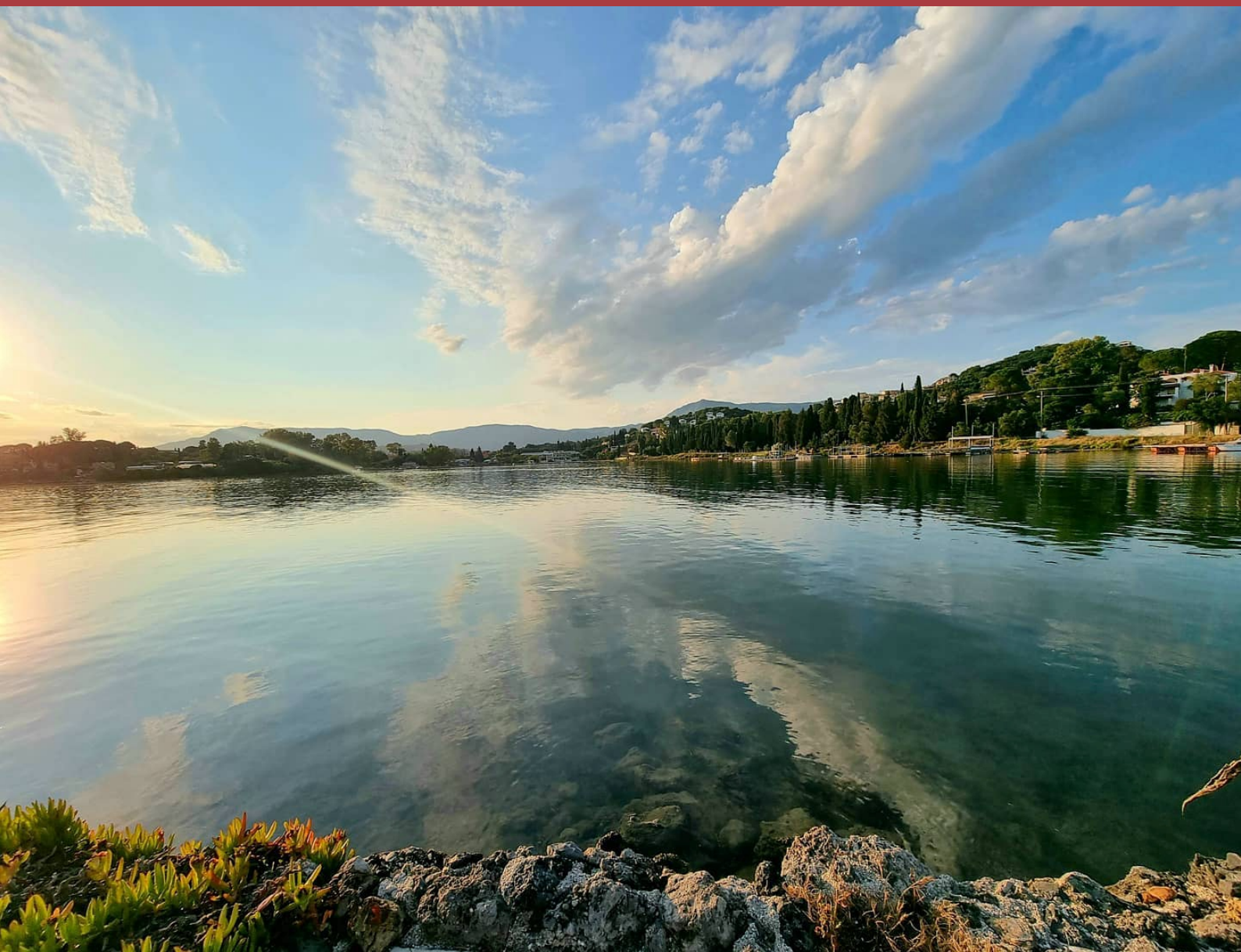


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MORPHOMETRIC ANALYSIS OF BICEPS MUSCLE TISSUE OBTAINED FROM RATS ACUTELY EXPOSED TO CARBON-TETRACHLORIDE

Milorad Antić¹, Vladimir Antić², Ivana Graovac¹, Jovana Čukuranović Kokoris¹, Ivan Ilić³, Miljana Pavlović¹, Vladimir Živković¹, Dušan Sokolović⁴

Skeletal muscles comprise around 40% of total body weight, and they are essential for locomotion and body posture. Under experimental conditions, mild damage occurring due to excessive reactive oxygen species production could be mimicked with acute exposure of rats to carbon-tetrachloride. The aim of the present study was to evaluate morphometric changes occurring in rat biceps muscle 24 h after the injection of carbon-tetrachloride (CCl₄). Biceps muscle tissue samples, obtained from control and CCl₄-damaged groups, stained with hematoxylin and eosin were used to measure muscle fiber area (MFA), muscle fiber perimeter (B), muscle fiber circularity (MFC) and muscle fiber roundness (MFR). The obtained data were compared using Students t-test for two independent samples. Morphometric analysis revealed that the parameters such as MFA, B and MFC were statistically significantly altered (increased) in the group exposed to CCl₄. At the same time, the MFR remained almost identical to that of the control group. The obtained results are in agreement with gross microscopic analysis and follow the tissue edema pattern. These data could be useful in future studies that are following changes in the skeletal muscles after CCl₄ application. *Acta Medica Medianae* 2023;62(3):5-10.

Key words: *biceps muscle, carbon-tetrachloride, edema, morphometry*

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Introduction

Skeletal muscles comprise around 40% of total body weight, and they allow body movement (locomotion) and maintain posture through processes of contraction and relaxation. These processes are dependent on morpho-functional organization of the skeletal muscle (1), which when disrupted might lead to poor and hampered locomotion. The process of contraction and

relaxation is highly dependent on cell energy sources (ATP) and on calcium ion concentrations (2). Muscle damage could be concomitant with some liver disorders (3), but also with some non-disease states which are associated with an increase in reactive oxygen species (ROS) production (4). The non-disease states also include excessive physical activity. Interesting feature of the skeletal muscles is that they are able to recover, both in structure and function, in a period of couple of weeks after their injury (5).

Carbon tetrachloride (CCl₄) is an industrial pollutant that is utilized for the production of paints and extinguishers. Half-life of CCl₄ in the working atmosphere is relatively long and can be somewhere between 30 and 100 years (4). It is a lipophilic organic solvent which has been in use for experimental research for decades. In experimental animals, single injection of a relatively high dose of CCl₄ is associated with a significant tissue damage arriving from excessive ROS production. These ROS are formed initially from the biotransformation of CCl₄ by the tissue cytochrome P450 (predominantly in liver). Molecules that are generated include trichloromethyl free radicals and trichloromethyl peroxide which further interact with various cellular structures. The generation of these highly reactive molecules occurs after body is exposed to

CCl₄, which can be either through inhalation or skin absorption (in working environment) or by an injection (in experimental animals). Damage is mainly seen in tissue highly expressing cytochrome P450, which include liver and kidneys (6), however, distant tissue damage is arriving from circulating ROS generated elsewhere.

The aim of the current study was to morphometrically investigate the changes occurring in bicep muscle myofibers in rats acutely exposed to carbon tetrachloride and to compare the obtained results with the ones obtained from the control group. Also, these findings will be brought in connection with the pathological appearance of the muscle tissue, as well as with biochemical findings.

Material and methods

Animals and housing

Male Wistar rats (250–300 g) were divided into groups of 6 animals (Institute of Biomedical Sciences, Faculty of Medicine, University of Niš, Serbia). Animals were housed under standard laboratory conditions at room temperature of 22 ± 2 °C. The humidity was 60%, and food and water were free. All experimental procedures, carried according to the Declaration of Helsinki and Europe Community Guidelines for the Ethical Use of Laboratory Animals (2010 EU Directive; 2010/63/EU), were approved by the local Ethics committee.

Experimental procedure

Seven days prior to the experiment, rats were randomly divided into two equal groups, each consisting of seven animals (total $n = 14$). Rats were treated with CCl₄ to induce acute tissue damage according to previously established protocols (4, 6, 7). Control animals, group I (vehicle control), received olive oil as a single dose 24 h prior to sacrifice in a volume of 10 ml/kg. Experimental group of animals, group II (CCl₄ control), were administered with CCl₄ dissolved in olive oil (1:1) in a dose of 1 ml/kg. One day after the injection, all animals were sacrificed by an overdose of ketamine (Ketamidol, 10%). After that, bicep muscle tissue was dissected, cleaned from surrounding tissue, and separated for histopathological analysis.

Tissue processing and staining

Muscle tissue specimens separated for histopathological study were immersed in formaldehyde solution (10%, w/v) for fixation. After this process, the tissue was dehydrated with increasing concentration solutions of ethanol (50-100%, v/v), and small tissue segments were cut and embedded in paraffin. Tissue sections, 4–5 μm thick, were obtained from

the paraffin molds and routinely stained with hematoxylin and eosin (HE). Tissue damage was scored following previously given scoring system scales (8), where the grade of damage were marked from 0 (absent) to 3 (severely present). Main parameters that were traced included muscle fiber and interstitial tissue edema.

Morphometric analysis

Morphometric analysis of HE stained tissue specimens was performed using ImageJ software (imagej.nih.gov/ij/). At least 10 images were captured of each tissue specimen using digital camera E-450 mounted on BH-2 microscope (Olympus). The magnification used for the tissue analysis and image capturing was 200x. All visible muscle fibers in each image were included in the analysis. The following parameters were measured: muscle fiber area (MFA), muscle fiber perimeter (B), muscle fiber circularity (MFC) and roundness (MFR).

Statistical analysis

The obtained data are presented as mean \pm SD. Comparison of the data was performed using Student's t test for two small independent samples (GraphPad Prism, version 7.0; USA). Probability values (p) less than 0.05 were considered to be statistically significant.

Results

Histopathological analysis

Bicep muscle tissue section obtained from a control group consisted of tightly packed homogeneous polygonal muscle fibers, with peripherally located nuclei and normal appearing cell membrane and cytoplasm (Figure 1 and Table 1).

Animals treated with CCl₄ displayed abnormal fiber morphology, including occasionally occurring cytokinesis and edematous (swollen) rounded muscle fibers (Figure 2 and Table 1). The stromal compartment was moderately large, with an infiltration of inflammatory cells, primarily neutrophils, lymphocytes, and macrophages, both around blood vessels and between muscle fibers.

Morphometric analysis

Morphometric analysis of the biceps muscle tissue pointed to a significant increase, compared to the control group, in MFA, B and MFC in rats exposed acutely to CCl₄ (Table 2). On the other hand, roundness of the muscle fibers (MFR) remained unaltered (Table 2).

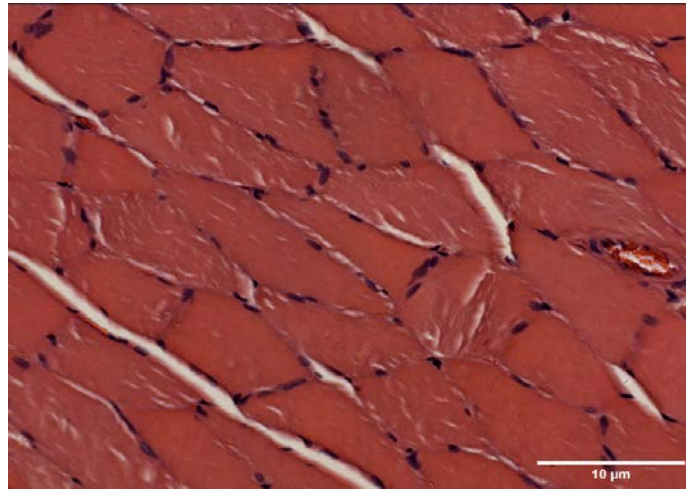


Figure 1. Histomorphological appearance of biceps muscle tissue obtained from rats of the control group

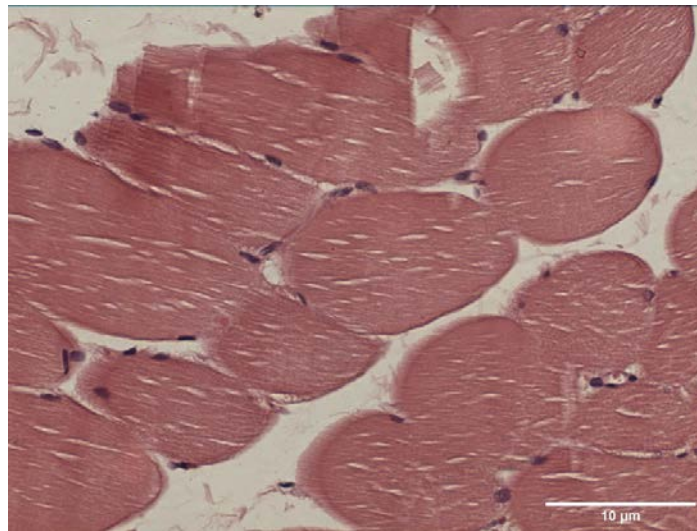


Figure 2. Histomorphological appearance of biceps muscle tissue obtained from rats treated with CCl₄

Table 1. Semiquantitative score obtained from the control and CCl₄-treated animals

Morphometric parameter	Control group	CCl ₄ group
Muscle fiber edema	0	2
Interstitial tissue edema	0	2.2
Cytoplasm degeneration	0	1.7

Table 2. Biceps muscle morphometric parameters measured in control and CCl₄-treated animals

Morphometric parameter	Control group	CCl ₄ group	p-value
Muscle fiber area (MFA; mm ²)	41.5 ± 6.3	68.9 ± 7.5	<0.001
Muscle fiber perimeter (B; μm)	26.3 ± 3.8	32.6 ± 2.4	<0.001
Muscle fiber circularity (MFC; mm)	0.74 ± 0.07	0.87 ± 0.06	<0.01
Roundness (MFR; mm)	0.7 ± 0.01	0.75 ± 0.09	>0.05

Discussion

The biceps femoris is one of the largest muscle in the hind-extremity of an animal and serves multiple functions including thigh abduction, hip extension, and knee flexion (9). Its damage following CCl₄ application has been previously proven using a panel of serum biochemical parameters such as lactate dehydrogenase and creatine kinase activity, as well as potassium ion levels (4). Cell and organelle membrane damage occurs after CCl₄ exposure due to a chain reaction which is initiated by the removal of a hydrogen atom from an unsaturated fatty acid by ROS (produced by xanthine oxidase) and free radicals formed after CCl₄ metabolism (7). These excessive ROS production leads to muscle antioxidant capacities depletion, which results in a more or less pronounced microscopic changes (4).

In animals exposed to CCl₄ there was a significant alteration in cytoplasm appearance (score 1.7; Table 1), which was not visible in the control group animals (score 0; Table 1). These microscopic changes might correspond to the damaged cytoplasm structures (proteins and lipids), as well as to the damaged organelles. These changes on the organelles are potentially irreversible, and could lead to cell death, especially if the organelles in question are mitochondria. On a previous occasion, an increase in MDA, a marker of lipid peroxidation induced by ROS, was noted in the muscle tissue of animals acutely exposed to CCl₄ (4). The ROS could arrive from different cells, and in the present situation these cells are mainly inflammatory ones such as neutrophils and macrophages, as well as mastocytes (4, 10). These cells are packed with enzymes capable of creating numerous ROS as a response to tissue injury and inflammation.

Using gross microscopic analysis the examination helped us reveal an expanded and rounded muscle fiber profile with eosinophilic cytoplasm (Figure 2), which markedly deviated from the findings in the control group (Figure 1).

The mentioned changes are the result of water influx into the cell and consequential cytosol dilution i.e. edema development (Miller MA, Zachary, 2017). If the process is not reversed this could lead to a decrease in ATP content and relatively fast switch to the anaerobic processes (11), which muscles could endure for some time due to their specific structure/function (2). After certain time point, when the intracellular lactate levels increase and pH and ATP decrease, cells suffer and more pronounced/irreversible changes occur (11). The described changes causing such massive disturbances would lead cell to oncotic necrosis (11).

Measured morphometric parameters could be perfectly used to estimate cell edema, since they describe cell features which are altered during cell volume expansion following water influx. These include MFA, which represents total skeletal muscle cell size (area), B, a total length of a cell membrane, and MFC, which describes a cell shape compared to a full circle. All three mentioned parameters were found to be statistically significantly increased in the group of animals treated with CCl₄. Also, the measured parameters (Table 2) correlate with the microscopic changes and score values for the corresponding groups (Table 1).

Conclusion

The present study revealed that acute application of CCl₄ provokes significant, potentially reversible, changes in the skeletal muscle appearance. This was determined based on the light microscopy analysis, and further corroborated using morphometric analysis. Detailed morphometric analysis confirmed the presence of significant muscle fiber edema with increased muscle fiber area, perimeter and circularity. Furthermore, the present analysis showed that the changes are only temporary, and could be potentially reversed since no muscle fiber necrosis or cell apoptosis were seen.

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doi: 10.5633/amm.2023.0301**MORFOMETRIJSKA ANALIZA TKIVA DVOGLAVOG
MIŠIĆA (*MUSCULUS BICEPS*) PACOVA AKUTNO
IZLOŽENIH UGLJEN-TETRAHLORIDU**

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Skeletni mišići čine oko 40% ukupne telesne težine i neophodni su za kretanje i držanje tela. U eksperimentalnim uslovima, blago oštećenje koje nastaje usled prekomerne proizvodnje reaktivnih vrsta kiseonika može se oponašati akutnim izlaganjem pacova ugljen-tetrahloridu. Cilj ove studije bio je da se morfometrijski procene promene koje se javljaju u dvoglavom mišiću pacova 24 sata nakon injekcije ugljen-tetrahlorida (CCl₄). Uzorci mišićnog tkiva bicepsa, dobijeni od pacova iz kontrolne grupe i grupe oštećene CCl₄, obojeni hematoksilinom i eozinom, korišćeni su za merenje površine mišićnih vlakana (MFA), perimetra mišićnih vlakana (B), kružnosti mišićnih vlakana (MFC) i zaobljenosti mišićnih vlakana (MFR). Dobijeni podaci upoređeni su korišćenjem Studentovog t-testa za dva nezavisna uzorka. Morfometrijskom analizom otkriveno je to da su parametri kao što su MFA, B i MFC statistički značajno izmenjeni (povećani) u grupi pacova izloženih CCl₄. U isto vreme, MFR je ostao skoro identičan onom u kontrolnoj grupi. Dobijeni rezultati u saglasnosti su sa mikroskopskom analizom i prate obrazac edema tkiva. Ovi podaci mogli bi biti korisni u budućim studijama koje prate promene u skeletnim mišićima nakon primene CCl₄. *Acta Medica Medianae* 2023;62(3): 5-10.

Ključne reči: dvoglavi mišić, ugljen-tetrahlorid, edem, morfometrija

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CENTRAL SENSITIZATION INVENTORY SCORES IN PATIENTS WITH NEUROPATHIC PAIN COMPARED TO HEALTHY SUBJECTS

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Central sensitization inventory (CSI) was designed as a tool for evaluation of the symptoms that have been found to be related to central sensitization (CS). The aim of this research was to examine differences in CSI scores between subjects with painful lumbosacral radiculopathy (PLSR). This study included 33 subjects with PLSR (mean age 54.12 ± 9.43 years, 6 (18.2%) women) and 30 healthy subjects (mean age 50.87 ± 5.64 years, 12 (40%) women). The Serbian version of the Central Sensitization Inventory (CSI) was used to examine the degree symptoms related to CS. In order to evaluate the emotional status, we used the Serbian version of the Depression, Anxiety, Stress Scale (DASS 21). The obtained results showed a significantly higher value of the CSI score in subjects with neuropathic pain ($t = -7.690$, $p = 0.000$). Statistically significantly worse DASS 21 values in subjects with neuropathic pain in all 3 subscales: depression subscale ($t = -2.437$, $p = 0.018$), anxiety subscale ($t = -3.597$, $p = 0.001$), stress subscale ($t = -3.982$, $p = 0.000$). The degree of expression of symptoms related to CS determined, as well as the degree of anxiety, depression and stress is significantly higher in subjects with PLSR compared to the group of healthy subjects. This should be kept in mind when designing a treatment plan for individuals with PLSR. *Acta Medica Medianae* 2023;62(3):11-16.

Key words: neuropathic pain, central sensitization, anxiety, depression, stress

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Introduction

In modern society, chronic pain is a major health problem (1). It frequently leads to impairment and can greatly impair one's capacity for physical activity (2). It is estimated that over 20% of Europeans suffer from chronic pain (3). A subtype of chronic pain is chronic neuropathic pain (4). It occurs as a result of a lesion or disease of the somatosensory nervous system, either peripherally or centrally (5). Radiculopathy is a condition caused by compression and/or irritation of the spinal nerve root, most often in the lumbosacral part of the spinal column (6). It is estimated that lumbosacral radiculopathy is present in 3% – 5% of the population and there is no major difference between the genders (7–9).

One of the important mechanisms in neuropathic pain is central sensitization (CS). According to International Association for the Study of Pain (IASP), central sensitization represents increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input (10). In other words, CS is characterized by increased sensitivity and responses to painful stimuli (11). It is hypothesized that CS represents an important factor in the development and maintenance of chronic neuropathic pain (12).

It is impossible to directly measure CS in humans (13). However, there are certain efforts to indirectly measure this phenomenon. Quantitative sensory testing and self-reported questionnaires are frequently used for this purpose in the literature (13). One of the most frequently used self-reported instrument is Central sensitization inventory (CSI). CSI was designed to evaluate severity of symptoms related to CS (14 – 18).

The phenomenon of central sensitization is associated with a great number of centers in the brain, and the limbic system is particularly important (19, 20). Limbic system has a role in the field of emotional learning and behavior in affective disorders (21). It is also considered to be an important center of the mutual relationship between pain and negative emotions (22, 23). It is well known that pain correlates with the

intensity of psychopathological symptoms - both with depression and with symptoms of anxiety.

Aim

The aim of this study was to examine the difference in symptoms of CS between patients with neuropathic pain due to lumbosacral radiculopathy and healthy subjects. The level of depression, anxiety, stress and length of sleep between the two tested groups was also compared.

Materials and Methods

This study was conducted at the University Clinical Center of Vojvodina in the period from 2019 to 2020. The Ethics Committee of the University Clinical Center of Vojvodina approved the implementation of this study (Decision No. 00-208).

The research included 63 subjects, 30 healthy subjects (HS) without pain and 33 subjects with painful lumbosacral radiculopathy (PLSR).

The criteria for inclusion in the HS group were the absence of any chronic or acute pain in the last month, the age of the subjects >18 years.

The criteria for inclusion in PLSR group were the presence of neuropathic pain from the lumbosacral radiculopathy according to the criteria suggested by Finnerup et al., duration of pain > 3 months, age of the subject > 18 years (24).

The criteria for exclusion from the research for both groups were subjects under 18 years of age, the presence of a malignant disease, patients who had spine surgery in the last 6 months, patients who did not understand the Serbian language, and for the PLSR group, pain that lasted less than 3 months.

The following questionnaires were used:

1. Central Sensitization Inventory (CSI) created for the purpose of assessing the severity

of symptoms of CS. It consists of part A and part B. The first part, which was used for this research, consists of 25 items related to symptoms of central sensitivity. The respondent evaluates the frequency of each item on a five-point Likert scale from 0 (never) to 4 (always). The total score ranges from 0 to 100, and higher values indicate a greater degree of central sensitivity symptoms (14, 15).

2. Depression, Anxiety, Stress Scale (DASS 21) consists of 21 questions and has three subscales of 7 items each. It is designed to evaluate emotional states such as depression, anxiety and stress. The respondent evaluates the level of exposure to each condition in the past week on a 4-point Likert scale. A higher score on this scale indicates a higher degree of depression, anxiety and stress (25).

3. Respondents reported the average length of sleep in the last month in hours.

The SPSS 24 package was used for statistical data processing. Frequency and percentage were used to describe the sample. Descriptive statistics were used to determine measures of central tendency (arithmetic mean), measures of variability (standard deviation). In order to determine differences between groups, Student's t and X² tests were used. In the applied tests, p < 0.05 was taken as significant.

Results

This study included 63 subjects, of which 30 were healthy subjects without pain, and 33 were subjects with PLSR (see Table 1 for more details). The largest number of subjects were men, although there was no significant difference between the examined groups (X² = 3.665, p = 0.093).

There was no significant difference in the values of body height (t = 1.749, p = 0.085) and body mass (t = -1.173, p = 0.246) between the studied groups.

Table 1. Demographic characteristics of the patient sample

	Healthy subjects	PLSR ¹	t/X ²	p
Age				
N	30	33		
Mean ± SD*	50.87 ± 5.64	54.12 ± 9.43	t = -1.680	p = 0.099
Gender				
Women n (%)	12 (40%)	6 (18.2%)	X ² = 3.665	p = 0.093
Men n (%)	18 (60%)	24 (81.8%)		
Body height (cm)				
N	30	33		
Mean ± SD*	171.9 ± 9.52	168.1 ± 7.9	t = 1.749	p = 0.085
Body weight (kg)				
N	30	33		
Mean ± SD*	75.5 ± 13.3	79.4 ± 13.4	t = -1.173	p = 0.246

1 Painful lumbosacral radiculopathy patients

Table 2. Patient-reported clinical variables

	Healthy subjects (n = 30)	PLSR ³ (n = 33)	t	p
DASS ¹ 21 - Depression	4.6 ± 5,8	8.9 ± 8	-2.473	0.018
DASS ¹ 21 – Anxiety	3.7 ± 3.6	8.8 ± 7.1	-3.597	0.001
DASS ¹ 21 – Stress	8.5 ± 5.5	15.8 ± 8.8	-3.982	0.000
Length of sleep (h)	6.9 ± 0.9	5.9 ± 1.3	-3.532	0.001
CSI ²	19.4 ± 8.7	40.2 ± 12.3	-7.690	0.000

1 Depression, Anxiety, Stress Scale,

2 Central sensitization inventory

3 Painful lumbosacral radiculopathy patients

As it turned out that there were no significant differences in age, gender, body height and body weight, we continued with the comparison of the remaining variables between the groups.

The following results showed that there was a significant difference in the depression, anxiety and stress subscale of DASS 21 between the studied groups (see Table 2 for more details).

Our data showed that PLSR patients slept one hour less on average than healthy subjects. This difference was statistically significant ($t = -3.532$, $p = 0.001$). Finally, we compared the CSI score values between the studied groups. PLSR group had, on average, more than double the CSI scores compared to the control group. This difference was highly statistically significant ($t = -7.690$, $p < 0.001$).

Discussion

It is believed that 7 – 8% of Europeans suffer from neuropathic pain with lumbosacral radiculopathy as one of the leading causes (26). Central sensitization is one of the mechanisms for maintaining neuropathic pain (15, 16). It is a physiological phenomenon in which neurons of the central nervous system become hyperexcitable (increased excitability), which leads to hypersensitivity to nerve impulses from the periphery (27). Lesions of the somatosensory nervous system could be the pain generator that has potential to push pain processing system into hyperexcited state (28).

The problem with CS is how to measure it. There is no possibility to measure directly CS in humans (13). In the literature, QST and self-reported questionnaire are the most frequently used methods. However, these methods have their flaws. Several specific factors have been found to confound the responses to QST and self-reported questionnaire: anxiety and depression, history of trauma, pre-morbid personality traits, fear of pain and low expectations of recovery,

sleep disorder, personal injury claim/compensation, genetic/familial transmission of CS predisposition, behavioural change in response to reactions of others, particularly spouse (13).

Our results showed more than twice the CSI scores in the group of subjects with neuropathic pain due to lumbosacral radiculopathy indicating that central sensitization symptoms are significantly pronounced in subjects with neuropathic pain. Other researchers found similar results (29 – 31).

These findings are important because patients with symptoms of central sensitization require specially adapted physical treatment as well as careful consideration of the drug therapy they will use. It is also important to note that even small changes and lesions seen on imaging can cause severe pain in these patients (32).

Our results indicated that there is a significant difference in the depression subscale between the groups, that is, that patients with neuropathic pain showed a higher degree of depression compared to healthy controls. Such results are expected, especially when the pain is moderate or severe, which is the case with neuropathic pain (33, 34). The reason for this may be a lower quality of life, a reduced work function or a greater need to use health care services (35). Also, it should be emphasized that depression and chronic pain are extremely related when it comes to elderly people. It is estimated that 13% of this population will have symptoms of both depression and chronic pain present at the same time (34). A number of authors suggest that neuroinflammation plays a key role in the pathogenesis of both conditions (36). In addition to depression, the group of patients with neuropathic pain also showed a higher degree of anxiety compared to healthy controls. Pain is essentially related to anxiety in its definition, which is the likely cause of these differences (36), and similar results are found by other authors (37, 38). We noticed that there were also significant differences between the groups of our respondents

in terms of stress. In this case, it was also confirmed that people with PLSR were significantly more susceptible to stress than healthy subjects. The connection between pain and stress is widely known (39). Pain is part of our homeostatic system and tells us that we are in danger (40).

The connection between pain and sleep should not be ignored. Chronic pain negatively affects sleep (41). One of the indicators of sleep quality is its duration (42). In our research, we used the average duration of the length of sleep in the last month. After the comparison, it was concluded that people with PLSR slept an hour less, on average, than the group of healthy subjects, and that there was a statistically significant difference between these groups in terms of this parameter. People with chronic pain are 18 times more likely to suffer from insomnia compared to the healthy population (43, 44). Other researchers also find results similar to ours (45). Also, it has been shown that anxiety and depression often occur together with chronic pain and insomnia (46).

The main limitation of the present study is relatively small sample size and in the future studies larger samples of patients with PLSR should be evaluated. The second limitation is usage of the self-reported questionnaire for evaluation of the CS. However, it is not possible to directly measure CS in humans, and CSI is useful as a screener of the CS presence (14 – 16, 18). In the future studies, we suggest combination of the self-reported instrument such as CSI and QST, as well as other methods (13).

Conclusion

The present study showed that the degree of central sensitization symptoms, depression, anxiety, and stress, as well as sleep problems were significantly more pronounced in the patients with chronic painful lumbosacral radiculopathy compared to healthy controls. These results should be kept in mind when designing a treatment plan for patients with painful lumbosacral radiculopathy.

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SKALA CENTRALNE SENZITIZACIJE KOD ZDRAVIH ISPITANIKA I ISPITANIKA SA NEUROPATSKIM BOLOM

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Skala centralne senzitivacije (engl. *Central Sensitization Inventory* – CSI) osmišljena je kao instrument za evaluaciju simptoma za koje se smatra da su povezani sa centralnom senzitivacijom. Cilj ovog istraživanja bio je da se ispita da li postoje značajne razlike u izraženosti centralne senzitivacije, stepenu anksioznosti, depresivnosti i stresa između zdravih ispitanika i ispitanika sa neuropatskim bolom, usled lumbosakralne radikulopatije (engl. *painful lumbosacral radiculopathy* – PLSR). Ovom studijom obuhvaćeno je 33 ispitanika sa PLSR (prosečne starosti 54,12 godina ± 9,43 godine; 6 (18,2%) žena) i 30 zdravih ispitanika (prosečne starosti 50,87 godina ± 5,64 godine; 12 (40%) žena). Srpska verzija Skale centralne senzitivacije (CSI) korišćena je za ispitivanje stepena simptoma povezanih sa CSI. Za procenu emocionalnog statusa koristili smo srpsku verziju Skale depresije, anksioznosti, stresa (engl. *Depression, Anxiety, Stress Scale* – DASS 21). Dobijeni rezultati pokazali su značajno veću vrednost CSI skora kod ispitanika sa neuropatskim bolom ($t = -7,690$, $p = 0,000$). Statistički značajno gore vrednosti DASS 21 dobijene su kod ispitanika sa neuropatskim bolom u trima supskalama: supskala depresije ($t = -2,437$, $p = 0,018$), supskala anksioznosti ($t = -3,597$, $p = 0,001$), supskala stresa ($t = -3,982$, $p = 0,000$). Stepenu izraženosti simptoma centralne senzitivacije, kao i stepenu anksioznosti, depresije i stresa, značajno je veći kod ispitanika sa neuropatskim bolom (PLSR) nego u grupi zdravih ispitanika. Ovo treba imati na umu prilikom dizajniranja plana lečenja osoba sa bolnom lumbosakralnom radikulopatijom (PLSR). *Acta Medica Medianae* 2023;62(3):11-16.

Ključne reči: neuropatski bol, centralna senzitivacija, anksioznost, depresija, stress

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APPLYING NEURAL NETWORKS TO HEALTH CARE QUALITY PARAMETERS

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For the purposes of monitoring and assessing the quality of care and treatment offered to patients and providing support for the activities related to health care, a quantitative indicator known as "indicator of quality in health care" is used. This study looked at the accuracy of forecasting case fatality rates using six distinct factors. Researching the relationship between the aforementioned factors (Death rate (percent) within 48 hours of admission, Surgery case fatality rate, The average length of hospital stay, The average number of pre-operative days, The average number of surgical procedures (anesthesia), The average number of nurses per occupied medical ward bed) and the prediction of the case fatality rate was the primary objective. Predictions of the case fatality rate will be made with the help of the Extreme Learning Machine (ELM) that will be built and utilized in the course of the research. Results from an ELM, a genetic programming (GP), and an artificial neural network (ANN) are contrasted and discussed. The accuracy of the computer models was assessed by comparing their predictions to empirical data and using a number of statistical measures. The results of simulations show that ELM may be used effectively in situations where the prediction of case fatality rates is required. *Acta Medica Medianae 2023;62(3): 17-23.*

Key words: case fatality rates, prediction, extreme learning machine

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Introduction

We face new complexities in improving the structure and management of health care delivery, for example, increasing the integration of processes in care delivery for patient-centered chronic disease management (1, 2), as health care systems in developed nations transition to a value-based, patient-centered model of care delivery (3). New technologies, including artificial intelligence, have the ability to handle non-traditional care settings, the changing healthcare workplace and workforce, and the introduction of new fragmented health information systems, all while providing cost-effective and appropriate treatment in real-time (3). There is a dearth of guidance on selecting appropriate methods that are tailored to the health care industry (2), despite

the fact that there is a plethora of solutions capable of addressing these health care management challenges due to the widespread use of artificial intelligence for making complex decisions across a wide range of industries.

Aging populations, illness complexity, medical treatment advancements, rising labor costs, and the growth of the health care industry are expected to cause global health care spending to approach \$8.7 trillion by 2020. It has been reported that many hospitals and other medical facilities cannot afford to upgrade their aging facilities and legacy equipment. Reportedly, decision-makers are shifting their focus to understanding and better aligning financial incentives for health care providers in order to assess the financial risk; population health management, including analyses of health, quality, and cost trends; and adoption of innovative delivery models for improved processes and coordination of care in order to make the transition to value-based care (1, 2).

Health care organizations need to be more strategically managed due to system interdependencies such as shifting environmental demands and competing objectives that may complicate the decision-making process (4). Most companies are risk-averse (5), and healthcare decision-makers may face cultural, technological, and risk-related obstacles when making high-risk decisions without a guarantee of a high return (5, 6). Both clinical (such as diagnosis, treatment and

therapy, medicine prescription and administration) and non-clinical (such as budget, resource allocation, technology acquisition, service additions/reductions, strategic planning) decisions need input from several parties (1, 7).

According to a white paper published by IBM, (8) health care providers are increasingly analyzing massive sets of routinely collected digital information in an effort to improve service and cut costs as the collection and digitalization of health care data (such as electronic medical records and DNA sequences) continues to rise. Concerns regarding the effectiveness of programs may be addressed, for instance, by analyzing clinical, financial, and operational data and making predictions about patients who are at risk. By 2025, the global market for health care predictive analytics is expected to rise from its 2015 value of \$1.48 billion at a CAGR of 29.3 percent (9). Equally promising is the forecasted growth of the artificial intelligence (AI) market for healthcare applications, which is expected to increase by 40% (Compound Annual Growth Rate) by 2021, leading to global revenue of \$811 million. The health care business is expected to be a major driver of the MLaaS market, which is projected to grow to \$5.4 billion by 2022 (10).

In a recent survey of AI's medical applications, artificial neural networks (ANN) were found to be among the most often used machine learning techniques (11). Several significant diseases, including cancer and cardiovascular disease, benefited from this approach. Applications of ANN in healthcare include clinical diagnosis, cancer prediction, speech recognition, hospital stay length prediction (12), image analysis and interpretation (13) (for example, automated electrocardiographic (ECG) interpretation used to diagnose myocardial infarction (14)), and drug development (13). Health care analytics have applications outside of patient care, including improved organizational management (15) and improved forecasting of key factors like cost or facility use (16). The use of ANN in decision support models has resulted in more efficient use of both medical professionals' and the healthcare system's time and resources (17).

Methodology

Though ANN has seen increasing use in recent years (18), there is still room for improvement in how well it can inform choices at different tiers of healthcare organizations. The motivation for this research was the lack of a thorough understanding of ANN's various applications in healthcare, and it is hoped that this work would be of assistance to scholars working to bridge the gap between organizational psychology and computer science. It may be challenging to keep up with the newest advances and trends in ANN applications (19), given the enormous number of reported uses and the complexity of the subject. Newcomers to the field of artificial

intelligence (AI) or ANN adopters may find the vastness and esoteric terminology of neural computing to be particularly challenging (19). Current literature evaluations of ANN applications are either too narrow in scope (i.e., they concentrate on data mining or AI approaches that may contain ANN but do not give insights particular to ANN) or too wide (i.e., they focus on a specific illness (20) or a certain kind of neural network (21)). The major goal of this scoping research is to provide a comprehensive examination of the many ANN applications in health care organization and decision-making at the micro, meso, and macro levels. Decisions about resource allocation or utilization can be made at three distinct levels: the individual patient's (micro) level, the group's (meso) level (e.g., the department's or organization's) level, where patient preference may be important but is not essential, and the public sector's (macro) level (22). We will examine the approach and context used, as well as identify the nature and extent of the applicable literature, by conducting this review. As Kononenko (2001) summarizes, the discipline of artificial intelligence (AI) that deals with machine learning provides essential resources for the intelligent processing of data. As electronic computers were widely available in the 1950s and 1960s, three major subfields of machine learning—statistical methods, symbolic learning, and neural networks—emerged (23). The physical sciences have found success in using ANN to tackle difficult problems, and more recently, scholars in organizational research have found success in using ANN as digital tools to speed up data collection and processing (24). ANN are flexible and useful modeling techniques because of their ability to extend pattern knowledge to new data, tolerating noisy inputs, and producing accurate and acceptable estimations (24). ANN belong to the larger family of flexible nonlinear regression and discriminant models, data reduction models, and nonlinear dynamical systems (25). In terms of their statistical similarities, ANN may be compared to generalized linear models, nonparametric regression, discriminant analysis, and cluster analysis (25). Its general structure as a statistical model is made up of basic, connected processing units that are trained on new sample data over and over again (24). Its use is most helpful in situations where the theoretical basis for prediction is unclear, such as when sample data exhibit complex interaction effects or do not meet parametric assumptions, when the relationship between independent and dependent variables is weak, when there is a large amount of unexplained variance in information, or when there is a lack of information. Single-layer perceptrons, multi-layer perceptrons, and radial basis function networks are examples of feed-forward neural networks, whereas examples of feed-back or recurrent neural networks include Competitive networks, Kohonen's self-organizing maps, and Hopfield networks (26). Data used in

this research is obtained by desk research of different national health systems in World Health Statistics 2022 (27), European Health for All database (28) and The World Bank Health, Nutrition and Population (29). ANN methodology applied is based on MATLAB ANFIS software modified to meet requirements of research scope (30). The research is a part of the project of applying ANN to the management of human

resources in healthcare and in the prediction of public health parameters with the aim of defining how to apply ANN to causal interpersonal and multifactorial relationships, carried out at the Department of Management of the Faculty of Mechanical Engineering in Niš. All relevant input and output parameters for this study are included in Table 1.

Table 1. Input and output parameters

Inputs	Parameters description
Input 1	Death rate (%) within 48 hours of admission
Input 2	Surgery case fatality rate
Input 3	The average length of hospital stay
Input 4	Average number of pre-operative days
Input 5	Average number of surgical procedures (anesthesia)
Input 6	Average number of nurses per occupied medical ward bed
Output	Case fatality rate

ANN

To process information, ANNs use processing units (nodes or neurons) that are coupled by a set of tunable weights so that signals may move both concurrently and sequentially across the network (14, 31). ANNs can contain anywhere from a single to numerous layers. In general, ANNs can be thought of as consisting of three tiers, or layers, of neurons: the input layer, which processes incoming data, the hidden layer, which is responsible for pattern extraction and does the bulk of the network's internal processing, and the output layer, which displays the results of the network's work.

According to a review written by Agatonovic-Kustrin and Beresford (2000), neurons in a neural network draw their energy from one another, and each neuron has a single output, a transfer function, and a weighted input. According to the authors, a single output is generated by the neuron after a transfer function has processed an activation signal based on a weighted sum of all inputs to the neuron. General network behavior is determined by transfer functions, learning rule, and network architecture (31). Nonlinear statistical modeling using ANN offers additional options to the standard approach of creating predictive models for dichotomous outcomes in medicine, logistic regression (32). The networks

can identify complicated non-linear correlations and interactions between dependent and independent variables, and users may do so with less formal statistical expertise. ANN may use both theoretical and empirical evidence to find answers to difficulties (31). Other benefits of ANN over conventional predictive modeling methods include its ability to learn inductively from training data and process non-linear functionality critical to dealing with real-world data, as well as its speed and simplicity of operation due to compact representation of knowledge (e.g., weight and threshold value matrices). Despite the fact that ANN do not need data-source knowledge, they still need big training sets because of all the projected weights (31). They may take a while to train, and getting varied results by using alternative weight initializations (32-36) is possible. While ANN have been put to good use, they still present challenges since we have very little understanding of how they learn or the scope of the information they contain (35). The literature identifies a number of problems with ANN, including their inability to explicitly identify potential causal relationships, their difficulty of use in the field, their susceptibility to over fitting, the fact that model development is empirical, which could mean it takes multiple attempts to develop an acceptable model, and the presence of methodological issues related to model development (37-42). Tu (1996)

outlines the pros and cons of utilizing ANN to predict medical outcomes, arguing that logistic regression models can be shared with more people whereas ANN models are less easy to understand and hence more challenging to implement. However, logistic regression coefficients may be made public for use by any user, while ANN connection weight matrices used for training a data set may be too vast and complicated for others to utilize, even if made publicly accessible (1, 36).

Results and discussion

Here we provide the ELM prediction model's performance results for predicting case fatality rates using the values from Table 1. The ELM model achieves an adequate level of prediction accuracy. Observe that the majority of the data

points lie on the diagonal. Thus, the ELM method's predicted values accord with the observed values to a high degree. An adequate coefficient of determination permits confirmation of this finding. Inaccurate estimates or projections are rare. Accordingly, it can be shown that the projected values have a very high degree of accuracy.

Performance comparison of ELM, ANN and GP

The prediction accuracy of the ELM models was compared to the prediction accuracy of the ANN and GP, which were used as benchmarks, to show the benefits of the proposed ELM technique more clearly and convincingly. The traditional statistical error indices (RMSE and R²) were employed for comparison. The findings from the forecasts are summarized in Table 2.

Table 2: Comparative performance statistics of the ELM, ANN and GP models for case fatality rate prediction in training ELM

		ANN		GP	
RMSE	R ²	RMSE	R ²	RMSE	R ²
0.1910	0.9926	0.2453	0.9878	0.3561	0.9743

Conclusion

One of the driving forces for the introduction of quality indicators in health care is the need to reduce cost, a priority in all sectors of the economy. This was accomplished via better process management. As a result, the efficiency of the healthcare system overall improves, as the chance for errors during treatment is greatly reduced, and the cost associated with duplicative efforts are cut down. It's important to note that process management in terms of health care quality indicators is a cornerstone of the idea of contemporary medicine. To put it simply, this basis is crucial to the practice of contemporary medicine. The high number of signs and factors that affect the case fatality rate makes precise forecasting of the case fatality rate in the future challenging. Therefore, this study offered a fresh approach to addressing the challenges of predicting the case fatality rate. In this approach,

we discard non-critical features of the input data.

Due to the many signs and factors that affect the case fatality rate, it is challenging to make an accurate prognosis of the case fatality rate in the future. Since this is the case, the findings reported here provide a unique approach to HHI forecasting that makes use of soft computing methods in order to circumvent the aforementioned challenges.

An efficient learning model based on ELM was developed to predict the case fatality rate with high accuracy. In comparison to the results of the ANN and GP, the accuracy of the forecasted values was assessed. It was shown via simulation that the ELM model had the best potential for estimating the case fatality rate. The ELM technique has potential use in estimating fatality rates and other applications where the fatality rate is relevant. This is true in the broad and the +narrow sense.

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PRIMENA NEURONSKIH MREŽA NA PARAMETRE KVALITETA ZDRAVSTVENE ZAŠTITE

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Za potrebe praćenja i procene kvaliteta nege i lečenja koji se nude pacijentima i pružanja podrške aktivnostima koje se odnose na zdravstvenu zaštitu koristi se kvantitativni indikator poznat kao „indikator kvaliteta u zdravstvenoj zaštiti“. Ova studija je razmatrala tačnost predviđanja stope smrtnosti slučajeva koristeći šest različitih faktora. Istraživanje odnosa između navedenih faktora (stopa smrtnosti (procenat) u roku od 48 sati od prijema, stopa smrtnosti hirurških slučajeva, prosečna dužina boravka u bolnici, prosečan broj preoperativnih dana, prosečan broj hirurških zahvata (anestezija), prosečan broj medicinskih sestara po zauzetom krevetu na medicinskom odeljenju) i predviđanje stope smrtnosti slučaja bili su primarni cilj. Predviđanja stope smrtnosti slučajeva urađena su uz pomoć mašine za ekstremno učenje (ELM), izgrađene i korišćene u toku istraživanja. Rezultati ELM-a, genetskog programiranja (GP) i veštačke neuronske mreže (ANN) bili su predmet poređenja i diskusije. Tačnost kompjuterskih modela procenjena je upoređivanjem njihovih predviđanja sa empirijskim podacima i korišćenjem niza statističkih mera. Rezultati simulacija pokazuju da se ELM može efikasno koristiti u situacijama kada je potrebno predviđanje stope smrtnosti. *Acta Medica Medianae 2023; 62(3): 17-23.*

Ključne reči: stopa smrtnosti, predviđanje, mašina za ekstremno učenje

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EVALUATION OF THE EROSION POTENTIAL OF FOURTEEN COMMERCIAL BEVERAGES BY MEASURING PH AND DETERMINING TITRATABLE ACIDITY

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Chemical factors that determine the erosive potential of food and beverages include pH value, mineral content, buffer properties (titratable acidity and buffer capacity), etc. The goal was to determine the pH and titratable acidity (TA) in fourteen commercially available beverages. Five carbonated soft drinks (among them two energy), two sports drinks, two fruit juices, two teas and three alcoholic drinks were evaluated. The initial pH was measured using a pH meter, and TA was determined by titration with NaOH. The pH and TA data were recorded as mean values of triplicate measurements \pm standard deviation. The pH values ranged from 2.51 (Guarana No Sleep) to 6.64 (green tea), and TA ranged from 0.54 ml (Coca-Cola) to 4.28 ml (orange juice) of NaOH to reach pH 5.5, and 1.08 ml (chokeberry juice) to 5.83 ml (orange juice) to reach pH 7.0. Literature data suggest that unsaturated substances with low pH and high TA have a high erosive potential. Drinks such as Guarana No sleep, Schweppes Bitter Lemon, Coca Cola and Sprite were found to have a pH below 3.0 and can be extremely erosive (Guarana have the highest TA) if consumed frequently, and with the habit of holding in the mouth. Also, regular and large consumption of drinks with a high TA (orange juice and Red Bull) could increase the risk of dental erosion, regardless of their pH above 3.0. *Acta Medica Medianae* 2023;62(3): 24-31.

Key words: dental erosion, commercial beverages, erosive potential, pH, titratable acidity

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Introduction

Tooth erosion, which implies the demineralization of teeth without the participation of bacteria (1), has become a real challenge for both researchers and clinicians in recent years. The complexity of erosive damage requires constant scientific and professional analysis from multiple aspects in order to prevent disease and preserve dental health in the long term.

Acidic substances, which may have an internal or external origin, or both at the same time, are mainly responsible for tooth erosion. The most common external factors of dental erosion are fruit juices, carbonated drinks, sports and energy drinks (2–4) whose consumption has increased sharply in recent decades. According to the latest data, the leading consumers of soft drinks (especially carbonated) are the United States of America with 154 liters of annual consumption per inhabitant. In Europe, the leading countries in the consumption of soft drinks are Belgium with 109, followed by Germany and Norway with 98 liters of annual consumption per capita. Europe tops the consumption of energy drinks, while in the USA sports drinks are the most popular (5).

Acidic foods, as well as acidic dietary supplements (acidic fruits, pickled vegetables, salad dressings, acidic candies, chewable vitamin C tablets, etc.) can also have an erosive effect (2). Apart from the fact that it can occur in chronic alcoholics, tooth erosion can be a problem for people who are involved in wine tasting, because the prolonged contact of acidic drinks and enamel can result in its damage (6, 7). In endogenous erosions, the main reasons for gastric acid

reaching the oral cavity are gastroesophageal reflux disease (GERD), vomiting during pregnancy, bulimia, anorexia nervosa, xerostomia and malabsorption syndrome (7).

The erosive potential of a substance (ability to cause dental erosion) depends on several chemical parameters, such as pH and buffer properties (titratable acidity -TA and buffer capacity, acid type, calcium and phosphate concentration (degree of saturation) and fluoride content (8). The pH value is an indicator of the initial concentration of hydrogen ions in the solution. Although it affects the degree of saturation (by determining the activity of PO_4^{3-} and OH^- ions), it represents an important independent factor of dissolution (9). According to many data from the literature, pH is a critical determinant of the erosive potential of beverages (10-12). Consumption of beverages with a higher concentration of available hydrogen ions ($\text{pH} < 4.0$) leads to an immediate softening of the tooth surface, which becomes susceptible to removal by abrasion and wear (8).

All acids, potential causes of erosive damage, are weak acids (except HCl) that are in an undissociated form at low pH values, but gradually dissociate with increasing pH (8). The following acids are found in food and drinks: phosphoric (cola drinks), citric (fruit, fruit juices, sports and energy drinks), acetic (vinegar, salad dressings, pickles), malic (apples, pineapples, grapes, orange juice, white wine), tartaric (grapes, champagne, red wine), lactic (cheese, yogurt, red wine) and ascorbic acid (vitamin C as a supplement to food and drink, some carbonated drinks - Sinalco, Izostar, etc.). Due to their gradual dissociation, weak acids act as buffers and thus oppose changes in pH value (13).

Although the pH value is an important factor of erosive potential, it does not provide information about the total acidity of the solution (drink or food), ie. about on the presence of undissociated acids. Titratable acidity, as a measure of solution buffering, is a strong indicator of higher concentrations of undissociated acid species in the erosive substance (8). The undissociated form of the acid is of great importance, because it has no charge and can diffuse more easily into the subsurface enamel layer ("near surface" layer). Once there, this species dissociates by acting as a proton (H^+) carrier in the enamel mineral and maintains an acidic (unsaturated) state that promotes further dissolution. This means that the pH of the environment will remain low for longer, and enamel dissolution will continue to progress (14).

In this research, the goal was to measure the pH and TA of fourteen beverages, which are commercially available to the Serbian consumer, based on which certain recommendations would be given regarding the consumption of beverages that can cause erosive damage to teeth.

Material and methods

To test the erosive potential of beverages, by measuring pH and determining TA, 12 commercially available beverages and two types of tea were used: five carbonated soft drinks (Coca-Cola, Schweppes, Sprite and two energy drinks: Guarana and Red Bull), two fruit juices (orange and aronia), two sports drinks (Aqua Viva Recharge Strong and Isodrinx isotonic Sports Drink), two teas (green and black) and three alcoholic drinks (beer, white and red wine). Four commercial packages were used for each drink. Table 1 shows the compositions of the tested beverages as listed on their packaging.

In line with the published protocol (3, 15), the pH was measured using a previously calibrated multifunctional electronic device CONSORT C830 (Consort bvba, Belgium), with the 50 ml of beverage placed in a beaker and stirred using a non-heating magnetic stirrer until a reaching stable reading. TA was calculated as the volume of a 0.9613 M NaOH solution required to increase the pH of each beverage to 5.5 and 7.0. The solution was added in aliquot of 0.3 ml while stirring with a non-heating magnetic stirrer until a stable pH reading was achieved. The values of pH and TA, respectively, were determined three times for each drink and an average value was calculated.

The pH was measured immediately after opening the packaging (bottle or can) at room temperature, while the prepared teas were cooled to a temperature of 22 °C.

Results

Initial pH values of the analyzed beverages and titratable acidity are expressed as mean values of triplicate measurements \pm standard deviation (Table 2).

Four drinks (Guarana No sleep, Schweppes Bitter Lemon, Coca Cola and Sprite) had a pH below 3.0. Seven drinks had a pH range of 3.0–4.0 (including Riesling white wine with a pH of 3.02) and three drinks had a pH above 4.0 (chokeberry juice $\text{pH}=4.04$). Green and black tea showed not much lower pH values than neutral (6.64 ± 0.02 and 6.61 ± 0.04).

Coca-Cola showed a rapid response when adding only 0.54 ml of NaOH to reach a pH of 5.5, which is the lowest TA value measured, and 1.73 ml to reach a pH of up to 7.0. Orange juice had the highest TA, with 4.28 or 5.83 ml of NaOH to reach equivalent pH values, followed by Red Bull (3.27 and 5.21). Due to the high pH of teas (above 5.5), their TA was calculated only up to pH 7.00 and was 0.13 ± 0.03 for green and 0.12 ± 0.02 for black tea.

Table 1. Compositions of the tested drinks as listed on their respective packaging

Beverages		Composition
Manufacturer		
1.	Coca-Cola	Water, sugar, carbon dioxide, caramel color (E150d), phosphoric acid, natural flavors including caffeine
	HBC – Srbija A.D. Zemun, Serbia	
2.	Schweppes Bitter Lemon	Water, high fructose syrup, lemon juice, carbon dioxide, lemon extract, citric acid, flavors, preservative potassium sorbate, antioxidant ascorbic acid, stabilizers E 1450 and E 445, color carotene.
	HBC – Srbija A.D. Zemun, Serbia	
3.	Sprite	Water, high fructose syrup, carbon dioxide, citric acid, acidity regulator sodium citrate, natural lemon and lime flavors, preservative sodium benzoate.
	HBC – Srbija A.D. Zemun, Srbija	
4.	Guarana No Sleep	Water, sugar, carbon dioxide, citric acid, taurine, guarana aroma, caffeine, vitamin mixture, preservative: sodium benzoate, color: E150d
	Knjaz Miloš A.D., Arandelovac, Serbia	
4.	Red Bull	Water, sucrose, glucose, citric acid, carbon dioxide, taurine, acidity regulators, caffeine, vitamins, flavors, colors (caramel, riboflavin)
	Red Bull, GmbH Fuschl am See, Austria	
5	Isodrinx Isotonic Sports Drink	Water, sugar, glucose, acidity regulator, citric acid, sodium chloride, sodium citrate, white emulsion (stabilizers E1450 and E445), calcium gluconate, preservative potassium sorbate, potassium gluconate, preservative sodium benzoate, magnesium citrate, aroma, color brilliant blue
	Nutrend, D.S.Chvalkovic, Czech Republic	
6.	Aqua Viva Recharge	Water, fructose, dextrose, citric acid, sodium citrate, potassium phosphate, magnesium carbonate, table salt, preservatives: E202 and E211, stabilizers E414 and E445, red orange flavor, sweetener E960 - steviol glycosides, colors E110 and E122
	Knjaz Miloš, A.D., Arandelovac, Serbia	
7.	Life Premium 100% voćni sok pomarandža	Water, concentrated orange juice, citric acid
	Nectar D.O.O. Bačka Palanka, Serbia	
8.	Chokeberry juice	Anthocyanins, proanthocyanidins, phenolic acids, flavanols, pectins, organic acids, proteins, carbohydrates, vitamin C, vitamin E, carotenoids, iodine, potassium, calcium and magnesium
	Loveberry, Valjevo, Serbia	
10.	Zaječarsko Pivo	Water, barley malt, corn grits, hop extract
	Heineken Srbija D.O.O. Zaječar, Serbia	
11.	Crnogorski Vranac Red Wine	Water, alcohol 12%, glycerol, organic acids, tannins, phenols, anthocyanins, flavan-3-ols, flavonols
	13. Jul Plantaže A.D., Podgorica, Montenegro	
12.	Royal Grozd – Rizling dry white wine	Water, alcohol 10.5%, lactic acid, malic acid, tartaric acid, citric acid, succinic acid, acetic acid and sulfates
	Vinarija Levač D.O.O Rekovac, Serbia	
13.	Green tea in filter bags	Caffeine, theophylline, theobromine, kaempferol, quercetin, chlorogenic acid, caffeic acid, gallic acid, catechin tannins, heterosides of terpene alcohols, fats, proteins, minerals, vitamin C, vitamin B
	Adonis pharmacy, Soko Banja Niš, Serbia	
14.	Black tea In filter bags	Caffeine, theophylline, theobromine, kaempferol, quercetin, caffeic acid, gallic acid, fats, proteins, minerals, fluorine, vitamin C, vitamin E
	Adonis pharmacy Soko Banja Niš, Serbia	

pH and titratable acidity (TA) measurement

Table 2. Initial pH and TA of the beverages

	Beverages	Initial pH \pm s.d.	TA \pm s.d. up to pH	
			5,5	7,0
1.	Coca Cola	2.56 \pm 0.06	0.54 \pm 0.05	1.73 \pm 0.07
2.	Schweppes Bitter Lemon	2.54 \pm 0.03	2.72 \pm 0.05	4.37 \pm 0.05
3.	Sprite	2.71 \pm 0.04	1.87 \pm 0.03	2.85 \pm 0.06
4.	Guarana No Sleep	2.51 \pm 0.03	3.24 \pm 0.03	4.96 \pm 0.05
5.	Red Bull	3.32 \pm 0.08	3.27 \pm 0.05	5.21 \pm 0.04
6.	Isodrinx Isotonic Sports Drink	3.43 \pm 0.02	1.55 \pm 0.03	2.41 \pm 0.05
7.	Aqua Viva Recharge (red orange) Strong	3.18 \pm 0.03	1.84 \pm 0.06	3.14 \pm 0.04
8.	Life Premium 100% orange juice	3.82 \pm 0.04	4.28 \pm 0.03	5.83 \pm 0.05
9.	Chokeberry juice	4.04 \pm 0.04	0.71 \pm 0.03	1.08 \pm 0.05
10.	Zaječar light beer	3.96 \pm 0.05	0.64 \pm 0.05	1.59 \pm 0.07
11.	Red wine <i>Kratošija</i>	3.49 \pm 0.05	1.82 \pm 0.04	2.34 \pm 0.03
12.	White wine <i>Rizling</i>	3.02 \pm 0.06	2.69 \pm 0.03	3.18 \pm 0.05
13.	Green tea	6.64 \pm 0.02	/	0.13 \pm 0.03
14.	Black tea	6.61 \pm 0.04	/	0.12 \pm 0.02

Discussion

Many authors pointed out the complexity of the erosive process in which, in addition to chemical parameters, physical factors are also important (flow rate - swishing or holding the drink in the mouth, frequent consumption - numerous short periods of acid exposure, temperature of the drink, adhesive ability). In addition, three biological factors are of great importance: the structure of the teeth, the influence of saliva, and the acquired pellicle. Whether the erosive potential of the beverage will succeed in manifesting itself through erosive damage to the teeth will depend on host factors and exposure conditions (2, 5, 16-18).

The pH measure of the substance acidity is an important indicator of dental erosion: as pH decreases, erosive damage increases, regardless of the way erosion is measured (9). At low pH, it is possible that some other influences are strong enough to prevent erosion, but also, erosion can progress in a solution with a relatively high pH in the absence of facilitating factors. This means that calcium and phosphate concentration, in

combination with pH, determine the degree of saturation with respect to tooth minerals. It has therefore been suggested that there is no fixed critical pH for tooth erosion (9). This value is calculated based on the concentration of calcium and phosphate in the erosive solution. Lussi et al. published critical pH values (pH_c) in relation to hydroxyapatite for various beverages, acidic food supplements and medications and found that they ranged from 3.9 to 6.5. From these data, the critical pH values of several beverages, whose erosive potential was determined in this paper, were extracted (Coca Cola 5.1; Sprite 6.5; orange juice 3.6; beer 5.0; red wine 5.1; white wine 5.1; and black tea 5.6) (19). Nevertheless, in this study the aim was to measure the initial pH and determine the TA, while the measurement of the concentration of calcium and phosphate was not in its focus.

The range of pH values was from 2.51 \pm 0.03 (Guarana No Sleep) to 6.64 \pm 0.02 (green tea). The obtained pH results for individual beverages are similar to reports of pH values of beverages published by other researchers, for example, pH of Coca Cola drink 2.56 compared to 2.45 (2), 2.67

(3), 2.55 (4); Red Bull energy drink pH was 3.32 compared to 3.30 (2), 3.81 (20), 3.35 (4); orange juice pH 3.82 compared to 3.56 (2), 3.60 (21); red wine pH 3.49 compared to 3.48 (21); white wine 3.02 compared to 3.60 (2).

Other authors, examining the erosive potential of the same beverage of different brands, found that the range of beer pH is 4.26–4.34 (22) (in the present study 3.96), white wine is 2.99–3.56 (23) (in the present study 3.02), red wine 3.43–3.68 (2) and 3.75–4.02 (23) (in the present study 3.49).

There was a smaller or larger difference in the pH values of the teas compared to the literature data, which can be explained by the differences in the type of tea (ready-made teas or those that are prepared immediately before consumption). In the present study green tea had a pH of 6.64 compared to 5.4 (24) and 6.75 (25) and black tea 6.61 compared to 5.0 (24) and 7.02 (25).

As for sports drinks, this study included domestic brands - Isodrinks Isotonic Sports Drink (pH 3.43) and Aqua Viva Recharge (pH 3.18), which could only be compared with sports drinks of those brands that are published in the literature, such as Gatorade with pH 3.17 (2) and 2.89 (4) or Isostar (pH 3.87) (2). Also, there are no data on testing the erosive potential of chokeberry juice.

Reddy et al. published an extensive study on calculating the pH of 380 commercially available soft drinks in the US (10). Based on a study of apatite solubility (11) indicating a logarithmic increase in apatite solubility as pH decreases under laboratory equilibrium conditions, Reddy et al. proposed to separate the chemical erosive potential of beverages into 3 zones: the zone of highly erosive beverages with pH <3, the zone of erosive beverages (pH from 3.0 to 3.99) and the zone of minimally erosive beverages (pH ≥4.0) (10). According to this recommendation, in present paper, all carbonated drinks except Red Bull would belong to highly erosive drinks, the erosive zone would include orange juice, sports and alcoholic drinks, as well as Red Bull, and minimally erosive drinks such as chokeberry juice, green and black tea.

According to Redi et al., knowledge of beverage pH is essential for the development of prevention in patients prone to erosive tooth damage. Their advice for erosion prevention is the elimination of extremely erosive drinks (pH <3.0), minimizing erosive drinks (pH 3.0–3.99), and substituting drinks with a (pH ≥4.0) (10).

Regardless of this emphasized theoretical approach to the solubility of apatite as a function of pH, a low pH value of an erosive substance (drink) does not necessarily mean enamel dissolution. As already mentioned, the erosive potential of the drink will also depend on the content of calcium and phosphate ions, which can be considered protective factors (2, 4, 20). One example is yogurt, which has a pH of around 4,

but is not erosive due to the high concentrations of these ions (2, 4).

From a chemical point of view, the type of acid in the erosive solution (drink) is also important. Apart from mono (acetic and lactic) and diprotic (malic and tartaric) acids, triprotic acids, which include citric and phosphoric, are particularly noteworthy. Citric acid can produce three hydrogen ions from each molecule that directly dissolves enamel minerals by reacting with carbonate or phosphate ions. Apart from hydrogen ions, the aqueous solution of citric acid contains acidic anions (citrate) and undissociated acid molecules. Given the number of hydrogen ions in the molecule, this acid will dissociate in three phases. On the other hand, the citrate anion can form complexes with calcium, removing it from the crystal surface. The activity of citric acid also depends on the pH. At lower pH values, this acid dissociates to provide hydrogen ions that directly attack the surface of the mineral, and at higher pH, the citrate ion extracts calcium from the surface of the crystal. At medium pH values, both mechanisms function. Citric acid thus shows a double harmful effect by demineralizing the tooth surface (8). Similar to citric acid, phosphoric acid also belongs to the class of triprotic, with three values of the dissociation constant, providing hydrogen ions at a lower pH and bonding with calcium in solution at a higher pH value. The difference is in the formation of complexes with calcium, which are stronger with citrate and have a three-dimensional shape of the molecule (8, 14).

The buffer properties of an aqueous solution represent a measure of resistance to pH changes and can be expressed through titratable acidity (the amount of base in mmol/L, required to raise the pH to a defined level of 5.5 and/or 7.0) and buffer capacity (determining how much base can withstand a solution without changing the pH value) (12, 14). This study focused on the determination of titratable acidity, a parameter of erosive potential that is considered more suitable than buffering capacity because it maintains longer the concentration of hydrogen ions available for interaction with the tooth surface, i.e. has a "closer" relationship with the concentration of undissociated acid (14). In relation to pH, Jensdottir et al. found a significant correlation between TA and dental tissue dissolution after long-term exposure to soft drinks (24 hours), while after short-term exposure (3 min) erosion was related to pH and not to TA. Therefore, it has been suggested that TA is a better indicator of erosive potential during longer erosive challenges and pH is more accurate for short exposure (26).

The TA values measured in this study were similar to the results of other authors (15, 27, 28), when comparing the values in milliliters of added base until reaching pH 5.5 or 7, 0. Contemporary literature suggests that TA should be calculated as a concentration in mmol/L, which is more chemically correct and allows for easier comparison (5, 8, 9). Therefore, the value levels

of this erosive potential parameter in the present research did not always correspond to the data from studies that used the recommended units (2, 4, 29, 30).

Conclusion

Although erosion is a multifactorial condition, which depends on many risk and protective factors, this research has pointed out some chemical aspects that are important for the erosive potential of some commercially available beverages. Literature data suggest that unsaturated substances with low pH and high titratable acidity have a high erosive potential. Drinks such as Guarana No sleep, Schweppes Bitter Lemon, Coca Cola and Sprite were found to have a pH below 3.0 and can be extremely erosive (among them is Guarana with the highest TA) if consumed frequently, and, for example, with the habit of holding in the mouth. Also, regular and large consumption of drinks with a high TA

(orange juice and Red Bull) could increase the risk of dental erosion, regardless of the fact that their pH is above 3.0. Therefore, knowledge of the beverages/food erosive potential (pH and TA) is very important and should be an integral part of the preventive strategy of erosive dental damage.

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EVALUACIJA EROZIVNOG POTENCIJALA ČETRNAEST KOMERCIJALNIH NAPITAKA PREMA PH VREDNOSTI I TITRABILNOJ KISELOSTI

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Hemijski faktori koji određuju erozivni potencijal hrane i pića uključuju pH vrednost, sadržaj minerala, puferska svojstva (titrabilnu kiselost i puferski kapacitet) itd. Cilj ovog rada bio je da se odrede pH vrednost i titrabilna kiselost (TK) četrnaest komercijalno dostupnih napitaka. Procenjavano je pet gaziranih bezalkoholnih pića (među njima dva energetska), dva sportska napitka, dva voćna soka, dva čaja i tri alkoholna pića. Početna pH vrednost napitaka merena je pH metrom, a TK je određena titracijom sa NaOH. Podaci o pH i TK zabeleženi su kao srednje vrednosti trostrukih merenja ± standardna devijacija. Vrednosti pH kretale su se od 2,51 (Guarana No Sleep) do 6,64 (zeleni čaj), a TK od 0,54 ml (Coca-Cola) do 4,28 ml (sok od pomorandže) NaOH da bi se pH dovela do pH 5,5 i od 1,08 (sok od aronije) do 5,83 (sok od pomorandže) do dostizanja neutralne vrednosti pH. Podaci iz literature sugerišu da nezasićene supstance sa niskom pH vrednosti i visokom TK imaju visok erozivni potencijal. Ustanovljeno je da pića Guarana No sleep, Schweppes Bitter Lemon, Coca-Cola i Sprite imaju pH vrednost ispod 3,0 (među njima Guarana ima najveću vrednost TK), te mogu biti izuzetno erozivna ako se konzumiraju često i uz naviku zadržavanja u ustima. Takođe, redovna i u velikim količinama konzumacija pića sa visokom TK (sok od pomorandže i Red bull) mogla bi povećati rizik od dentalne erozije, bez obzira na to što je njihova pH vrednost iznad 3,0. Poznavanje erozivnog potencijala (pH i TK) pića/hrane veoma je važno i treba da bude sastavni deo preventivne strategije erozivnih oštećenja zuba. *Acta Medica Medianae* 2023;62(3):24-31.

Ključne reči: erozija zuba, komercijalna pića, erozivni potencijal, pH, titrabilna kiselost

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CHARACTERISTICS OF OVARIAN TUMOR CHANGES IN THE PEDIATRIC POPULATION

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On average, a third of all adnexal masses in girls originate from the ovary. The clinical presentation is non-specific. The treatment trend is ovary-preserving surgery. The aim of the work was to present the characteristics of ovarian masses in the population of girls aged up to 18 years. This retrospective study was conducted by analyzing the medical records of female newborns and girls up to 18 years of age. In the neonatal group, aged up to 12 months, 44.4% had right-sided and 55.6% left-sided ovarian masses. Cystectomy was performed in 6 (66.7%), while ovariectomy and salpingo-oophorectomy were performed in 1 (11.1%) baby each. In the group of girls aged 1–18 years, there were 63% right-sided and 34% left-sided changes. One girl had bilateral. The largest number of girls underwent cystectomy 24 (63.2%). Of the non-neoplastic changes, there were 9 (23.6%) follicular cysts, 8 (21.1%) simple cysts, 6 (17.1%) haemorrhagic corpus luteum cysts, while of the neoplastic benign changes there were 11 (28.8%) mature teratoma, and 4 (11.4%) serous cystadenoma.

The incidence of ovarian tumor changes in the population of girls up to the age of 18 is very low. Non-neoplastic changes occur much more often. *Acta Medica Medianae* 2023;62(3):32-41.

Key words: tumor changes, ovary, neonates, girls

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Introduction

Available literature data are scarce regarding ovarian tumour changes in pediatric population in comparison to adult population, and a lot still remains unknown. There are almost no official guidelines on the management of ovarian tumours in children, so more clinical studies are necessary in order to improve the treatment quality (1).

On average, one third of all adnexal masses in young girls originate from the ovaries, but they also may originate from fallopian tubes and other pelvic organs. These changes are predominantly non-neoplastic, with cystic changes being the most common ones. They are followed by neoplastic benign changes, while neoplastic malignant changes are extremely rare (2).

Clinical manifestation is nonspecific and may often mimic other pathological conditions with similar symptomatology, thus making diagnostic procedure more difficult. The most common presenting symptom is abdominal pain, although these tumours may be utterly asymptomatic. Imaging techniques are leading diagnostic methods in diagnosing these changes, while laboratory analyses, such as value of tumour markers assessment, may be useful in differentiating benign from malignant tumours (3).

From a historic perspective, the main principle in treating all adnexal masses was oophorectomy. Having in mind the fact that these tumours are predominantly benign at this age, oophorectomy was often not necessary, resulting in an increased risk of infertility and other complications. This is why the treatment trend in recent years has been ovarian-sparing surgery aiming at healthy ovarian tissue preservation (4).

Aim of the paper

The aim of the paper was to demonstrate characteristics of ovarian tumour changes in the population of girls up to 18 years of age, and to point out their specificities related to age.

Material and methods

This retrospective study was conducted by analyzing medical records of female newborns and girls up to 18 years of age, treated at the Clinic for Pediatric Surgery, Orthopedics and Traumatology, Clinical Center Niš, in the period from January 1, 2017 to October 10, 2022.

A criterion for study inclusion was the diagnosis of ovarian tumour changes. All the patients underwent a complete clinical, laboratory, ultrasound, radiological examinations and imaging, as well as nuclear magnetic resonance imaging (MRI) when surgical treatment was indicated after the tumour had been diagnosed and specimen sent for pathohistological analysis (PH). The patients were divided into two groups according to their age. The first group comprised infants (0–1 year of age), and the second group comprised patients aged between 1 and 18 years. Based on medical history, we analyzed the side and size of the tumour, type of tumour and performed surgical intervention as well.

Upon collecting data, a unique database was created in EXCEL, followed by statistical analysis and data processing by using SPSS software package. Descriptive statistics (mean value) and a measure of variability (standard deviation) were used in the study. The Student’s t-test was used to analyze how significant the differences were. The results are shown in tables and graphs.

Results

The study included 9 babies who had a surgery intervention. The oldest baby was 80 days old, and the youngest 5 days old. Average age was 32.11±23.42, with the median of 30 days (Table 1).

In 4 (44.4%) babies, changes were located on the right side and in 5 (55.6%) babies on the left side (Graph 1).

Table 2 shows distribution of performed surgical interventions. In the majority of babies, i.e. 6 (66.7%), cystectomy was performed and one newborn baby girl underwent ovariectomy and salpingo-oophorectomy (11.1%). In one patient, autoamputation of the cyst was detected intraoperatively (11.1%).

The most common pathohistological (PH) finding was simple cysts, in 8 (88.9%) babies, while haemorrhagic pseudocyst was present in 1 baby (11.1%).

Girls up to 18 years

The study included 38 girls in whom surgery intervention was performed. The oldest girl was 17, and the youngest 5 years old. Average age of all the girls was 14.37±2.91 years, with the median of 15 years (Table 3).

The greatest number of changes was evidenced in 24 (63%) girls, located on the right side, while in 13 girls (34%) there were left-sided changes. One girl had a bilateral change (Graph 2).

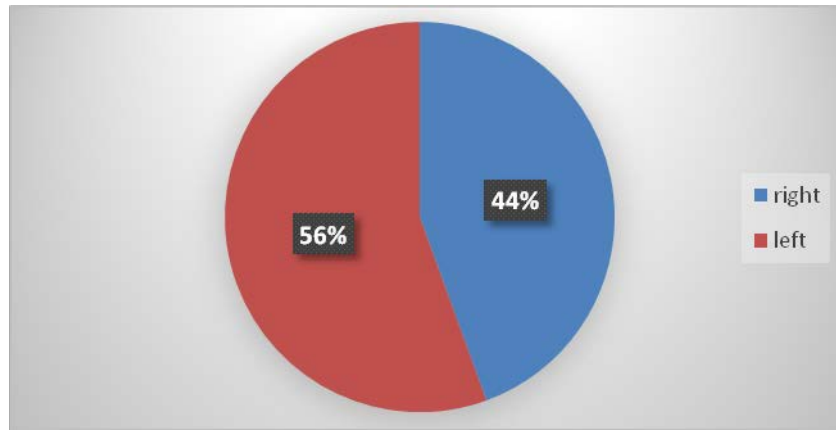
Table 4 shows the distribution of surgical interventions performed. In the majority of girls, i.e. 24 (63.2%), cystectomy was performed, a combination of ovariectomy and puncture in 4 girls (10.5%), adnexectomy in 3 (7.9%), partial ovariectomy in 2 (5.3%) patients, and there was autoamputation of the cyst in one girl.

A total of 21 girls had non-neoplastic ovarian tumours: follicular cysts in 9 (23.6%), simple cysts in 8 (21.1%), haemorrhagic corpus luteum cysts found in 6 (17.1%) girls, while, out of neoplastic benign lesions, the most common ones were a mature teratoma in 11 (28.8%) and serous cystadenoma in 4 (11.4%) girls (Table 5).

The cysts were divided into three groups according to their size. Small-sized cysts up to 5 cm were seen in 16 (47.1%), medium-sized 5–10 cm in 15 (44.1%), and big-sized over 10 cm in 3 (8.8%) girls (Table 6).

Table 1. Distribution of ovarian tumors according to age in neonatal population

Characteristics		
	Girls n (%)	9 (100,0)
Age	$\bar{x} \pm SD$	32,11±23.42
(days)	Me (Iq)	15 (29)
	Min-Max	5-80



Graph 1. Distribution of ovarian tumors according to predominant side of occurrence in newborns

Table 2. Distribution of performed surgical interventions in neonatal population

Type of surgery	n	%
Cystectomy	6	66.7
Ovariectomy	1	11.1
Salpingo-oophorectomy	1	11.1
Autoamputation	1	11.1
Σ	9	100.0

Table 3. Distribution of ovarian tumor changes according to age in a population of girls aged from 1 to 18 years

Characteristics		
	Girls n (%)	38 (100,0)
Age	$\bar{x} \pm SD$	14.37 \pm 2.91
(years)	Me (Iq)	15 (3)
	Min-Max	5-17



Graph 2. Distribution of ovarian tumor changes according to the predominant side of occurrence in a population of girls aged between 1 and 18 years

Table 4. Distribution of performed surgical interventions in a population of girls between 1 and 18 years of age.

Type of surgery	n	%
Cystectomy	24	63.2
Ovariectomy	4	10.5
Partial ovariectomy	2	5.3
Puncture	4	10.5
Adnexectomy	3	7.9
Autoamputation	1	2.6
Σ	38	100.0

Table 5. Distribution of PH findings of ovarian tumor changes in a population of girls aged between 1 and 18 years

PH finding	n	%
Follicular cysts	9	23.6
Haemorrhagic corpus luteum cysts	6	17.1
Mature Teratoma	11	28.8
Simple cysts	8	21.1
Serouscystadenoma	4	11.4
Σ	38	100.0

Table 6. Distribution of ovarian tumor changes according to the size in a population of girls aged 1–18 years

Cysts	n	%
Small (up to 5 cm)	16	47.1
Medium (5–10 cm)	15	44.1
Large (>10 cm)	3	8.8
Σ	34	100.0

Discussion

Ovarian tumours are rare in the pediatric population. The literature data report the incidence of 2.6 cases per 100,000 girls, and they represent approximately 1% of all childhood malignancies. About 10–20% of all ovarian tumour masses have malignant potential (5,6). The World Health Organization has recommended classification of ovarian tumours into two big groups: non-neoplastic and neoplastic tumours. Non-neoplastic lesions, such as functional cysts and benign tumours, are most common tumour masses in the pediatric population (7).

The most frequent symptom of ovarian tumour is abdominal pain (in 57% of patients), while palpable abdominal or pelvic masses are present in 46% cases. They may be accompanied by nausea, vomiting, weight loss, obstipation, urinary infection and dysuria symptoms.

They may clinically be completely asymptomatic, or presented as the manifestation of acute abdominal pain caused by tumour mass torsion. Accompanying endocrine changes, such as precocious puberty, abnormal vaginal bleeding, virilization or masculinization, may also be the first manifestation of hormonally active tumours (3).

In every palpable abdominal mass in lower abdomen, the initial and gold standard in diagnostics is abdominal ultrasound which provides information on tumour mass size, localization, and the nature of tumour by distinguishing a cystic, solid or mixed tumour. Computed tomography and magnetic resonance imaging provide additional information on tumour nature and they show if the tumour has spread (8).

Neonatal ovarian cysts are most common abdominal masses in female newborns. The exact pathogenesis is still unknown. There are some data that point out the influence of maternal placental gonadotropins, especially in the third trimester, that stimulate fetal ovaries and thus ovarian cysts are formed (9). It has been reported that maternal risk factors, such as diabetes mellitus, Rh isoimmunization, and preeclampsia, are associated with an increase in gonadotropin

levels, resulting in an increased risk of cyst formation (10). Most neonatal cysts cannot be diagnosed before the third trimester, because in this period the hypothalamic-pituitary-ovarian axis develops, so that fetal cysts identified before the third trimester are believed to arise from the other organ of origin (mesenteric cysts, meconium pseudocyst, simple hepatic cysts, ureterocele). There is no gold standard in the management of neonatal ovarian cysts. Since they are classified into simple and complex, based on ultrasound image, and since simple cyst may progress into complex cysts during pregnancy, or torsion in utero, some authors point out the significance of prenatal treatment of simple cysts by in utero aspiration in order to preserve ovarian tissue and prevent possible complications. Diguisto et al. in a randomized controlled trial emphasized the importance of in-utero aspiration and its association with increased incidence of in-utero involution of the cyst and a reduced neonatal oophorectomy (11). Also, an advantage of in-utero aspiration is a possibility of establishing the diagnosis by analyzing cyst content. Lecarpentof et al. in a retrospective study included a total of 42 newborns with intraabdominal cystic masses and analyzed biochemical content of the fluid sampled by in-utero aspiration of the cyst content. They concluded that estradiol levels of 1000ng/l or higher diagnostically confirm ovarian cyst with 100% sensitivity and 100% specificity in comparison to other diseases that cause intraabdominal cystic masses (12). On the other hand, there are some disadvantages of in-utero aspiration, such as re-accumulation of the cyst, infections, preterm delivery, and impossibility to perform some procedures because of fetal position or dry aspiration (11, 13). The most severe complication of the cyst is torsion with subsequent ischemia and necrosis, increased risk of the adnexa or ovary loss, and potential adhesions to surrounding tissues that may result in intestinal or urinary obstructions. In a large meta-analysis with 92 non-randomized studies, Tyraskis et al. reported that cysts measuring 40mm or more have higher risk of torsion, and surgical treatment

is required more in complex than simple cysts (14). In our study, during a five-year period, there was a total of 9 newborns with ovarian cysts, out of them there were eight cysts verified as simple cysts by ultrasound and one cyst was identified as hemorrhagic pseudocyst. Considering the size of abdominal cavity, each cyst larger than 30mm is believed to be large, so our opinion is that every cyst of 30mm in diameter or more should be treated surgically in order to prevent torsion and the loss of vital ovarian tissue. Literature data reveal the tendency of spontaneous regression of cysts measuring less than 20mm, so continuous ultrasound monitoring is necessary, as well as timely reaction if the cyst progresses in size. Since the diameter of all the cysts in our study was over 30mm, all the newborns were indicated for surgical treatment. In 6 of them cystectomy with ovarian tissue preservation was performed, in two babies torsion was reported, so oophorectomy, that is salpingo-oophorectomy, was performed, and in one patient a free auto-amputated cyst originating from the right ovary was found intraoperatively. The advantage of such a treatment is that surgery enables complete removal of the cyst with optimal preservation of ovarian parenchyma where possible and purposeful, and adhesion adhesiolysis as well if diagnosed. Also, timely diagnosis significantly shortens the parents' anxiety level (15).

In another group of patients (1–18 years) ovarian tumour mass was diagnosed in 38 patients. Changes were mostly observed on the right ovary (63%), while in one girl both ovaries were affected. There is no logical explanation why the changes are mostly right-sided, but very often such a pain may imitate appendicitis, so ultrasound examination is of vital importance in establishing the diagnosis and in choosing optimal treatment option (that is, an incision on abdominal wall is made for both ovaries and uterus to be extracted).

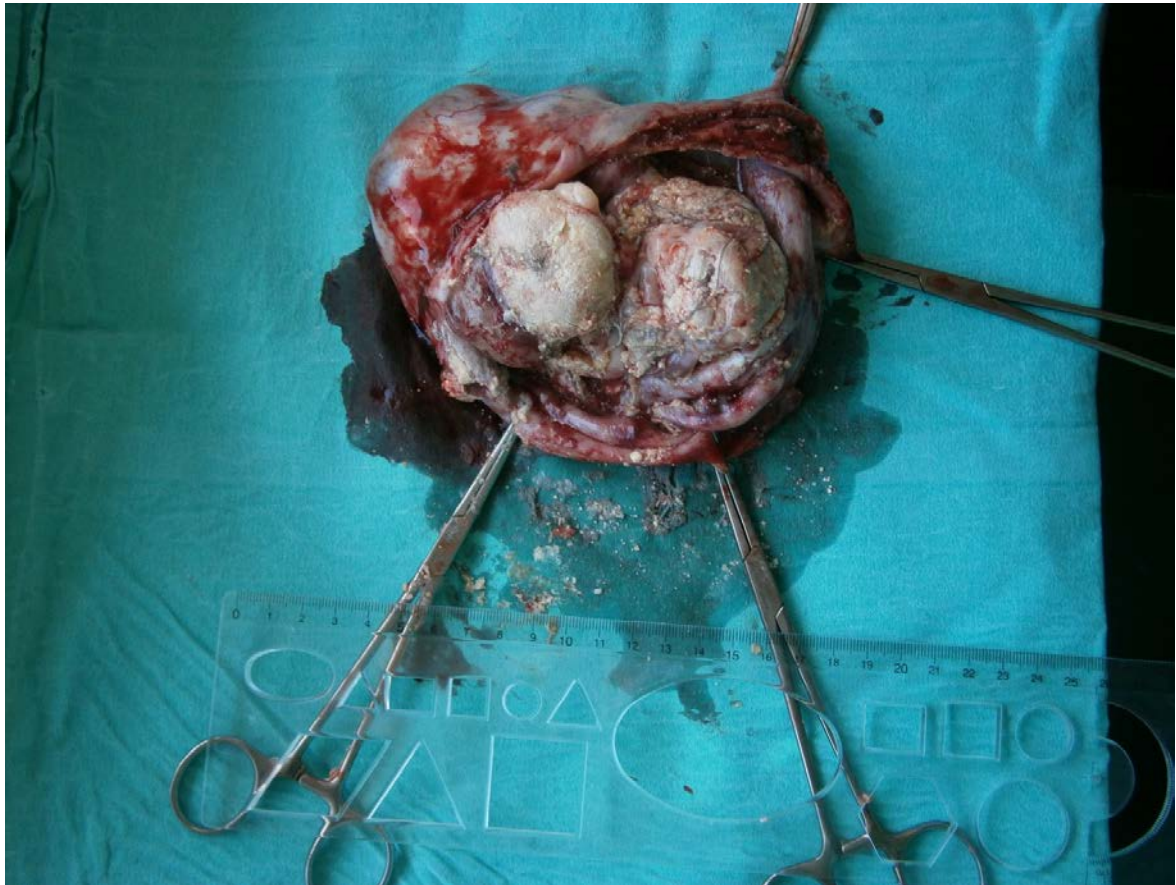
Average age of girls was 14.37 years, so it can be concluded that the majority of already described tumour changes occur in puberty, with an important role of hormones in etiology (7). Most ovarian cysts occur during puberty and they are known as functional cysts. They may be classified as: a) follicular cysts that occur as a result of physiological fluctuation in hormone levels, they may produce estrogen, and they represent half of non-neoplastic ovarian tumors, and b) corpus luteum cysts that are formed following ovulation, they may be filled with blood and they are active in secreting hormones. Very similar to pathoanatomically functional cyst are simple cysts that are endocrine-inactive. There was a total of 21 (60%) girls with non-neoplastic ovarian changes, 7(20%) of them had simple cysts, 8 (22.9%) follicular cysts, 6 (17.1%) corpus luteum cysts, and in one girl the presence of both follicular and corpus luteum cysts was verified pathohistologically. It is known that the incidence of developing ovarian cysts in early childhood and pre-pubertal period is extremely low because of

low hormonal activity (16). Our results are not in accordance with the results of Zhang et al. who analyzed a total of 521 patients operated at the Gynecology Hospital of Fudan University, Shanghai, China, where 92 girls had non-neoplastic ovarian lesions, making up percentage distribution of 17.7% (17). On the other hand, Sadhegian et al. diagnosed non-neoplastic lesions in 76.9% patients in a smaller study population (18).

Out of neoplastic benign lesions, the incidence of mature cystic teratomas is the highest (Figure 1). They are neoplastic benign changes that contain elements from all three germ cell layers (endoderm, mesoderm, and ectoderm). They are commonly referred to as 'dermoid cysts' in cases when ectodermal component is predominant (19). They account for more than 50% of all ovarian tumours in pediatric population and are mostly asymptomatic, but abdominal pain or palpable mass may be present. Macroscopically, they are well-differentiated encapsulated masses filled with thick mass, usually unilocular, with characteristic protuberance within known as the Rokitanski nodule that may contain all elements of ectodermal origin (bones, teeth, hair, thyroid tissue, brain tissue, etc.) (20). There were 10 (28.6%) girls with pathohistologically (PH) verified diagnosis of mature teratoma, similar to the results of Cassa et al. who report percentage of mature teratomas in 39.6% patients out of a total of 102. Far less common are cystadenomas (2.8%), in our population they were present in 11.4% of patients. On the other hand, Zhang et al reported even 16.5% cystadenoma cases (17,21). Cystadenomas are mostly benign epithelial neoplasms, 75% of them are serous, and remaining 25% are mucous cystadenomas commonly seen in adult population, they very rarely occur in children (22).

Based on the size, tumour changes have been classified into 3 groups (small, medium, big). The distribution of small and medium tumours was similar, while there were considerably fewer changes regarding the size over 10cm. Some authors emphasize a positive correlation between big tumours and higher risk of malignancy, so in a retrospective study by Rogers et al., out of a total of 129 children treated at the Hospital for Sick Children in Toronto, Canada, the diameter of all malignant ovarian tumours was greater than 8 cm (23). Depoers et al. developed a predictive score system for detecting ovarian malignancies in order to avoid unnecessary adnexectomy, and this system may be applied in confirmed ovarian tumours with negative aFP and HCG. They classified patients into three groups according to the size of tumour (up to 65mm, 65–130mm, >130mm) and the presence of cystic tumour component: low risk, middle-risk, and high-risk patients. Patients at low risk could undergo ovarian-sparing surgery. Patients at middle risk require additional diagnostic procedures, such as an MRI, while patients at high risk should be proposed a radical surgical treatment (24).

Figure 1. Mature teratoma, nonneoplastic benign tumour change which in its composition can have elements of the all 3 germ layers



A fundamental imperative in treating all ovarian tumour masses is to preserve the remaining ovarian tissue (17,25,26). This was a leading principle in our study; in 24 (63.2%) girls cystectomy was performed, in 2 (5.3%) partial ovariectomy. In cases of torsion and subsequent necrosis with almost no healthy ovarian tissue left, and in suspected malignant neoplasms, there was no possibility for ovarian preservation, so in 4 (10.5%) girls ovariectomy was performed, and adnexectomy in 3 (7.9%) patients. Although ovariectomy was a basic option in treating torsion from a historic point of view, the importance of detorsion even in necrotic ovary has been emphasized recently, in order to increase the rate of fertility preservation, since follicular activity may be reestablished (27). Ovarian fixation for ovarian torsion is still being debated. Some authors believe that fixation may disturb ovaries-fallopian tubes relation, while others advocate ovarian fixation in case of the absence of contralateral ovary, recurrence, or an elongated ovarian ligament (28).

We believe that this study still has some limitations. All the cases are from one center experience only, the number of participants is low, and this can affect the final outcome of the study. Also, the study is a retrospective one, all the data

were collected from existing medical records, so there was no adequate follow-up of operated patients. In the light of all aforementioned considerations, it is necessary to conduct a prospective study aiming at postoperative follow-up of patients to evaluate ovary function and its effect on fertility.

Conclusion

In conclusion, it can be said that the incidence of ovarian tumours in a population of girls up to 18 years of age is very low. In a neonatal population non-neoplastic tumour changes, that is follicular cysts, are most common. It is believed that placental gonadotropins play a key role in the pathogenesis of tumour changes. In a population of girls aged 1–18 years non-neoplastic lesions are predominant tumour changes, while mature teratomas are most common neoplastic benign tumours, but cystadenomas are not so common. The majority of these changes occur in puberty, with hormonal influence in their pathogenesis. The imperative in surgical treatment is healthy ovarian tissue preservation, and this should always be kept in mind

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KARAKTERISTIKE TUMORSKIH PROMENA JAJNIKA U DEČJEM UZRASTU

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U proseku, trećina svih adneksalnih masa kod devojčica vodi poreklo od jajnika. Klinička prezentacija je nespecifična. Trend lečenja predstavlja hirurgija čiji je cilj očuvanje jajnika.

Cilj rada jeste prikazati karakteristike tumorskih promena jajnika u populaciji devojčica uzrasta do 18 godina.

Ova retrospektivna studija sprovedena je analizom medicinske dokumentacije ženske novorođenčadi i devojčica uzrasta do 18 godina.

U neonatalnoj grupi, uzrasta do 12 meseci, bilo je 44,4% desnostranih i 55,6% levostranih promena. Kod šest beba (66,7%) urađena je cistektomija, dok je ovariektomija i salpingo-ooferoektomija urađena kod po jedne (11,1%) bebe. U grupi devojčica od jedne godine do 18 godina bilo je 63% desnostranih i 34% levostranih promena. Jedna devojčica imala je obostranu promenu. Kod najvećeg broja devojčica urađena je cistektomija – 24 (63,2%). Od neneoplastičnih promena bilo je devet (23,6%) *Cystis follicularis*, osam (21,1%) *Cystis simplex* i šest (17,1%) *Corpus luteum haemorrhagicum et cysticum*; od neoplastičnih benignih promena bilo je jedanaest (28,8%) *Teratoma maturum* i četiri (11,4%) *Cystadenoma serosum*.

Incidencija tumorskih promena jajnika u populaciji devojčica do 18 godina vrlo je niska; znatno češće se javljaju neneoplastične promene. *Acta Medica Medianae* 2023; 62(3): 32-41.

Ključne reči: tumorske promene, jajnik, neonatus, devojčice

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A PLAN FOR DEALING WITH THE OCCURRENCE AND SPREAD OF INFECTIOUS DISEASES CAUSED BY THE CORONAVIRUS AND THE ROLE OF THE OCCUPATIONAL MEDICINE SERVICE

Ivona Milenković^{1,2}

The plan for the application of preventive measures against occurrence and spread of infectious diseases is valid for all workplaces and in the work environment of the organization and is implemented in order to prevent epidemics of infectious diseases and eliminate risks to safe and healthy work of employees. The aim of this paper was to point out the importance of the existence of a plan for the implementation of preventive measures against occurrence of infectious diseases and the role of the occupational medicine service in the implementation of this plan. The results of the paper present the experiences in the implementation of preventive measures in the event of an epidemic of infectious diseases caused by the coronavirus and the method of treatment of patients with this disease. The views and activities of the occupational medicine service in the case of work ability assessment in persons after a previous illness and declaring the disease caused by the coronavirus an occupational disease were presented. It was concluded that it is extremely important to act according to the established plan. *Acta Medica Medianae 2023; 62(3):42-46.*

Key words: *infectious disease, ability to work, COVID-19, occupational disease*

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Introduction

Work organizations should prepare a plan for dealing with natural disasters and other major disasters in emergency situations. The health service has an extremely important role in the event of danger in the case of infectious diseases and the implementation of preventive measures aimed at preventing the outbreak of an epidemic (1).

Preventive measures are implemented on the basis of the plan of application of measures that is valid for all workplaces in the organization's working environment. Preventive measures are applied to prevent the occurrence and spread of infectious diseases and to eliminate risks for the safe and healthy work of employees and persons who find themselves in the working environment. This plan is an integral part of the Act on risk assessment, which is adopted in accordance with

the law and regulations in the field of occupational safety and health (2, 3).

The plan for the implementation of measures must contain:

1. preventive measures and activities to prevent the outbreak of infectious diseases,
2. responsibility for the implementation and control of the implementation of preventive measures and activities, and
3. measures and activities for handling in the event of an outbreak of an infectious disease.

The following participate in the implementation of these measures:

1. organization,
2. person for safety and health at work,
3. employees, and
4. managers of the organization.

Prevention measures can be general or special. All employees must be regularly informed by written instructions and instructions with all relevant information, procedures and obligations regarding the protection of health and safety from infectious diseases (4, 5). Employees are obliged, without exception or improvisation, to comply with the measures that have been ordered.

The aim of the work

To point out the importance of developing a plan for dealing with natural disasters and other major disasters and emergency situations and the role of the health service, occupational medicine

service and persons safety and health at work in the event of an infectious disease.

Material and methods

The subject of the work is the health institution of the Community Health Centre team, which actively participated in the implementation of measures to prevent the occurrence and spread of the infectious disease caused by the coronavirus, in the detection and treatment of sick persons and in the evaluation of the work capacity of persons who have suffered from the disease (6).

As part of the work methodology, legal regulations from this area were used (Statute of Work Organization, Law on OSH, Rulebook on Preventive Measures for Safe and Healthy Work to Prevent the Occurrence and Spread of Epidemics of Infectious Diseases, Act on Risk Assessment, etc.).

Results

The health institution implemented preventive measures and activities aimed at preventing the epidemic of infectious diseases caused by the coronavirus, performed clinical examinations and treated the sick.

Recommendations for preventing the transmission of the coronavirus infection.

Health workers have the right to dignified work, which includes dignity, equality, fair income and safe working conditions. In the context of the COVID-19 pandemic, together with the right to a safe working environment, health workers have duties and responsibilities regarding the protection of safety and health at work in accordance with international labor standards (7), as well as to follow WHO recommendations for patient safety (8). They include:

- adherence to established occupational health and safety procedures, avoiding exposing oneself or others to safety and health risks and participating in OSH training organized by the employer;
- using existing protocols for patient assessment, triage and care;
- rapid implementation of established reporting procedures in the field of public health regarding potential and confirmed cases;
- caring for patients with respect and compassion, while ensuring their dignity and maintaining confidentiality;
- providing or repeating accurate information in the field of infection prevention and control and public health to patients and the public;
- proper dressing, use, removal and disposal of PPE;
- monitoring yourself for signs and symptoms of COVID-19 and reporting any unprotected exposure to SARS-CoV-2, whether at

work or outside, to the person for contact and information in the field of occupational health or the occupational health service and voluntary quarantine;

- consulting the occupational health service if they notice signs of excessive stress or mental health problems that may require support; and

- informing the immediate superior of any situation where they have reasonable justification to believe that it represents an immediate and serious danger to life or health.

Results of clinical examinations of patients The Community Health Center in Vlasotince employs 210 workers, of which 153 are in primary health care, 14 are in dentistry and about 50 workers are financed from the local self-government budget. According to the structure of employees, there are 35 doctors and 5 dentists, and another 18 doctors on the project, 120 nurses and about 40 non-medical staff.

The Vlasotince Health Center provides health care for the population of this municipality, which includes about 30,000 inhabitants.

The first patient was reported in March 2020 and by December 2020, 258 cases were confirmed positive for the tested PSR material.

The COVID clinic was opened on March 18, 2020 and from the beginning, it has been working continuously. Twenty samplers (nurses, laboratory technicians and sanitary inspectors) were trained. Testing began on April 7, 2020. After taking the samples, they were packed and taken to the Institute of Public Health in Leskovac, from where they were transported to the laboratories. All doctors participated in the running of the clinic, several doctors worked in shifts, which depended on the number of patients who came for examination.

The work was organized so that after the examination of the patient in the COVID outpatient clinic, blood was taken for the laboratory according to the doctor's instructions and referred to Ro imaging, where positive patients and patients with suspicion of COVID-19 virus infection had access from the outside to the Ro service, so that the entrance to the central building was reduced to a minimum, all for the sake of enabling the rest of the staff to work smoothly with patients who did not have respiratory problems.

Patients with a more severe clinical picture were referred to the COVID hospital in Leskovac and kept there for treatment. The patients were driven by the COVID driver or the entire emergency service team depending on the case. The director and head nurse of the Community Health Center were in charge of the COVID clinic.

Pediatrics worked as a COVID clinic because there was a possibility of separating sick and healthy children, so children up to the age of 18 were examined and admitted to pediatrics and, if necessary, referred to the laboratory in the COVID clinic.

The COVID clinic is located in a separate building in the Health Center and it is possible that the roads from the green to the red zone do not interfere. A space for interventions for COVID patients has been organized, as well as a special space for taking swabs for PCR testing. There is good cooperation with ZZJZ as well as with RFZO in Leskovac. Each patient was monitored from the onset of the first symptoms until the end of the treatment and return home.

Discussion

The assessment of work ability is a very delicate job for an occupational medicine specialist given that COVID-19 is an infectious disease that the health service has not encountered until now and that there are many unknowns regarding the onset of the disease itself, the course of the disease as well as the complications that might occur after the end of the disease.

When assessing work ability, it is important to wait for the patient's complete healing and rehabilitation. During the assessment of working capacity, the occupational medicine specialist encounters the following types of assessment:

- when establishing an employment relationship (issuance of employment certificate);

- during periodic and targeted inspections of persons working at workplaces with special working conditions;

- in the event of disease complications (respiratory system, cardiovascular system, neurological system, hematopoietic system, etc.);

- when processing the requests of the sick for obtaining pension and disability insurance;

- when changing a workplace within the same company if the new workplace differs from the previous one and qualifies as a workplace with increased risk;

- when working in conditions of extreme physical exertion (sports activities, military service, etc.); or

- at the request of the court for the assessment of working capacity, etc.

When evaluating work capacity, a medical specialist must know well the psychophysical condition of a person who has suffered from an infectious disease, the state of function of

damaged systems and organs after the disease, as well as the requirements of the workplace, and then harmonize all of that.

A special problem is the requirement to recognize this disease in professionals in the acute phase. The World Health Organization has proposed that in case of infection with COVID-19 due to exposure at work, this disease should be recognized as an occupational disease, so the right to compensation, treatment and rehabilitation that applies to any occupational disease should be respected.

In our opinion, this recommendation should be accepted in the future list of occupational diseases when it comes to health personnel who fall ill with this disease during work, for employees of the police, employees of homes for the elderly and similar professions.

In other industries, it can hardly be said that employers are responsible for the occurrence of this disease among employees due to the lack of an effective vaccine, lack of other protective means, non-compliance with regulations, indiscipline and the like. It will be a big challenge for the occupational medicine service in the future if a new list of occupational diseases is created. It may happen that such cases are also the subject of forensic medical expertise. Until now, in our country, this disease has not been recognized as an occupational disease in any case.

Conclusion

The organization should draw up a plan for the implementation of measures for dealing with the occurrence of an infectious disease, which must necessarily contain preventive measures and activities to prevent the occurrence of an epidemic, responsibilities for the implementation of this plan and measures in the event of an epidemic of an infectious disease. The participation of the health service plays a significant role in the development of this plan and its implementation.

The occupational medicine service should be involved in the development of criteria for assessing the work ability of employees who have suffered from an illness, as well as criteria for recognizing this illness as an occupational illness.

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PLAN ZA POSTUPANJE U SLUČAJU POJAVE I ŠIRENJA ZARAZNE BOLESTI I ZAZVANE KORONA VIRUSOM I ULOGA SLUŽBE MEDICINE RADA

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Plan primene preventivnih mera za sprečavanje pojave i širenja zaraznih bolesti važi za sva radna mesta, kao i u radnoj okolini organizacije, a sprovodi se u cilju sprečavanja epidemije zaraznih bolesti i otklanjanja pojave rizika, kako bi se ostvarili bezbedni i zdravi uslovi za rad zaposlenih lica. Ovaj plan je sastavni deo Akta o proceni rizika, koji se donosi saglasno sa rizikom i propisima iz oblasti bezbednosti i zdravlja.

Cilj rada bio je da se ukaže na značaj postojanja plana primene preventivnih mera za sprečavanje zaraznih bolesti, kao i na ulogu službe medicine rada u realizaciji ovog plana.

U delu rada posvećenom rezultatima istraživanja prikazana su iskustva u sprovođenju preventivnih mera prilikom pojave epidemije zarazne bolesti izazvane virusom korona i način tretmana obolelih.

U diskusiji su izneti stavovi i aktivnosti službe medicine rada u slučaju ocenjivanja radne sposobnosti osoba posle preležane bolesti.

U zaključku je konstatovano da je od izuzetnog značaja napraviti plan za postupanje u slučaju pojave i širenja zarazne bolesti izazvane virusom korona. Takođe, ukazano je na ulogu službe medicine rada u tretmanu osoba koje su preležale bolest. *Acta Medica Medianae 2023; 62(3): 42-46.*

Ključne reči: zarazna bolest, radna sposobnost, COVID-19, profesionalno oboljenje

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FEATURES OF THE INNATE IMMUNE RESPONSE DURING THE SARS-COV-2 INFECTION

Tanja Džopalić¹, Milica Veljković², Marko Bjelaković³, Branislav Jovanović⁴

First reports of the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the disease caused by the virus—coronavirus disease 2019 (COVID-19), were announced in late December 2019. Ever since, the disease has taken more than 6 million lives worldwide. COVID-19 is considered as dominantly respiratory and vascular disease which pathogenesis could be explained by hyperactivation of the immune response. Innate immunity receptors are responsible for the first contact with the virus and subsequent activation of transcription factors leading to the production of the high amounts of interferons (IFNs) and proinflammatory cytokines (IL-1 β , IL-6, TNF, etc.). Such an inflammatory response limits viral replications. However, SARS-CoV-2 have developed several ways to avoid immune protection by the host. Dysregulated secretion of these cytokines may lead to cytokine storm and PANoptosis, a life-threatening condition.

This review article aims to describe the main characteristics of the innate immune response during the SARS-CoV-2 infection. *Acta Medica Medianae* 2023;62(3):47-53.

Key words: SARS-CoV-2, COVID-19, inflammation, cytokines, receptors

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Introduction

First reports on the specific pneumonia cases of unknown etiology were announced in December 2019 by Chinese Center for Disease Control and Prevention. Later, in January 2020, the causative agent was identified and labeled as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by the virus as coronavirus disease 2019 (COVID-19). The disease had a devastating influence on demography in the world that resulted in more than 6 million deaths worldwide (1). After infecting the host cells, SARS-CoV-2 is subject to genetic mutations over time. Therefore, different variants of virus have been described: Alpha (B.1.1.7) - first appearance in the United Kingdom in 2020;

Beta (B.1.351) – in South Africa since 2020; Gamma (P.1) - in Brazil since 2021; Delta (B.1.617.2) - in India since December 2020; Omicron (B.1.1.529) - in South Africa since 2021.

The main mode of SARS-CoV-2 transmission is through a respiratory system and viral droplets. Another way of infection is upon a contact with contaminated surfaces. There are epidemiological reports indicating the presence of SARS-CoV-2 on plastic and stainless steel for up to three days, millboard for up to a day (2, 3). Other studies showed the presence of live virus in feces of patients with SARS-CoV-2 infection indicating possible fecal-oral transmission (4).

Epidemiological data has shown that individuals of all ages can be infected with SARS-CoV-2 infection. However, elderly and patients with certain medical comorbidities (cardiovascular disease, chronic lung disease) are at great risk of developing severe disease (5).

COVID-19 is considered as dominantly respiratory and vascular disease since SARS-CoV-2 primarily affects the respiratory and vascular systems. However, function of other organs may be disturbed by the infection as well (Figure 1).

The pathogenesis of COVID-19 disease most likely can be explained by overactivation of the immune response. This review article aims to describe the characteristics of the innate immune response during the SARS-CoV-2 infection.

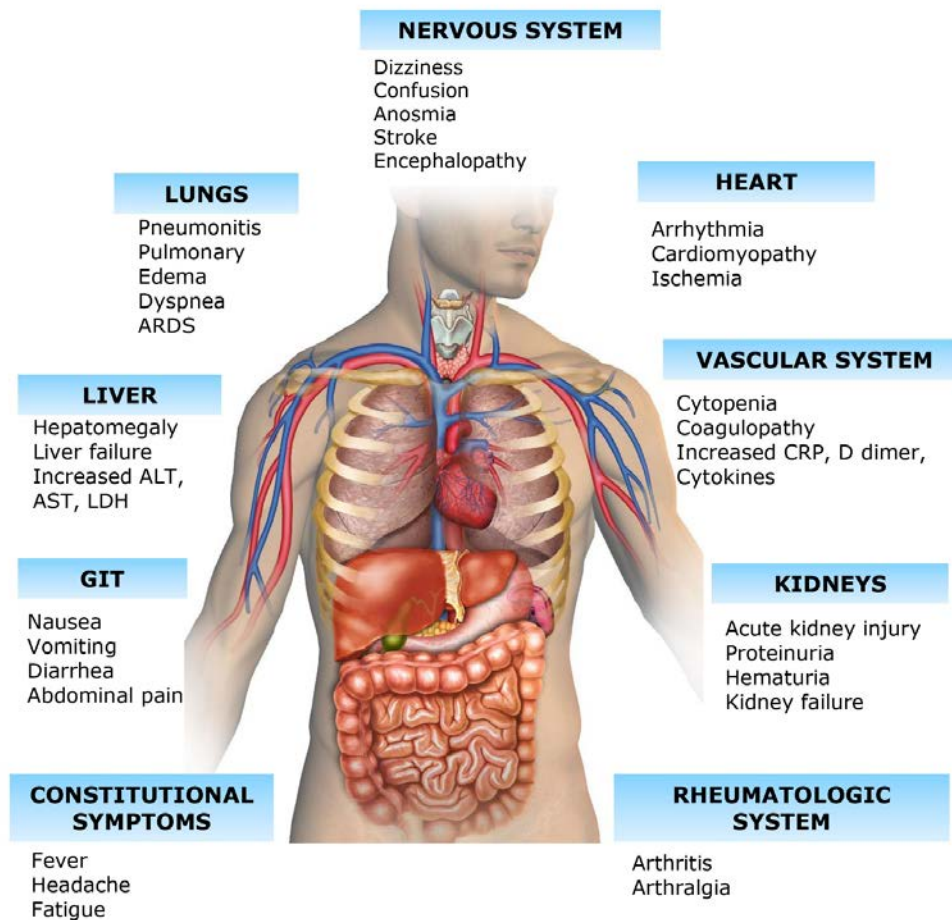


Figure 1. The most common clinical manifestations of COVID-19

COVID-19 is considered as dominantly respiratory and vascular disease. However, infection can also affect other major organ systems, such as nervous system, gastrointestinal tract (GIT), hepatobiliary, cardiovascular, renal, rheumatologic, etc.

ARDS, acute respiratory distress syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein. (Adapted from <https://medlineplus.gov/anatomy.html>)

SARS-CoV-2 receptors

The SARS-CoV-2 virus consists of the positive single-stranded (ss) RNA genome and it is classified into the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, and genus *Betacoronavirus* (6). The most important proteins for viral replication, structuring, pathogenicity and binding with cellular receptors are expressed on its surface. They are termed as spike (S), envelope (E), membrane (M), and nucleocapsid protein (N) (7). The virus also produces certain open reading frames (ORFs) responsible for encoding the accessory proteins significant in viral pathogenesis (8). The SARS-CoV-2 enters the host cells binding to specific receptors. Namely, as the most important protein mediating membrane fusion and viral penetration, S glycoprotein binds to angiotensin-converting enzyme 2 (ACE2), its crucial receptor with the

highest binding affinity.

S-protein forms trimmers on the surface of the virus, in the form of S1 and S2 subunits with receptor-binding protein (RBD), where RBD directly interacts with the ACE2 (9). After binding to the receptor, proteolysis of the S protein takes place upon which viral membrane and target cell merge.

During this process, S1 subunit recognizes and binds to the receptor, while S2 subunit mediates the fusion, after which the viral RNA enters the cell (10).

Recent studies have described several membrane proteins that function as ACE2 cofactors or alternative receptors. Most of them are expressed on plethora of the cells, such as epithelial cells, platelets, alveolar epithelium, dendritic cells, hepatocytes, etc. (11–16). The most notable ones are presented in Table 1.

Table 1. ACE2 cofactors or alternative receptors for SARS-CoV-2

Receptor	Cell expression
ACE2	Epithelial cells, macrophages, platelets, endothelial cells, smooth muscle cells, many other cells
Neuropilin 1 (NRP1, CD304)	Nerve cells of the brain and nasal cavity, endothelial cells
Chondroitin sulfate	Most of the cells
CD147 (Basigin)	Highly expressed on cells of the immune system
GRP78	Different cells
CD206	Macrophages, monocytes, dendritic cells
CD249	Epithelial cells, macrophages, platelets, endothelial cells, smooth muscle cells, many other cells

SARS-CoV-2 and PRRs

The main route of the SARS-CoV-2 entry into human organism is through the respiratory tract, but contact with an infected surface is important as well (17). Innate immunity receptors responsible for the first contact with the virus belong to the pattern recognition receptors (PRRs) -Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and inflammasomes (18). In general, these receptors are mainly expressed in dendritic cells (DCs) and macrophages where they recognize microbial nucleic acids and upon engagement of myeloid differentiation protein 88 (MyD88) and TRIF adaptor molecules, activate the transcription of type I and type III interferons (IFNs) as well as nuclear factor κ B (NF- κ B)-dependent proinflammatory cytokines and chemokines with the consequent induction of death of the infected cell (19, 20).

TLRs are a family of transmembrane receptors consisting of ectodomains with multiple leucine-rich repeats (LRRs), linked by a transmembrane domain to a conserved cytosolic domain called the Toll/IL-1 receptor homology (TIR) domain (18). To date, 28 TLRs have been identified in vertebrates, of which humans possess only 10 (TLR1-10) (21). Bearing in mind the function and position in the host cell, there are two main groups of TLRs: [1] *Cell membrane* TLRs - expressed on the surface of the cell, and they include TLR1, 2, 4, 5, 6, and 10; and [2] *Intracellular* TLRs - expressed within the host cells on the organelle bio membranes like endoplasmic reticulum (ER), endosomes, and lysosomes, and they include TLR3, 7, 8, and 9 (22). These intracellular TLRs are responsible for viral recognition, i.e. their pathogen-associated molecular patterns (PAMPs) such as single strand

(ss)-RNA, double strand (ds)-RNA or CpG-DNA (20). Regarding SARS-CoV-2 infection, to date there no data confirming direct involvement of any type of human TLRs. Namely, *in vivo* murine studies have described that the SARS-CoV-2 E protein recognition by macrophage TLR2 mounts inflammatory responses (23). *In silico* studies suggest that TLR1, TLR4 and TLR6 are the receptors with the highest affinity for SARS-CoV-2 S protein binding (24). Additionally, chromosomal TLR7 anomalies have been described among the young individuals with the severe forms of the disease. Such a finding indicates a protective role of TLR7 during the viral infection (25).

NLR family of proteins represent a group of PRRs responsible for the initiation of innate immune response during the cellular injury and stress (26). The best described member of the family, NLRP3 inflammasome, mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1 β /IL-18 and cleavage of gasdermin D, which forms pores in the plasma membrane leading to pyroptotic cell death (27). Several studies have suggested that NLRP3 senses SARS-CoV2 infection (28, 29). Their common findings are reflected through the increased levels of IL-1 β and IL-18 in plasma, which correlated with disease severity and mortality in patients with COVID-19.

SARS-CoV-2 and cytokines

Numerous evidence suggest that COVID-19 morbidity and mortality are related to high amounts of both IFNs and proinflammatory cytokines (30, 31). In general, their role is reflected through clearing the infection and maintaining cellular homeostasis. However, dysregulated production of proinflammatory cytokines may lead to a cytokine storm, a life-

threatening condition. In the context of SARS-CoV2 infection, this excessive production of cytokines may induce PANoptosis (32). This condition is defined as programmed cell death pathway dependent on PANoptosomes – a complex consisted of caspase(s) with or without inflammasome components (32). Synergism of IFN- γ and TNF induces a lethal shock syndrome in mice, similar to a cytokine storm detected in some patients with severe COVID-19 (33). PAMPs, DAMPs and pathogens may trigger PANoptosis and this very process is most probably responsible for multiorgan damage in COVID-19 patients.

Viral evasion strategies

One of the main functions of innate immunity is to induce an inflammatory response that will limit viral replications. However, SARS-CoV-2 has developed several ways to avoid such immune protection by the host. Namely, it may inhibit IFN production through the expression of several viral proteins that block IFN signaling pathways (34). The SARS-CoV-2 alters myeloid response with an excess of circulating immature monocytes, neutrophils and myeloid progenitors. This condition is known as emergency myelopoiesis and it is observed among patients with mild to severe COVID-19 (35). During this stage of the disease, myeloid cells produce high amounts of inflammatory cytokines which lead to vascular permeability and organ failure (36). Hypercoagulation, followed by arterial and venous embolism is also often detected among COVID-19 patients (37). It is presumed that the virus alters the vascular endothelium during inflammatory process and activates the cells included in the release of coagulation factors (von Willebrand factor, factor VIII) (38). In addition, severe

COVID-19 is accompanied by a high titer of autoantibodies specific for nuclear antigen, T and B cell antigens, chemokines, and cytokines which all together activate mechanisms responsible for tissue damage and organ failure (39, 40).

Conclusion

The COVID-19 pandemic resulted in a loss of more than 6 million lives worldwide (1). Despite numerous preventive efforts and rapid advances in basic and translational science, the infection still remains a global threat.

Wide spectrum of receptors and effector molecules, with IFN signaling, cytokine production and cell death, makes the innate immune system the first line of defense against SARS-CoV-2 infection. However, the virus itself developed strategies to avoid these protection mechanisms, leading to hyperactivation of the innate immunity with consequential hyperinflammation, cytokine storm, severe diseases and mortality. Therefore, many treatment strategies targeting innate immune response have been introduced. Such a therapy balances between inflammation and immunomodulation preventing excessive pathological inflammation (32).

As the SARS-CoV-2 infection persists, there is a need for additional knowledge of the COVID-19 immunopathogenesis and hence development of new therapeutics.

Acknowledgements

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Pregledni rad

UDC: 616-097:[616.98:578.834
doi: 10.5633/amm.2023.0307**KARAKTERISTIKE UROĐENOG IMUNSKOG ODGOVORA
TOKOM SARS-COV-2 INFEKCIJE***Tanja Džopalić¹, Milica Veljković², Marko Bjelaković³,
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Prva saznanja o infekciji akutnim respiratornim korona virusom (SARS-CoV-2) i bolesti izazvanog virusom – COVID-19, objavljena su krajem decembra 2019 godine. Od tada je bolest uzela više od šest miliona života širom sveta. SARS-CoV-2 smatra se dominantno respiratornim i vaskularnim oboljenjem, čija se patogeneza može objasniti hiperaktivacijom imunskog odgovora. Receptori urođenog imuniteta odgovorni su za prvi kontakt sa virusom i naknadnu aktivaciju transkripcionih faktora, koji dovode do proizvodnje velikih količina interferona (IFN) i proinflamatornih citokina (IL-1 β , IL-6, TNF itd.). Takav inflamatorni odgovor ograničava replikaciju virusa. Međutim, SARS-CoV-2 je razvio nekoliko načina da izbegne imunski odgovor domaćina. Neregulisano lučenje ovih citokina može dovesti do razvoja citokinske oluje i PANoptoze, stanja opasnih po život. Ovaj pregledni članak ima za cilj da opiše glavne karakteristike urođenog imunskog odgovora tokom infekcije izazvane SARS-CoV-2 virusom. *Acta Medica Medianae* 2023;62(3):47-53.

Ključne reči: SARS-CoV-2, COVID-19, inflamacija, citokini, receptori

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ATOPIC DERMATITIS IN CHILDREN

Katarina Harfman Mihajlović^{1,2}, Hristina Stamenković^{3,4}

Atopic dermatitis represents a non-infectious, multifactorial inflammatory and chronic dermatosis. Pruritus is the main symptom. The pathophysiology of atopic dermatitis is very complex and includes genetic disorders, a defect in the epidermal barrier, an altered immune response and a disruption of the skin's microbial balance, all of which damage the epidermis, causing itchy skin lesions. The prevalence varies, but it is generally present in 30% of children, and 85% of cases manifest before the age of 5. The triad of atopic diseases consists of atopic dermatitis, allergic rhinitis and asthma. A retrospective analysis of the documentation of the children who were treated at the Pediatric Clinic of the University Clinical Center Niš from 2018 to 2019 focused on anamnestic data, clinical manifestations, and laboratory parameters obtained from the medical records of the subjects. The total number of examined children was 40, among them, 21 were male and 19 female. They were divided into 3 age groups, from 2 months to 7 years. Anamnestic data indicate a connection between AD and other disorders of atopic diseases, allergic rhinitis, asthma, respiratory infections, and milk allergies. *Acta Medica Medianae* 2015;54(3):54-60.

Key words: atopic dermatitis, asthma, allergic rhinitis

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Introduction

Atopic dermatitis (AD) is a non-infectious, multifactorial chronic inflammatory dermatosis with pruritus as the main symptom. It includes erythema, xerosis, erosions and excoriations, oozing, and lichenification.

The chronic course of the disease, which shows a high degree of relapses and the involvement of the whole family in the treatment process, greatly affects the quality of life (1).

It involves genetic changes, a defect in the epidermal barrier resulting in an altered immune response and disruption of the skin's microbial balance, all of which damage the epidermis and

cause itchy skin. Repeated scratching initiates a self-perpetuating cycle of scratching, which can have a significant impact on subsequent disruption of the skin barrier.

The basis for the manifestation of different phenotypes and endotypes of atopic dermatitis is provided by numerous changes at the genetic level (2).

Etiopathogenesis

The multifactorial etiopathogenesis of AD includes gene mutations, inadequate response of the immune system to the factors from the external environment, and defects in the epidermal barrier.

Prevalence varies, but AD is generally present in 30% of children, and in 85% of cases the disease manifests itself before 5 years of age. The triad of atopic diseases is represented by AD, allergic rhinitis and asthma (3).

AD is one of the main risk factors for developing asthma - more precisely, children with AD are more likely to develop it than children without AD.

Patients with specific IgE antibodies to common environmental allergens (extrinsic AD) present at the age of 2–4 years and diagnosed with eczema have a higher risk of progression to allergic rhinitis and asthma than those with eczema without IgE sensitization (intrinsic AD). IgE sensitization is the main risk factor for the progression and persistence of asthma, but it affects the early onset and severity of AD (4).

There are different forms of the disease that are manifested by different primary changes that are not in accordance with the classical concept of AD, which begins in childhood.

The most important factors that can contribute to the manifestation of the disease and its relapse are skin exposure to detergents, soaps, woolen and synthetic materials, aeroallergens such as dust mites from house dust, animal hair, and allergens in children in food (egg whites, milk, soy, peanuts, walnuts, almonds, wheat flour, fish, crustaceans) (5).

Atopic diseases show a genetic connection. In monozygotic twins, the concordance rate is 80% compared to 30% in dizygotic twins.

Genetic polymorphisms found in AD are responsible for mediators that trigger atopic inflammation.

This dermatosis can be caused by various external and internal factors that can act independently or together (6).

The stratum corneum with all its structural components and the stratum granulosum are two essential structures of the epidermal barrier (Figure 1).

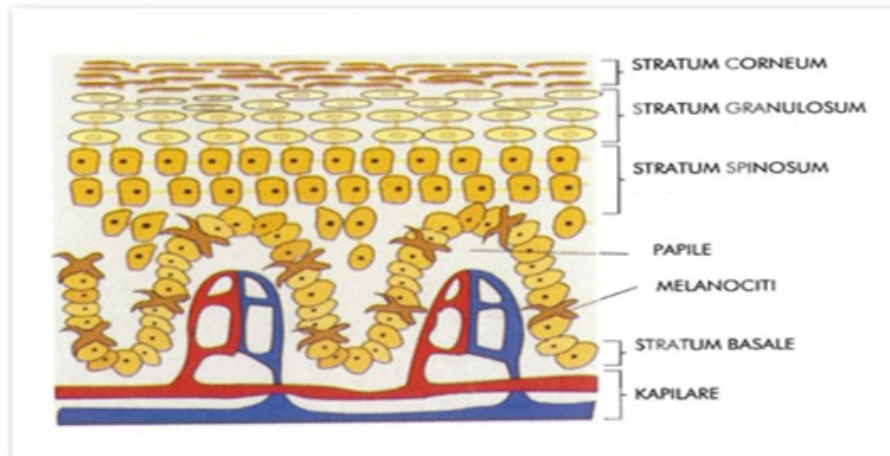


Figure 1. Review of skin layers

The histopathological image in acute dermatitis with vesicles is characterized by a change that includes spongiosis, acanthosis, parakeratosis that occurs in the epidermis and vasodilatation, edema, and lymphatic-histiocytic infiltration in the dermis.

IL-4 and IL-13 are two types of cytokines that play an important role in the production of chemokines, dysfunction of the skin barrier, suppression of antimicrobial peptides (AMP) and allergic inflammation, as confirmed by studies (7).

Evidence for the importance of DNA methylation has been shown by recent studies and an association between umbilical cord blood methylation at 5'-C-phosphate-G-3' IL-4R sites and the development of eczema at 1 year of age of the baby.

Neurons that express the histamine-H1 receptor and histamine-H4 receptor result in histamine activation, which can cause itching as well as an allergic reaction. H1 antihistamines are prescribed as therapy in the treatment of itching after the appearance of urticaria, but their effect is limited in the treatment of chronic pruritus in patients with eczema.

The lipid matrix consists of lipids such as ceramides, long-chain free fatty acids (FFAs) and

cholesterol. The matrix is organized in lamellar bodies and located between corneocytes. When epidermal differentiation occurs, precursor lipids are located in lamellar bodies within the upper cell layers of the epidermis and extruded into the extracellular domain. The enzymatic treatment that follows this process produces the main classes of lipids, which are necessary to maintain the integrity of the epidermal barrier.

Altered lipid composition was observed in skin with changes and skin without lesions. More precisely, long-chain EO ceramides are very important because they are covalently bound to keratin proteins and cover the surface of each corneocyte. Levels of long-chain ceramides are reduced in AD patients who are colonized with *Staphylococcus aureus* compared to those not colonized.

By examining AD, we came to the knowledge that *S. aureus* is frequently present in patients with a more severe form of the disease, while *S. epidermidis* presents in those with a milder form of the disease. *S. aureus* colonizes the skin of patients with AD and its role is crucial in the development and progression of the disease.

Colonization of *S. aureus* can result in the expansion of B-cells independently of T-cells, which also affects regulation of pro-inflammatory

cytokines, such as TSLP, IL-4, IL-12 and IL-22. This stimulates mast cell degranulation.

AD patients have a lower number of bifidobacteria in their intestines and a higher number of staphylococci compared to healthy individuals. Overgrowth of pathogenic bacteria, such as *Escherichia coli* and *Clostridium difficile*, is thought to be associated with lower concentrations of beneficial bacteria, reduced induction of regulatory T (Treg) cells, and increased intestinal permeability.

A specific or altered microbial composition in the intestines prevented the activation of Th2-immunity and stimulated regulatory immunity, producing regulatory dendritic cells and Treg cells. However, further studies are needed to reach a conclusion on how dysbiosis affects the function of the epidermal barrier and the development of AD (8).

In AD, epidermal lipids are altered both qualitatively and quantitatively.

It is known that affected individuals have a lack of natural moisturizing factors (urea and amino acids) and impaired epidermal lipid metabolism. This explains why their skin is more prone to dryness and has an impaired barrier function.

It has been proven by previous studies that sensitivity to allergens in early childhood and children with AD is more likely to later develop allergic rhinitis and asthma. The effect of aeroallergens on AD is great because there is no correlation between allergic rhinitis and AD (9).

At this time, there is very little information about the factors that influence the onset of asthma and allergic rhinitis in children with AD. However, based on several studies, it can be said that they are preceded by immunoglobulin E (IgE) sensitization.

The preclinical phase (phase 0) of AD begins already in childhood. Chronic inflammation of the skin occurs, but without any evidence of IgE sensitization.

In this phase, sensitization to allergens occurs. These allergens are most often from food and from the external environment, which leads to the classic IgE-related phenotype of AD. This results in chronic inflammation of the skin, suitable for *Staphylococcus* colonization (10).

Key elements in the pathophysiology of asthma development are infiltration of the bronchial mucosa with eosinophils and bronchial hyperreactivity. It has been proven that patients with AD can have hyperreactivity even without a diagnosis of asthma. The triggers for the development of bronchial hyperreactivity have not been fully explained. A positive family history of eczema, a younger age at the onset of the disease, the clinical picture of AD and multiple exposure to allergens certainly increase the risk.

AD is caused by complex pathogenetic mechanisms and therefore represents a multifactorial heterogeneous disease. The skin in these patients is extremely dry and dull, with

increased permeability. It is constitutionally irritable, and pruritus is the earliest symptom of the disease (due to elevated histamine)—it is regularly present and can be so strong that the vicious cycle of itching-scratching-itching occurs (11).

Clinical manifestations

It is clinically characterized by erythema, edema, vesicles and wetting in the early stage of the disease, and lichenification, desquamation and hyperkeratosis in the later chronic stage.

Skin manifestations change continuously, with periods of exacerbation and remission, and are usually associated with various provocative factors (12).

Typical predilection sites, as well as clinical manifestations, change during the life of AD patients. As a result, AD is classified into 4 stages:

1. Atopic dermatitis in infants—Eczema infantum

Babies can get symptoms as early as 2–3 months of age. The rash usually appears suddenly, making the skin dry, cracked, and itchy. It typically appears on the face—especially on the cheeks and scalp (known as the "cradle cap"), knees and elbows, while the central part of the face remains unaffected.

A yellowish layer of seborrheic, hard scales on the scalp in the first months of life in babies is usually an early presentation of AD. These scales can rarely be found on the folds and in the ankle area. In severe cases, the disease becomes generalized (Figure 2).

In babies, atopic skin is also very rarely found in the diaper area. Although diaper rash may look similar, that area is too wet for AD to occur. The changes are of the exudative and eczematous type and consist of vaguely limited beaches with erythema, edema, papulovesicles, wetting and crusts. In 50% of the babies, the symptoms disappear spontaneously at the end of the second year of their lives.

2. Childhood atopic dermatitis—Besnier's Prurigo presents a clinical picture from 3 to 11 years of age. Predilection places for the appearance of changes on the skin are the cubital and popliteal folds, on the neck and around the joints.

There are two potential scenarios—that the changes have developed as a transition from the previous form to a chronic form or that they have occurred suddenly. They represent ill-defined plaques with lichenification and desquamation.

3. Atopic dermatitis of adolescents and young adults—usually, a dry rash with scaly patches that itch appears first. The skin is often uneven, thickened, and rough to the touch.

The inner sides of the elbows and knees, neck, wrists, ankles and/or the folds between the buttocks and thighs are most commonly affected.

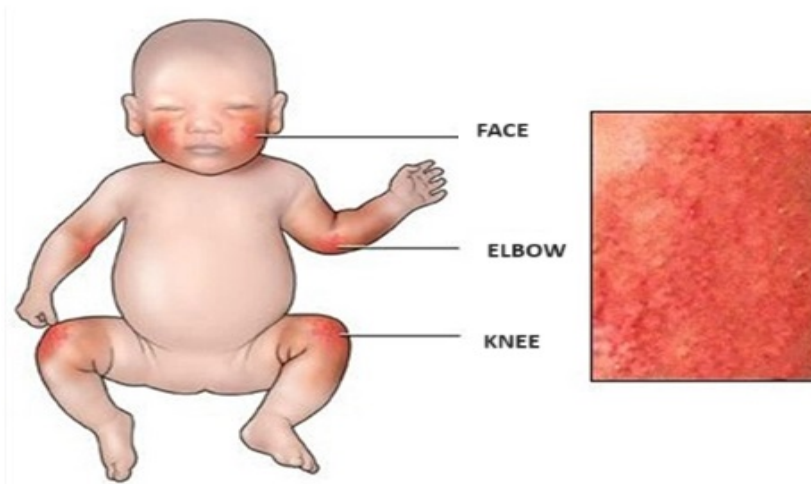


Figure 2. Predilection sites for AD in infants

In addition, pruritic nodules, scratching marks, chronic eczema of the hands and feet, inflammation around the eyes, which corresponds to the clinical picture of diffuse atopic dermatitis, are often found as well.

4. Atopic dermatitis in adulthood remains active in a small number of patients and rarely occurs for the first time. It is characterized by lichenified plaques that appear on the entire skin, affecting the face, folds, and anorectal region (13).

The course of the disease is chronic, accompanied by improvements and reactivation of the disease. In most patients, remission occurs after puberty or by the age of 30. In a smaller number of patients, the chronic relapsing course is maintained throughout life.

Complications are caused by an infection with staphylococci (colonization) and viruses (reduced cellular immunity) (14).

Aim of the Research

The aim of the study was the examination of the frequency of atopic dermatitis in children and its association with other disorders belonging to the type of atopic diseases.

Respondents and Methods

A retrospective analysis of the documentation of children who were treated from 2018 to 2019 at the Children's Internal Medicine Clinic of the Niš University Clinical Center was performed.

Anamnestic data, clinical manifestations, and laboratory parameters obtained from the medical records of the subjects were analyzed.

The value of specific IgE to inhalant and nutritional allergens in examined children was also assessed for each age group.

Results

The total number of examined children was 40—21 male (53%) and 19 female (47%).

Three groups of patients were divided into three age groups, ranging from 2 months to 7 years:

Group 1: Children under 2—10 children

Group 2: 2-year-olds—19 children

Group 3: 2-7-year-olds—11 children

Anamnestic data indicated the association of AD with other disorders belonging to the type of atopic diseases. Out of 40 children, 25 had comorbidities—in 9 cases AD came with asthma, in 6 with allergic rhinitis, in 8 with respiratory infections, and in 17 with milk allergy.

This indicates that an average of 27.25 out of 40 subjects with atopic dermatitis suffered from another atopic disease.

The value of specific IgE was tested in the groups of 2-year-olds and children under 2—59% to milk (Figure 3).

It was also tested in the group of children from 2 to 8—43% to mites, 39% to gluten, 15% to animal hair, and 3% to grass (Figure 4).

There were no elevated values for other allergens in the examined patient groups. In 32% of children in the 2–8 age group, immunoglobulin class A values were decreased, whereas other values did not show deviations in all age groups.

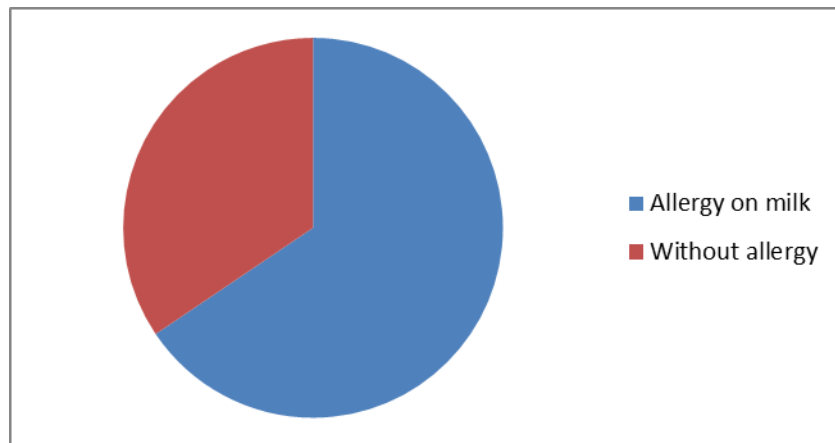


Figure 3. Allergy on milk in children from 2 to 8 years old
*59% of children have allergy on milk

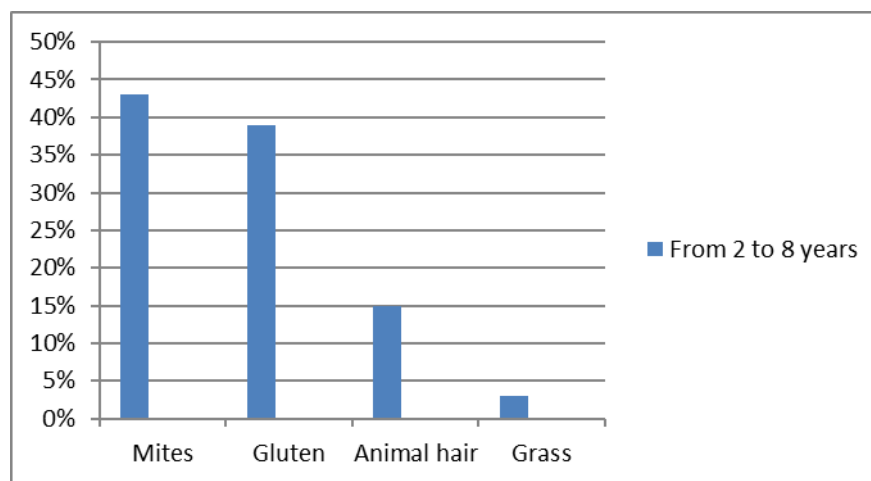


Figure 4. IgE value to specific inhalant and nutritional allergens

Discussion

Atopic dermatitis is closely related to asthma and allergic rhinitis. Although there is a general consensus that the existence of a diagnosis of atopic eczema increases the chances of developing asthma and rhinitis, the risk of developing these diseases should be determined in different populations according to precisely determined methods, which was the aim of this research (15).

Based on the questionnaire of the International Study of Asthma and Allergy in Childhood (ISAAC), a survey which included all schoolchildren of the city of Cartagena (Murcia) was conducted in Spain. The association between the severity of atopic dermatitis and asthma, and allergic asthma and allergic rhinitis was analyzed. The conclusion was as follows: it was shown that schoolchildren with atopic eczema have a three-fold higher risk of developing allergic rhinitis (OR: 3.33; 95% CI: 2.45–4.54), a 4-fold higher risk of developing asthma (OR: 3.85; 95% CI: 2.74–5.42) and a fivefold risk of allergic asthma (OR: 4.91; 95% CI: 3.17–7.59) compared to school-age children without atopic eczema. Thus, a direct

connection between AD and allergic rhinitis and asthma was observed (16).

In a German longitudinal study of atopy in 1,300 children, it was found that patients with atopic dermatitis are at a higher risk of developing asthma at the age of 7 years. However, patients with atopic dermatitis and no wheezing during the first 3 years of life are not at increased risk of developing wheezing or bronchial hyperreactivity at the age of 7 years. Atopic dermatitis and asthma are thought to be related, but atopic dermatitis does not precede asthma, whereas allergic rhinitis is a risk factor for asthma and may precede asthma.

A cross-sectional study in five Mexican cities with a sample of almost 15,000 children aimed to determine the prevalence of asthma, allergic rhinitis and atopic dermatitis in six- and seven-year-olds. In order to determine the prevalence of allergic diseases and their symptoms, parents had to fill in the questionnaire of the International Study of Asthma and Allergy in Childhood. Ninety-five percent of confidence intervals (CI) were estimated for proportions. Of the total sample, 7,466 (52.5%) were boys, and 7,463 (47.5%)

were girls. Overall, the prevalence of asthma and exercise-induced asthma was 6.1% (95% CI = 5.7%–6.5%) and 2.1% (95% CI = 1.9%–2.3%), respectively (17).

Conclusion

Studies have shown that dysregulation of innate and acquired immunity plays a key role in the occurrence of AD. However, recent genetic

and molecular research has focused on the fact that what precedes the appearance of the disease is a disruption of the skin barrier function. The etiology of AD emphasizes the important role of

disruption of the epidermal barrier, which leads to increased epidermal permeability leading to pathological inflammation of the skin and percutaneous sensitization to allergens.

Therefore, most new treatment strategies aim to strengthen specific aspects of the skin barrier or skin inflammation. Several studies have shown that in the prevention of AD, the early use of emollients in high-risk infants is necessary. This may have wider implications in terms of halting the progression of atopic comorbidities, including food allergies, asthma and allergic rhinitis.

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Pregledni rad

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DOI: 10.5633/amm.2023.0308**ATOPIJSKI DERMATITIS***Katarina Harfman Mihajlović^{1,2}, Hristina Stamenković^{3,4}*¹Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija²Dom zdravlja Ražanj, Ražanj, Srbija³Univerzitetski klinički centar Niš, Klinika za pedijatriju, Niš, Srbija⁴Univerzitet u Nišu, Medicinski fakultet, Katedra Pedijatrija, Niš, SrbijaKontakt: Katarina Harfman Mihajlović
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Atopijski dermatitis je hronična, nezarazna, multifaktorijalna upalna dermatoza sa pruritusom kao glavnim nalazom. Patofiziologija atopijskog dermatitisa je kompleksna. Uključuje genetske poremećaje, defekt epidermalne barijere, izmenjen imuni odgovor i poremećaj mikrobne ravnoteže kože, koji oštećuju epidermis izazivajući lezije kože sa svrabom. Prevalencija varira, ali je generalno prisutan kod 15% – 30% dece, a 85% slučajeva manifestuje se pre pete godine života. Atopijski dermatitis, alergijski rinitis i astma čine trijadu atopičnih bolesti. Retrospektivnom analizom dokumentacije dece koja su lečena na Klinici za pedijatriju Univerzitetskog kliničkog centra Niš u periodu od 2018. do 2019. godine analizirani su anamnestički podaci, kliničke manifestacije i laboratorijski parametri dobijeni iz medicinske dokumentacije ispitanika. Ukupan broj ispitivane dece je 40 – 21 ispitanik bio je muškog pola, a njih 19 bilo je ženskog pola. Podeljeni su u tri grupe po uzrastu (od dva meseca do sedam godina). Anamnestički podaci ukazuju na postojanje udruženosti AD sa drugim poremećajima po tipu atopijskih bolesti – astmom, alergijskim rinitom, respiratornim infekcijama i alergijama na mleko. *Acta Medica Medianae 2015; 54(3):54-60.*

Ključne reči: atopijski dermatitis, astma, alergijski rinitis

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SOCIAL MEDICINE APPROACH IN RESOLUTION OF THE PROBLEM OF CONTAMINATION OF PUBLIC AREAS WITH DOG FECES AND ITS PUBLIC HEALTH RELEVANCE

Marko Ristić¹, Dragan Nikolić², Nemanja Jovanović³, Tamara Ilić³

Since dogs and humans share their living environments and effectuate very close contacts with each other, with dog owners not caring adequately for their pets, there is a possibility of transmission of particular parasitic diseases from dogs to humans. In view of the fact that dogs are carriers and definitive hosts of a large number of zoonotic endoparasites, their feces may represent a source of infection for humans and a real threat to their health. That is the reason for raising the level of awareness among citizens about the necessity of executing zoohygienic measures in dog breeding, as well as about the significance of causally planned dehelminthization and anti-ectoparasitic treatments. Further, it is essential that dog owners perform regular coprological examinations, immediately after getting the dog and four times a year afterwards, abiding by the principle of shifting the preparations used based on their different chemical formulations. A timely diagnosis of intestinal parasites in dogs and proper treatment of infected animals enable the prevention of these infections in humans. Contamination of public areas with intestinal parasites from dog feces represents a public health problem that should be kept under control using the appropriate social medicine approach. Such an approach would typically involve health education measures related to the control of parasite transmission in the environment, provision of the guidelines to dog owners for prevention of the occurrence, persistence, and spread of zoonotic diseases, and health education of medical and veterinary professionals, pet owners, and the wider community regarding the preservation of the health of both humans and pet animals. An increased level of knowledge and corrected attitudes and behaviors among individuals would certainly contribute to more effective prevention of zoonotic diseases. *Acta Medica Medianae* 2023;62(3): 61-69.

Key words: dog, intestinal parasites, diagnosis, prevention, zoonotic diseases

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Introduction

A large number of dogs worldwide are infected with intestinal parasites, the developmental forms of which are eliminated via feces into the environment, which, due to their zoonotic potential, represent a constantly present threat, especially to pre-school and school children

(1, 2, 3). Dog feces is one of the main sources of contamination in public areas in urban environments, which thus become the primary infection sites for urban human populations (4, 5). There is a pressing need for raising human awareness about this complex problem; a joint action of all the segments of the veterinary and medical professions, pet owners, and people who visit public parks would constitute the prerequisite for sustainability for the concept "One Health", which includes protection of the environment and the health of both humans and animals (6).

In urban parks, the main areas for children to play, petsto walk, people to relax and enjoy themselves, there is a close cohabitation of owners' dogs with free-roaming dogs, where pet dogs are exposed to new parasitic infections. The presence of a large number of these animals in limited urban areas results in the continual contamination of public green areas, parks, and sand playgrounds with adult and developing forms of parasites from the dog's feces (6, 7, 8).

Regarding the geographical distribution and clinical relevance, *Toxocara canis*, hookworms and *Trichuris vulpis* are the most widely distributed dog helminths, but the importance of these pathogenic agents is oftentimes underestimated by veterinary doctors, human medicine doctors, and the community (9). Depending on the severity of dog infection, we should not overlook the eggs of *Dipylidium caninum* and *Taenia* spp., the trematodes of *Alaria alata*, and cystic forms of the *Giardia intestinalis* protozoan, *Amoeba* spp., and *Cryptosporidium* spp. as potential sources of human infection (10, 11, 12).

Changes in living conditions and nutrition of dogs have as a consequence more diverse and more complex health problems in dogs. The very close cohabitation of people and dogs creates opportunities for transmission of certain diseases from dogs to humans. It is therefore necessary that dog owners keep and feed their pets in an appropriate manner, as well as be acknowledged, at least at a basic level, about the etiopathogenesis of parasitic infections that affect their animals. Insufficient knowledge of these problems or an inadequate level of information often cause an unfounded fear of transmission of certain parasites from dogs to humans or, on the other hand, a neglect of the danger of potential zoonotic infections (13).

The aim of this paper is to suggest the need for a comprehensive insight into the problem and the realization of health education measures that would make possible the education of doctors, doctors of veterinary medicine, pet owners, and all those who visit public parks. An improved level of knowledge, together with positive motivation, could possibly help in the eradication of parasitic infection in public places and consequently reduce the degree of overall contamination with feces from infected dogs.

Epidemiological significance of canine intestinal parasites

Pathogenic action of zoonotic endoparasites from the dog feces primarily threatens dog owners, dog breeders who exercise insufficient care about dehelminthization of their broods, children who do not wash their hands after contacts with animals or have a habit of geophagia, farm workers and grocery sellers (especially in semi-rural and rural areas, with lots of free-roaming dogs who defecate on the agricultural land), when unwashed or insufficiently washed food can be a significant source of infection for humans (13). Parasite eggs may enter the organism via inhalation as well, usually in the summer when, in urban environments, dog feces on the streets become dry (14).

The contact of people with soil is one of the routes of spread for intestinal dog parasites. These causative agents reach the soil with canine and human excretions and are able to persist in soil for a long time, making it a potential infection

reservoir (15). Geohelminths are able to survive in soil the longest; they reach the organism of a susceptible individual through wounds in the skin or visible musoca (*Ancylostoma caninum*, *Uncinaria stenocephala*, and *Strongyloides stercoralis*) causing a cutaneous larva migrans (CLM) (16, 17), which may persist in human tissues for as long as several years (18, 19). The infections occurring in this way are mostly sporadic, but in extraordinary circumstances and situations (mass disasters, catastrophes, wars), the disease may occur in the form of epidemics as well (15).

A timely diagnosis of intestinal parasites in dogs and a proper treatment of infected animals make possible the prevention of infections in humans. In humans, as non-specific (paratenic) hosts, after penetrating the bowel wall, *T. canis* larvae migrate (by hepatopulmonary and somatic routes) to the liver, lungs, brain (VLM) and eye (OLM); they there remain active without any morphological changes for more than a year, until they are blocked by the inflammatory reaction and granuloma formation. Clinically, in the cases of OLM, there are vision disorders, endophthalmitis, strabismus, and even blindness (19).

Human infections with hookworms (*A. caninum*, rarely *U. stenocephala*) and rhabditids (*S. stercoralis*) from dog feces tend to occur when their eggs are excreted in a warm, humid soil or sand, where infective larvae develop from the eggs and penetrate the unprotected skin of the arms, legs, feet, gluteal region or back, after the skin comes into contact with contaminated soil or sand. In their passage through the skin, the larvae produce migratory pruritic dermatitis (17, 20).

In a study performed to examine the parks in the territory of Niš municipality (21, 22), two parasites with a zoonotic potential have been diagnosed: the nematode *Capillaria aerophila* in the specimens of dog feces (8–14%), and trematode *Alaria alata* in the specimens of dog feces (22–38%), soil (2%) and sand (16%). *Alaria alata* causes human larval alariasis, which in people produces multi-organ disorders— inflammatory, hemorrhagic, granulomatous, and necrotic lesions in the bowels, lymph nodes, liver, spleen, pancreas, adrenal glands, kidneys, lungs, heart, brain, and spinal cord (23). In the last decade, interest in this trematode and its potential zoonotic significance has been on the rise since this parasite has been diagnosed in Europe and in a number of countries neighboring Serbia, such as Croatia, Romania, and Bulgaria (24, 25).

Capillaria aerophila is the cause of human pulmonary capillariasis, which manifests in the infected with the onset of acute bronchitis and bronchiolitis, asthma, and a productive cough. This parasite has been diagnosed in foxes in suburban environments in Serbia (26, 27), which serve as an infection source for dogs; human infections have also been reported in Serbia, which has a special medical significance.

The intestinal parasites from soil and sand diagnosed in dogs represent a serious hazard and an essential potential that may harm human health, especially the health of children aged 3–5 years. Human infections with intestinal parasites hosted by dogs occur mostly by the fecal-oral route (by petting owned dogs and stray dogs, especially on their posterior parts where infective developmental forms of the parasite are located; by touching the mouth with unwashed hands after playing with dogs, especially by children; by playing on the soil and in sand ponds in the parks, around playground slides and swings). If humans are exposed to this sort of risk via contaminated soil and sand or by direct contact with dogs, there is a degree of probability that human infection with these pathological agents of parasitic etiology will actually occur (13).

There are numerous cases of parasitic zoonoses, the causative agents of which are transmitted to people in public areas via contaminated dog feces, as documented by the literature data for the territory of Serbia. Lalošević et al. (28) have reported about a case of respiratory capillariasis in a woman from Sremska Kamenica, resembling a bronchial carcinoma. It is a zoonosis caused by the nematode *C. aerophila*, which circulates among wild carnivores, from which it is transmitted by the fecal-oral route to domestic carnivores (stray dogs and owned dogs) and further to people. Gvozdrenović et al. (29) described a case of a familial epidemic of cryptosporidiosis in which three immunocompetent patients had abdominal cramps and pain in the muscles. Their fecal samples did not contain any blood or mucus, but were very loose and demonstrated cryptosporidium oocysts. After symptomatic therapy, the complaints were cured after 10–17 days. After they completed an epidemiological survey, the authors could not ascertain the source of the infection. There was also the case of a little girl, aged 4.5 years, in whom toxocariasis was detected by indirect immunofluorescence testing. In this patient, the complaints consisted of elevated body temperature, abdominal pain, changes on the skin in the form of an allergic reaction, and 43% eosinophilia. After two months of therapy with albendazole, this clinical case was resolved successfully, as demonstrated by the control examination two years later (30). A retrospective analysis of the findings of examination of feces samples from healthy individuals without any digestive symptoms for the presence of *Giardia lamblia* in the period 2004–2014, positive findings were reported in 574 individuals, with an equal gender ratio, aged on the average 33.76 ± 12.93 years, and especially in those who had a professional contact with food. The highest prevalence was reported in 2005 (4.9%) and the lowest prevalence was in 2014 (0.57%) (31). Perić et al. (32) reported about two Serbian patients in whom there were no anamnestic data about earlier travelings abroad, and in whom cutaneous

larva migrans was diagnosed in 2016. The first described patient was a 72 year-old man from Western Serbia, in whom the changes involved the thoracic part of the body. The second patient was a 31 year-old man from Central Serbia with changes involving his right arm. This syndrome usually occurs in people who travel to or stay in subtropical and tropical countries, especially those who frequent beaches. The lesions primarily affect the feet, gluteal region, and other parts of the body in direct contact with contaminated surfaces (soil and sand).

Since humans are non-specific hosts for most of the above mentioned parasites, these organisms cannot complete their developmental cycle up to the adult stage in the human organism. Regarding differential diagnosis, clinicians have much difficulty with some of the dog parasites (*T. canis*, *C. aerophila*, and *A. alata*), the larval stages of which, on their migration route, are halted in particular tissues and organs (liver, lungs, kidneys, heart, lymph nodes) producing cystic formations. Encysted parasitic larvae, after a period of time, may calcify, necrotize, or degenerate, and their presence may confound even the most experienced diagnosticians. Insufficient information and education about what may be etiologically expected can result in serious mistakes in making a valid diagnosis (13).

The intense process of urbanization of Serbian cities leads to the expansion of city belts into the suburbia and holiday settlements inhabited priorly only by foxes. Therefore, a close contact of stray dogs and owned, household watchdogs with foxes has been made possible. Such a contact produces a significant change in the parasitic fauna of dogs, which subsequently eliminate these new infective agents via their gastrointestinal tract onto publicly accessible surfaces, creating a source of infection for humans (27). In Serbia, similar to many other European countries, vaccination of foxes against rabies has been regulated by law since 2010 (administered via oral route, using baits). As a result of that, rabies has been successfully controlled, but the number of foxes has increased, thus increasing the prevalence of parasites for which foxes represent a source or reservoir of infection. In the chain fox – stray dog – owned dog – human, it is just an additional factor of increased risk and probability of human infection with intestinal parasites of wild and domestic carnivores (13, 33). Stray dogs, which freely roam the city parks, represent the most serious threat to human health. It is therefore essential that this problem be resolved in the long term by adopting a strategy that would regulate the number of abandoned dogs, including mandatory parasitological control of public areas likely to be infested (34). Since foxes represent a source of numerous parasitic zoonoses for both stray and owned dogs, an appropriate social medicine approach is necessary in the resolution of this

pressing public health and ecological problem in urban environments.

A suggestion for a program of health education measures for the resolution of the problem of contamination of public areas with dog feces

In accordance with the Guidelines of the ESCCAP (European Scientific Counsel Companion Animal Parasites) of 2021 and based on the results of performed investigations of public parks in the territory of Niš, in which four most prevalent endoparasites have been identified (*T. canis*, 36.66–38%; *ancylostomatidae*, 24.66–32%; *T. vulpis*, 20–28%; and *A. alata*, 28%) and contamination of developmental forms of endoparasites has been detected in 38–46% of soil samples and 40% of sand samples (13, 22), a suggestion has been defined by the program of health education measures, categorized into three groups, through which the public health problem of contamination of public areas with dog feces has to be approached.

I Recommendations by doctors of veterinary medicine for the purpose of controlling parasite transmission in the environment

For the parasites, the eggs, larvae, or proglottids of which are excreted by feces, the control and elimination of the pre-parasitic stages in the environment are essential for the reduction of the risk of infection in other susceptible animals or people. The populations of foxes and stray dogs in rural and urban environments may represent additional sources of infection for infectious agents that parasitize dogs. Infections of intermediary or paratenic hosts (birds, rodents, snails, and common earthworms) may contribute to the prolonged survival of the pre-parasitic stages in the environment (13).

Most of pre-parasitic stages are very resilient to degradation under the impact of environmental factors (they live from several months to several years). Freshly excreted developmental forms of many parasites can be directly infective (for instance, the eggs of *Taenia* spp. and *Echinococcus* spp). Other parasites, such as nematode eggs, require from several days to several weeks at appropriate temperatures (usually above 16 °C) to transform into the infective stage (35).

That is the reason why it is vital to prevent initial contamination with parasites by implementing comprehensive parasite control programs, which will be based on local epidemiological information and knowledge.

a. Safe disposal of animal feces is essential.

It is necessary to forbid disposal of animal feces in toilets or in compost intended for use in olericulture. In the cultures or regions where laws

do not prohibit this, feces can be disposed of in the collections of home waste.

It is necessary to encourage the measures which would facilitate feces removal, providing appropriate waste bins and special bags to be used for this purpose. Since it is very complicated to control cat defecation in the open, cat parasite control should be the focus of special attention.

Local authorities, especially in urban areas, should adopt and implement the laws that would regulate the control and safe disposal of feces.

b. Adoption and implementation of the laws/regulations by appropriate bodies, which would put under control the population of wild carnivores

c. Implementation of regular dehelminthization of infected animals is necessary in order to reduce contamination of the environment. In appropriate cases (e.g., dogs with persistent clinical signs or suspicion of resistance), it is necessary to perform regular coprological tests four times a year.

d. Implementation of extreme measures for decontamination of very contaminated areas, including removal of contaminated soil or sand or covering it with concrete or asphalt (in very crowded dog breeding kennels, parasite eggs retain their vitality for months or even years in the soil).

e. In breeding kennels or households with several or more animals, a strict treatment and quarantine for new animals is recommended, which is essential in order to avoid bringing in infected animals.

f. Children's playgrounds should be adequately fenced in order to prevent the entry of animals, especially cats.

i. Sand ponds should have protective covers during periods when they are not used by children.

ii. Sand in sand ponds should be regularly replaced (at least once or twice a year) if they are open and probably contaminated with feces.

g. Contaminated areas should be made accessible to sunlight in order to reduce the level of contamination – since drying up and ultraviolet light are very deleterious to parasite eggs, reducing their number.

h. Recommendations for dog owner education

i. At dog reviews, shows, or festivals, discussion forums for dog owners and cynophiles, in the form of open classes.

ii. Pet food distributors in pet shops should be engaged to distribute information materials since it is not sufficient to love a dog or other animal but to know its associated health risks and the transmission of these to the owners.

iii. Creation of a dog owner database, with e-mail addresses to which relevant information will occasionally be sent about dog vaccinations, or treatments against endo- or ectoparasites.

iv. At least twice a year (in the spring and the fall) discussion forums should be organized in hunting associations, debating this issue and handing out education leaflets.

v. Distribution of educational brochures and leaflets about parasitic diseases to pet shops and dog groomers.

vi. Each dog owner and cynophile should get the brochure "One dog, one health"; TV information campaigns should also be organized about the issue of parasitic diseases.

vii. In rural environments, doctors of veterinary medicine should leave the brochure in each visited household or at gathering places in villages.

viii. Level of awareness of the people should be raised whenever possible about the ever increasing number of dogs, possibly causing the problems to future unconscious dog owners.

II Recommendations of doctors to dog owners for the purpose of preventing the occurrence, maintenance, and spread of zoonotic diseases

a) Adequate personal hygiene practices, especially hand washing after contact with pets and before meals;

b) Minimizing exposure of children to potentially contaminated environments, educating them about personal hygiene principles (e.g. regular nail trimming), stressing the importance of adoption of such habits;

c) Wearing gloves when working in the garden;

d) Washing of raw fruits, vegetables, and mushrooms before consuming them;

e) Control of parasitic infections affecting pets through repeated treatments and/or regular diagnostic testing;

f) Prevention of infections (wherever possible), reducing the risk of infections affecting pet animals;

g) Regular disposal of pet feces for the purpose of reducing environmental contamination with parasitic infective stages. Pet feces should not be disposed of in recyclable waste;

h) Regular and complete dog care, aimed to reduce the risk of contamination with helminth eggs;

i) Footwear change in order to prevent living space contamination (professional breeders or those who work in dog shelters for abandoned animals should leave their shoes and work clothes at their workplaces);

People in regular contacts with animals which potentially can transmit zoonotic parasites should be well acknowledged with the risks; the health risks are considerably higher when pregnant women are concerned, or in those already affected with other diseases or immune system suppression. These records should be made available to doctors of human and veterinary medicine without any need for the

personal or familial medical records of the patients. Bearing this in mind, special attention should be paid to:

- immunocompromised individuals, such as the elderly, diabetics, those with HIV infection, those undergoing organ transplantation, those receiving immunosuppressive chemotherapy, or those who receive treatments for autoimmune diseases;

- other susceptible groups, such as pregnant women, babies, or those with developmental disorders;

- people exposed to professional risks, such as farmers, workers in breeding kennels for dogs and cats, and hunters.

III Recommendations of doctors regarding health education of medical and veterinary professionals, pet owners, and the community as a whole

a) The protocols and recommendations for the control of parasitic infections should be clearly communicated to veterinary and paraveterinary professionals and consistently implemented.

b) It is necessary to encourage cooperation between the professions of human and veterinary medicine, whenever possible, since the link between them is extremely important from the point of view of public health, especially in the domains of timely detection, effective treatment, and prevention of zoonoses.

c) Professional brochures, leaflets, and posters placed in veterinary clinics, pharmacies, and shops for pet food and other supplies and equipment are very useful tools in health education. In the era of modern technology and social networks, educational activities can be undertaken electronically as well (web sites). There are also the billboards or visible advertisement boards placed in parks frequented by both people and animals, as well as written educational materials related to the safe removal of one's pet's feces after a defecation in a public area as a potential source of zoonotic parasites.

d) The importance of continued regular and planned dehelminthization or joining the "pet program" should be publicly clarified for all veterinarians, veterinary technicians, and other professionals for animal protection and literally promoted.

e) Through communal, municipal, and republican institutions and communal police, programs should be created for mandatory anti-parasitic treatments, with strict recording about that in the republic's health records (pet passports), and the possibility of checking that by the authorities (communal policemen).

f) It should be regulated by law that each owner who visits public areas with his pet should be obliged to possess and always carry with him the pet passport, which contains the proper evidence of dehelminthization with a veterinary seal and signature.

g) Only responsible ownership of dogs or cats can facilitate care for public health and encourage the acceptance of these animals as human companions.

h) Pet owners should be informed and well acknowledged about potential health risks of parasitic infections, not only for the pets but also for themselves and their families.

IV Educational recommendations for dog owners

Brochures such as "One dog, one health", through which the broader public is informed and educated, should be distributed to public health centers and institutes, to privately owned laboratories and pharmacies, and made available to patients who own and love dogs.

Brochures should be distributed to all dog breeders who report about new litters and at entry to dog shows via the Cynological Association of Serbia.

V Educational recommendations to those who do not own dogs

Brochures should be distributed to parents in children's daycare institutions, in schools, and

to patients who regularly visit public health centers and institutes for sanitary examinations.

Preschool and school children should be adequately educated since they represent the riskiest category of the population (in view of their wishes and resolutions to own dogs).

Stray dogs are becoming regular inhabitants of schoolyards and playgrounds, visited during weekends (when not in use) also by parents with small children, who may step into dog feces and thus come into contact with potentially infective material; that is the reason why broader public should be appropriately informed about this problem.

Community service workers whose occupation is to deal with urban hygiene and urban green infrastructure should be educated about the possibility of infection and the proper way to deal with feces in public areas.

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SOCIJALNO-MEDICINSKI PRISTUP REŠAVANJU PROBLEMA KONTAMINACIJE JAVNIH POVRŠINA FECESOM PASA I ZNAČAJ ZA JAVNO ZDRAVLJE

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Pošto čovek i pas dele životni prostor i ostvaruju veoma blizak kontakt, a mnogi vlasnici ne brinu adekvatno o svojim ljubimcima, postoji mogućnost prenošenja izvesnih parazitskih oboljenja sa psa na čoveka. S obzirom na to da su psi nosioci i pravi domaćini velikog broja vrsta zoonoznih endoparazita, njihov feces može predstavljati izvor infekcije za ljude i opasnost po njihovo zdravlje. Stoga, nužno je podizanje svesti građana o neophodnosti sprovođenja zoonoznih mera u odgoju pasa i značaju kauzalne planske dehelmintizacije i antielktoparazitskog tretmana. Takođe, neophodno je da vlasnici sprovode redovne koprološke preglede, neposredno nakon nabavke psa i četiri puta godišnje, u toku njihovog boravka kod vlasnika, uz poštovanje principa smene preparata na bazi različitih hemijskih formulacija. Pravovremena dijagnostika crevnih parazita kod pasa i ispravan tretman inficiranih životinja omogućuju prevenciju pojave infekcija kod ljudi. Kontaminacija javnih površina intestinalnim parazitima iz fecesa pasa predstavlja problem javnog zdravlja, koji je neophodno staviti pod kontrolu odgovarajućim socijalno-medicinskim pristupom. Ovaj pristup podrazumeva aktivno i plansko sprovođenje Predloga zdravstveno-vaspitnih mera, koje se odnose na kontrolu transmisije parazita u životnoj sredini, pružanje smernica vlasnicima pasa u sprečavanju pojave, održavanja i širenja zoonoznih oboljenja i zdravstveno-vaspitu edukaciju osoblja medicinske i veterinarske struke, vlasnika kućnih ljubimaca i šire društvene zajednice u cilju očuvanja i poboljšanja zdravlja ljudi i životinja. Povećan nivo znanja i korigovani stavovi i ponašanje pojedinaca doprineli bi efikasnijoj prevenciji zoonoza. *Acta Medica Medianae* 2023;62(3):61-69.

Ključne reči: pas, intestinalni paraziti, dijagnostika, preventiva, zoonoze

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PAIN MANAGEMENT OF SENSORIMOTOR POLYNEUROPATHY IN COVID-19 INFECTIONS

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A 73-year-old man has tested positive for SARS-CoV-2. On the tenth day of the disease, a symmetrical distribution of spontaneous pain and dysesthesia occurs in both feet. Initial pain management, ordered by general practitioner was not effective, so he was referred to the pain specialist. The new therapy included combination of anticonvulsants, antidepressants and adjuvant therapy with partial, but satisfactory reduction in pain. Education/reeducation of general practitioners would accelerate the detection and symptomatic treatment in the early stages of COVID-19. An even better suggestion would be to refer these patients to pain medicine specialists. *Acta Medica Medianae* 2023;62(3):70-74.

Key words: *peripheral neuropathy, COVID-19, chronic pain, pain management, treatment outcome*

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Introduction

Since December 2019, an unknown, fast spreading cause of viral pneumonia, called Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2), has spread from China to the rest of the world. Initially, it was assumed that respiratory symptoms predominate, but, over time, a significant clinical spectrum of complaints appeared (1). Depending on the severity of the clinical picture, neurological manifestations were recorded in 36.4% to 45.5% of patients infected with SARS-CoV-2. The most common manifestations of the central nervous system (CNS) were headache, cerebrovascular disease and epileptic seizures, while damage to the peripheral nervous system (PNS) was manifested

by ageusia, anosmia, polyneuropathy (2, 3). Although their pathogenesis has yet to be fully elucidated, neurological symptoms have been attributed to the neuroinvasive potential and neurotropic characteristics of SARS-CoV-2 during and after infection (3, 4). We focused on the therapeutic approach to neuropathic pain in the patient with COVID polyneuropathy, examined and treated as an outpatient.

Case report

In October 2020, a 73-year-old man has tested positive for SARS-Cov-2, with a mild clinical picture: fever (up to 38.4°C), cough, headache, without myalgia, pneumonia or unpleasant smell and taste. On the 10th day of the disease, a symmetric distribution of spontaneous pain and dysesthesia (tingling, burning) occurred in both feet. The intensity of the pain gradually increased to maximum rated 8/10 on the Numerical Pain Rating Scale (NPRS). The complaints were especially expressed at night, accompanied by sleep disturbances and anxiety. After COVID infection sanation, the pain (6/10 on NPRS) persisted for the next few months, despite analgesic therapy (paracetamol, ibuprofen and thioctic acid with topical application of capsaicin cream). This therapy was prescribed by a general practitioner. The patient's personal medical record stored data of medically regulated hypertension, prostatic hyperplasia, a non-smoking as well as no alcohol consumption status. After 6 months, during his first neurological examination, the following evoked positive symptoms were

revealed: bilateral distal hyperalgesia, mechanical and thermal allodynia as a result of a pathological increase in nerve excitability. Dysesthesia and paroxysmal pain in the affected region indicated the presence of spontaneous positive symptoms. All upper extremity reflexes were intact; however, patellar and Achilles reflexes were absent. His physical examination was unremarkable. Pain DETECT questionnaire (PD-Q) score was 31/38, DN4-questionnaire 6/10. Electromyoneurography (EMNG) examination showed distal sensorimotor, dominantly sensory polyneuropathy (PN). In addition to the possible association of COVIDinfection with the clinical picture, we also searched for all common causes of PN: inflammatory, endocrinological, toxic, nutritional deficiencies, tumors, neurodegenerative causes. Oncomarkers: total prostate-specific antigen (tPSA), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), Beta-2-microglobulin; immunological tests: IgG, IgA, IgM, complement components C3 and C4, rheumatoid factor (RF), antineutrophil cytoplasmic antibodies (ANCA), antinuclear antibodies (ANA), anti-cyclic citrullinated peptide antibodies (antiCCP) and thyroid status—all were normal. Viral panel (HIV 1 and 2 antibodies, p24 antigen, HBs antigen,

Borrelia burgdorferi antibodies), serum micronutrient levels (folic acid, vitamin B12) and serum protein electrophoresis were also normal. Lumbar MRI, performed without contrast, did not reveal any compression of the nerve root and spinal cord. Nerve biopsy and lumbar puncture were not performed. His physical exam was unremarkable.

Considering the severity of the reported pain, a new analgesic therapy was introduced, which included a combination of analgesic (anticonvulsant-pregabalin in a dose of 300–600 mg daily, and antidepressant-duloxetine 60 mg/day), neuroregenerative agents (nucleotides and B vitamins, once a day). In the meantime, physical treatment (kinesitherapy and magnetotherapy) was started. After 4 months, partial but still satisfactory pain remission was reported, NPS 4/10 with significant improvement in sleep and quality of life.

It is interesting that the patient overcame a second infection with COVID-19 a year and a half after the first infection, and after a full vaccination with the Sinopharm COVID-19 vaccine. There was no worsening of neurological complaints.

Discussion

Distinguishing features of the COVID-19 infection are the genetic diversity and rapid evolution of the virus. Although the precise pathophysiological mechanism of both central and peripheral neurological lesions has not been established yet, the characteristic "cytokine storm" is considered the main mediator of this viral infection (5). In a study of 35 autopsies performed on patients with COVID-19 infection, no signs of

direct viral invasion of muscle and nerve fibers were detected. PN is considered to be a consequence of inflammatory and immune damage associated with cytokine release (6).

To our knowledge, the literature reports a small prevalence of peripheral sensorimotor PN associated with COVID-19. In a recent study conducted on 1760 patients with neurological symptoms of COVID-19, Rifino et al. (7) identified 31 (22.6%) patients with clinically evident involvement of the PNS: 17 patients with Guillain-Barré syndrome, 9 myopathy and critical illness neuropathy (CRITICAL Illness MYopathy and/or Neuropathy-CRIMYNE), 2 brachial plexopathy and 3 peripheral polyneuropathy cases. Some cases of hospital-treated motor PN as a complication of COVID-19 have also been recorded (8, 9).

Neuropathic pain is difficult to treat effectively. The initial pain therapy for our patient had no literature confirmation. Paracetamol is not recommended in the treatment of chronic nociceptive and neuropathic pain neither as monotherapy nor as polytherapy. On the other hand, it is believed that approximately 40% of patients with neuropathic pain take NSAIDs (nonsteroidal anti-inflammatory drugs), although there is insufficient data on their effectiveness for this purpose. Commonly used ibuprofen is a non-selective inhibitor of cyclooxygenase (COX-1) that inhibits the production of proinflammatory prostaglandins (PG) and thus exerts an analgesic and anti-inflammatory effect in acute inflammatory nociceptive pain or during exacerbation. Although there is a hypothesis about the immunological pathogenesis of neuropathic pain in Covid-19 PN followed by increased PG production, where NSAIDs might find their place, their use is still not recommended in chronic neuropathic pain (10).

Alpha lipoic acid (ALA), also known as thioctic acid, is a powerful endogenous and exogenous antioxidant. In addition to the known effect on increasing the level of endogenous antioxidants (glutathione and coenzyme Q10), it reduces the production of proinflammatory cytokines (interleukin, chemokines, tumor necrosis factor- α -TNF- α). The hypothesis that the entry of SARS-CoV-2 into the cell could be slowed down or prevented if ALA is used at the same time, could contribute to the therapeutic potential of this agent in Covid PN (11).

Capsaicin is a selective, potent, high-affinity agonist for the transient receptor potential vanilloid type 1 (TRPV1) ion channel complex. Since it has not shown interactions with other commonly used analgesics, it can be combined with them. According to the Special Interest Group on Neuropathic Pain of the International Association for the Study of Pain, capsaicin is recommended as a second-line drug for the treatment of peripheral neuropathic pain (12). The guidelines of the German Society of Neurology (German Society of Neurology) advise the 8% capsaicin dermal patch as a first-line option for

localized neuropathic pain and as a second-line option for neuropathic pain of any cause (13).

The winning therapeutic combination for Covid PN in our case was the following: anticonvulsants, antidepressants and nutritional agents. Although there are no clinical trials studying the use of antiepileptics pregabalin (PGB) or gabapentin (GBP) for neuropathic pain associated with SARS-CoV-2, they are still traditionally used. Anticonvulsants bind with high affinity to the $\alpha 2\text{-}\delta$ -subunit of neuronal calcium channels in peripheral and central nociceptive neurons, thereby reducing the influx of calcium ions. According to the recommendations, they are used as first-line therapy: GBP in a daily dose of 1200–3600 mg, divided into three doses, and PGB, in a daily dose of 300–600 mg, divided into two doses. Gabapentin is approved for the treatment of peripheral neuropathic pain and pregabalin for peripheral and central neuropathic pain (14).

Combination of anticonvulsants with tricyclic antidepressants (amitriptyline) and serotonin and noradrenaline reuptake inhibitors-SNRI (venlafaxine and duloxetine) are the first-line treatment of peripheral neuropathic pain.

Duloxetine is a balanced neuromodulator of pain. Its analgesic effect results from potentiation of descending nociceptive inhibitory pathways by inhibition of presynaptic reuptake of serotonin and norepinephrine, two monoaminergic neurotransmitters. Common side effects (nausea, headache, dry mouth, insomnia, constipation) are classified as mild to moderate and are less common than tricyclic antidepressants, making them more acceptable. The initial dose is 30 mg daily, with an increase in the dose over a period of 7 to 14 days. The target dose to be achieved initially should be 60 mg, and the maximum dose is 120 mg once daily in the morning (13, 15).

In our case, as in many other cases of COVID infection, the administration of group B vitamins had adjuvant support. Vitamins B1, B6 and B12 contribute to analgesia in different ways: they regulate nerve conduction/excitation and selectively inhibit conduction in sensory nerves. Prolonged inhibition of pain is caused by the interaction of vitamins with intraspinal and supraspinal receptors in various systems, releasing endogenous opioids or inhibiting non-opioid neurotransmitters (γ -aminobutyric acid). On the other hand, data of their antiviral effect on the replication of the Sars-CoV-2 virus contribute to the positive effect of B group vitamins (16, 17).

Conclusion

Although a clear mechanism of neurological manifestations of SARS-CoV-2 has not yet been established, our example confirms the effect of a standard pharmacological approach to neuropathies: anticonvulsants and antidepressants, topical analgesics and nutritional agents. In patients recovering from COVID-19 infection, persistent neuropathic pain has a negative effect on their quality of life, physical functioning, and emotional status. Our experiences suggest that neuropathic pain is underdiagnosed in primary health care, therapy is usually carried out with inadequate drugs, and when specific drug therapy is included, it is often underdosed. Therefore, the education/reeducation of general practitioners considering pain therapy would accelerate the detection and symptomatic treatment in the early stages of COVID-19. An even better proposal would involve referring these patients to pain medicine specialists, which would enable a multidisciplinary approach and thus provide a chance for a better outcome in all aspects as well as prevent the emergence of SARS-Cov-2 post-viral burden.

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TERAPIJA BOLA SENZOMOTORNE POLINEUROPATIJE U INFEKCIJI IZAZVANOJ COVID-19 VIRUSOM

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Sedamdesettrogodišnji muškarac oboleo je od SARS-CoV-2 sa blagom kliničkom slikom. Desetog dana bolesti javljaju se simetrična distribucija spontanog bola i dizestezija u oba stopala. Inicijalna terapija bola, ordinirana od strane primarne zdravstvene zaštite, nije dala zadovoljavajući efekat. Nova terapija, ordinirana od strane specijaliste medicine bola, podrazumevala je kombinaciju antikonvulziva, antidepresiva i adjuvantne terapije. Time je izvršena delimična, ali ipak zadovoljavajuća redukcija bola. Edukacija/redukacija službe primarne zdravstvene zaštite u pravcu terapije bola ubrzala bi detekciju i simptomatsko lečenje u ranim fazama infekcije izazvane COVID-19 virusom. Još bolji predlog podrazumevao bi upućivanje ovih bolesnika specijalistima terapije bola. Acta Medica Medianae 2023;62(3):70-74.

Ključne reči: periferna neuropatija, COVID-19, hronični bol, terapija bola, ishod

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XANTHOGRANULOMATOUS PYELONEPHRITIS AND DIAGNOSTIC APPROACH: A CASE REPORT

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Xanthogranulomatous pyelonephritis (XGPN) is an uncommon chronic granulomatous process that can result in significant destruction of renal parenchyma and propagation of inflammation into adjacent pararenal tissues. The presented patient had xanthogranulomatous inflammation of renal pelvis, peri- and paranephritic tissue, with formation of a large tumor-like mass which was in close relation to the base of urinary bladder. The findings of pathognomonic foamy macrophages and multinucleated giant cells showing diffuse positivity for CD68 confirmed the precise diagnosis. Having in mind that XGPN can mimic various clinically and pathologically benign and malignant conditions, a multidisciplinary diagnostic approach is required. Sometimes, careful clinical, imaging, nuclear and histopathological examinations are necessary to determine the type and degree of renal damage, which will dictate surgical approach, especially if nephrectomy is not planned. *Acta Medica Medianae 2023;62(3):75-80.*

Key words: xanthogranulomatous pyelonephritis, diagnostic approach, histopathology

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Introduction

Xanthogranulomatous pyelonephritis (XGP) is an unusual, unilateral and very rare form of chronic granulomatous pyelonephritis due to infection (*E. coli*, *Proteus*) or stones. Nephrolithiasis, most often with the staghorn-type calculus, is not a prerequisite, nevertheless remains a well-established predisposition for XGP (1). Kidney involvement in XGP can be diffuse, segmental or focal with inflammation localized within the cortex of the kidney (2, 3). The precise pathogenetic mechanism remains incompletely understood in XGP. Associations between XGPN and calyceal stones and staghorn calculi, with pyelo-ureteral junction obstruction, ureteropelvic duplication, ureteral schistosomiasis and tumours

(including renal cell and urothelial carcinomas) are well known, but obstruction may be incomplete, and renal parenchymal destruction and subsequent renal impairment occur secondary to the chronic inflammation and macrophage infiltration (3 - 5). The combination of obstruction and infection is presumed the primary initiator, resulting in an interstitial pyelonephritis, followed by chronic granulomatous immune response which is the result of incomplete eradication of the inciting agent (5). Granulomatous inflammation is a response induced primarily by bacteria presence within the granulomas, in both intra- and extracellular locations. The failure to completely degrade the bacterial products provokes a chronic inflammatory response, but also suggests a limited/incomplete host immune response (6).

XGPN starts from the renal pelvis and calyces, spreading to the renal parenchyma, extends beyond the kidney into the perinephric and pararenal spaces, and finally, adjacent organs such as the liver, spleen, duodenum, pancreas, and great vessels can be involved if left untreated (7–9). The typical gross pathological appearance is that of an enlarged kidney with capsular thickening and replacement of parenchyma with yellow tissue, with necrosis and a dilated pelvicalyceal system containing stones/debris and variable volumes of pus. The microscopic examination of a XGPN shows three distinct zones centered by a calyx. The inner zone consists of leukocytes, lymphocytes, plasma cells, histiocytes

or macrophages, and necrosis. In the middle zone, we can see granulation tissues surrounded by hemorrhage. Giant cells, cholesterol clefts, and fibrous tissues are characteristics of the outer zone. The pathognomonic feature in XGPN is the presence of lipid-laden foamy macrophages (xanthoma cells) that give a yellow color to the tissue (10, 11).

In this case report, we present a patient with XGPN that presented as pseudotumor on radiological and gross pathological examination, with an emphasis on diagnostic dilemmas.

Case report

A 61-year-old female presented with abdominal pain and leukocytosis. The native graphics of urinary tract detected a calcium intensity shadow measuring 21 x 16 mm on the left side, at the height of L2/L3. Distally, ipsilaterally, at the level of the transverse process of L4, there was another shadow of calcium density with a diameter of 6.5 mm. As well as a soft tissue shadow, from the lower pole of the left kidney to the base of the urinary bladder, probably a cyst or tumor was detected with the largest diameter of 12 cm. CT scans of left kidney showed multiple cystic changes of diameter from 25 mm to 150 mm and compression on the blood vessels without infiltration, as well as a calculus of diameter 20 mm with hydronephrosis grade III-IV. Static renal scintigraphy with ^{99m}Tc-DMSA detected that the distal pole of the left kidney was displaced laterally and irregularly shaped. The

cortical parenchyma was markedly reduced and scarred. A large number of photo-deficient fields were observed which according to their scintigraphic characteristics corresponded to calculus and cysts. Individually, the left kidney participated with 27% and the right with 73% in the total renal function (Figure 1A).

The gross pathology showed pyonephrotic left kidney 20.5 x 14 x 8 cm, with the staghorn-type calculus in the pelvis and calculus in the proximal part of the ureter with complete obstruction. The renal parenchyma was atrophic with a thickness of 1 mm, with the exception of the lower pole where it was up to 15 mm focally. Tumor-like adrenal gland tissue, 92 x 55 x 37 mm was detected in the peripelvic space and extended below the lower pole of the kidney. A cavity 105 x 75 mm, filled with blood and pus was localized in peripelvic fat, and was in contact with the yellow tumor-like mass. Microscopic examination showed renal parenchymal destruction with obstructive uropathy and XGPN in pelvis and peripelvic adipose tissue, including sections sampled from a tumor-like mass. XGPN was characterized by a granulomatous mixed inflammatory infiltrate with fibrosis and cholesterol clefts in the background (Figure 1B). The inflammatory infiltrate was composed of xanthomatous histiocytes with foamy cytoplasm, which showed diffuse positivity for CD68, neutrophils, lymphocytes, and plasma cells (Figure 1C, 1D). Microscopic findings included the presence of abscess in perinephritic fat.



Figure 1A. Static renal scintigraphy with ^{99m}Tc-DMSA, posterior projection, markedly reduced cortical parenchyma of the left kidney with scarring changes

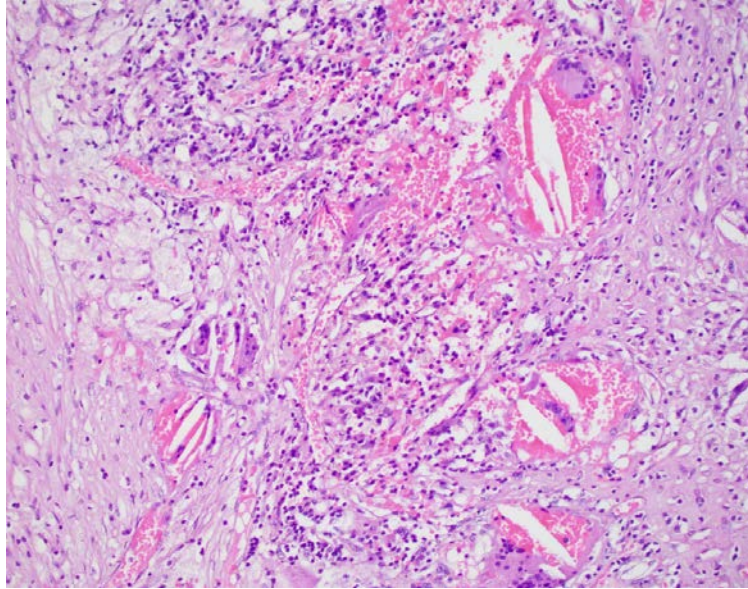


Figure 1B. Cholesterol clefts, xanthomatous histiocytes, and granulomatous inflammatory infiltrate in xanthogranulomatous pyelonephritis (H&E, original magnification $\times 20$)

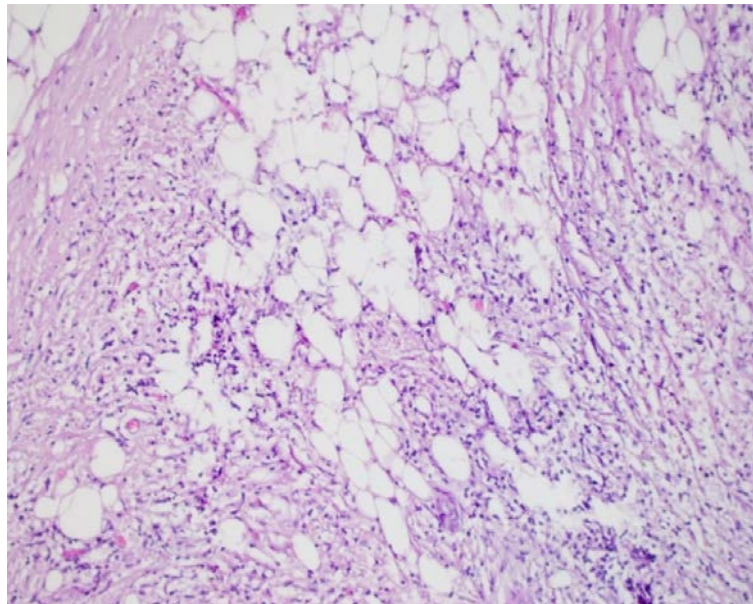


Figure 1C. Fibrous background and xanthogranulomatous inflammation in peripelvic adipose tissue (H&E, original magnification $\times 20$)

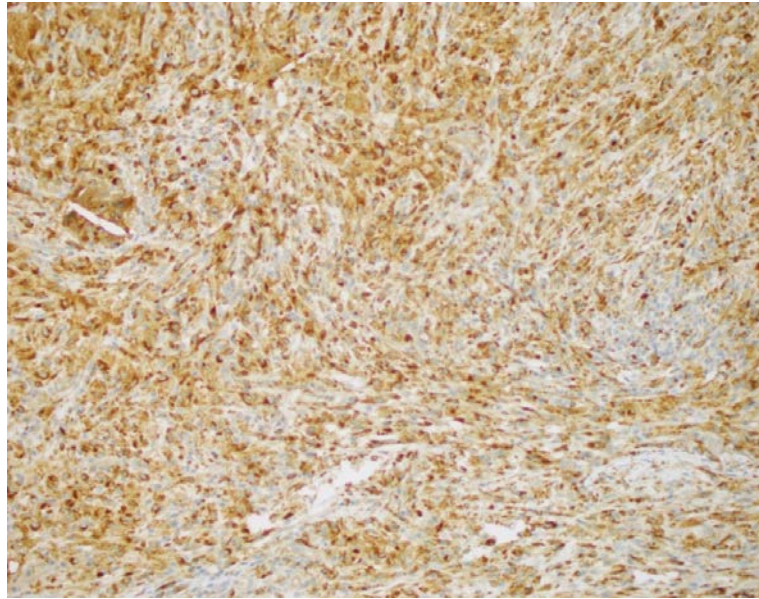


Figure 1D. The xanthomatous cells and multinucleated giant cells are positive for CD68 (IHC staining, original magnification $\times 20$)

Discussion

XGPN is well known as the “great imitator” or pseudotumor because of the overlap of imaging features with other conditions in kidney, including renal cell carcinoma (RCC), urothelial carcinoma, tuberculosis and malakoplakia, which can lead to serious misdiagnosis. In some cases, the presence of synchronous XGPN and renal malignant tumor creates an additional diagnostic dilemma (12, 13).

The diagnostic algorithm includes conventional radiographs of the abdomen, ultrasound, and CT for DDg with tumors especially in focal/segmental form of XGPN and in detection of associated complications (14).

Therefore, early identification and treatment are required to decrease the morbidity and mortality associated with this condition. Although antibiotics can be given in acute infection, the treatment of choice for XGP is nephrectomy for a nonfunctional kidney (15). ^{99m}Tc -DTPA and ^{99m}Tc -MAG3 can show the degree of residual renal function, and this finding will dictate surgical approach, renal salvage or nephron-sparing surgery if feasible, especially in the setting of focal XGP (16, 17).

On the one hand, multiple yellow nodules around calyces may form a mass and be infiltrative which may be suspicious of neoplastic proliferation. In our case, XGPN with extension of xanthogranulomatous inflammation to the peri- and paranephritic tissue forms a large tumor-like mass, which was the main Ddg dilemma intraoperatively and during macroscopic examination.

The most important diagnostic challenge is misinterpretation of foam cells in XGPN as clear cells in clear cell RCC (cRCC). Characteristic histology and immunohistochemistry stains usually lead to the right diagnosis. Our staining showed diffuse cytoplasmic CD68 positivity in foamy macrophages and multinucleated giant cells, and on the other hand, characteristic immunohistochemical finding in cRCC is expression of Vimentin, CD10, and RCC (12). Foamy macrophages can be seen in papillary RCC, especially in type 1, with characteristic localization in cores of tumor papillae, and ICH findings are similar with cRCC because of their origin from proximal tubules epithelium (18).

Malakoplakia is a rare histiocytic disease that is clinically presented as a single or multiple white-yellow soft raised plaques on the mucosal surface in the pelvis, ureter and most often in the urinary bladder. Microscopy finding detected foamy epithelioid histiocytes with PAS+ granular eosinophilic cytoplasm in lamina propria, some lymphocytes and occasional giant cells (19).

Conclusion

In evaluation of XGPN, it is necessary to integrate the clinical presentation of the disease with the findings of radiological and nuclear diagnostic methods, and differentiate with respect to other malignant and non-malignant conditions. Multidisciplinary diagnostic approach, including radiological, nuclear, and histopathological examinations, is necessary in XGPN especially if nephrectomy is not planned.

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Prikaz bolesnika

UDC: 616.61-002-073
doi: 10.5633/amm.2023.0311**KSANTOGRANULOMATOZNI PIJELONEFRITIS I
DIJAGNOSTIČKI PRISTUP: PRIKAZ SLUČAJA***Filip Veličković^{1,2}, Marina Vlajković^{1,2}, Miloš Stević^{1,2}, Sanja Veličković³,
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Ksantogranulomatozni pijelonefritis (KSGPN) je redak hronični granulomatozni proces, koji može dovesti do značajne destrukcije bubrežnog parenhima i propagacije zapaljenja u susedna pararenalna tkiva. Prikazani bolesnik imao je ksantogranulomatozno zapaljenje bubrežne karlice, perinefritičnog i paranefritičnog tkiva, sa formiranjem velike tumorolike mase, koja se pružala do baze mokraćne bešike. Nalaz patognomoničnih penastih makrofaga i multinuklearnih džinovskih ćelija, koje su bile difuzno pozitivne na CD68, potvrdio je preciznu dijagnozu. Budući da KSGPN može oponašati različita klinički i patološki benigna i maligna stanja, potreban je multidisciplinarni dijagnostički pristup. Ponekad su neophodna pažljiva klinička, radiološka, nuklearna i patohistološka ispitivanja, kako bi se utvrdio tip i stepen oštećenja bubrega, što će odrediti hirurški pristup, posebno ako nefrektomija nije planirana. *Acta Medica Mediana* 2023; 62(3): 75-80.

Ključne reči: ksantogranulomatozni pijelonefritis, dijagnostički pristup, histopatologija

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A 36-YEAR-OLD FEMALE PATIENT TREATED WITH AN OCCLUSAL SPLINT: A CASE REPORT

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Valdeta Osmani²

This article describes a case of a 36-year-old female patient who sought help due to long-lasting pain in the temporomandibular joint (TMJ) region. Clinical examination revealed pain on the left side in the region of TMJ, and pain on opening, chewing, and palpation. Also, deviation to the right, and clicking on opening were present. A painful sensation on the left masseter muscle was detected on palpation. The diagnosis was established according to the functional analysis, clinical examination, and obtained anamnestic data. The decision was stabilizing occlusal splint to be administered in combination with exercises, physiotherapy, and symptoms-relieving analgesic treatment if needed.

This protocol aimed to alleviate the painful symptomatology and enable relaxation of the muscles of mastication to some degree, before orthodontic treatment. This would obtain a solid basis for stable orthodontic treatment outcomes.

Based on the case of the presented patient, it can be concluded that temporomandibular joint disorders (TMJD) can be treated with occlusal splint therapy in combination with exercises and physiotherapy. *Acta Medica Medianae* 2023;62(3):81-87.

Key words: temporomandibular joint (TMJ), temporomandibular disorders (TMD), stabilization splint therapy, orthodontics

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Introduction

Temporomandibular disorders (TMD) are relatively present in everyday practice. Patients are used to living with that kind of pain, which usually is accepted as common and normal. TMJD (temporomandibular joint disorders) are often misdiagnosed and patients are experiencing similar mild symptoms for long period, to the point when those symptoms are not tolerable. Chronic pain might cause a decrease in quality of life, triggering many health concerns for patients and a great social burden. Nociceptive and emotional

behaviors are reliant and share overlapping neural mechanisms. Consequently, TMD (temporomandibular disorder) pain due to inflammation might correspondingly induce emotional disturbance (1). Of immense importance for our practice is accurate diagnosis and treatment planning of our patient's condition (2, 3, 4, 5).

As orthodontic practitioners or future orthodontic practitioners, we should consider that orthodontic treatment is neither the cause nor cure for TMD, relying on reports of the current systematic reviews (6, 7).

The proper management will include using reversible and conservative modalities such as medications, physical therapy, oral appliances, and self-care (8, 9).

A thorough clinical interview and physical examination to detect TMD signs and symptoms before the establishment of orthodontic therapy is mandatory (5).

Unilateral pain present in the TMJ region and sensations in the soft tissues around the joint, that increases during mandibular movements may often be symptoms of TMD (2, 3, 5).

As muscles are holding the body in a given position, this location awareness is primarily relayed to the cortex in order of proprioception. According to TMJ, there has been an overall consensus that the symptoms of TMJ are not

usually in the joint itself, but also in the body's neuromuscular system, primary the masseter and temporalis, medial and lateral pterygoid (10).

Internal derangements of the temporomandibular joint are defined as a change in normal anatomic relationships so that the articular disc is displaced from its position between the head of the condyle and the glenoid fossa. The internal derangement can be functional or anatomic disturbance between the condyle, the disc, and the glenoid fossa. A more common derangement is the joint's functional (neuromuscular) derangement that often results from trauma. Those trauma-initiated displacements of the articular disc, especially the anterior displacement of the disc due to the spasm of the superior head of the lateral pterygoid muscle, will generally result in symptoms like clicking, popping, pain, and other functional problems in the masticatory system (11).

The five most common etiologic factors that have been the topic of research are the occlusal condition, trauma, emotional stress, deep pain input, and parafunctional activity such as bruxism and clenching (2). Functional conditions resulting from occlusal interferences are found in clinical practice usually and regularly. In orthodontic practice, unilateral crossbite conditions may cause a functional one-sided shift, which if not treated on time might be, but not necessarily cause a TMJ problem (12).

Case Presentation

A 36-year-old female patient came to the University Dental Clinique "St. Pantelejmon", Skopje at the Department of Orthodontics, complaining of pain in her left side around the TMJ joint region. The pain was increasing on the mouth opening. Also, the patient reported that she had already consulted several doctors before coming to our department and got an explanation that the condition was not curable and was suggested medication treatment only, which reportedly did not help. Medical history revealed prior orthodontic treatment, and the same was performed 2-years before for unilateral crossbite treatment. Also, she reported existing bad habits like holding the phone with her shoulder while speaking and chewing gum frequently.

Clinical diagnostics

On palpation, a painful sensation was present in the left masseter muscle region approximately near the TMJ, and a painful sensation while palpating the left TMJ region. A painful sensation on the mouth opening was present, also the pain was present during mastication. Protrusive and lateral movements were painful as well. A click was present on the left side on opening. The questionnaire given to the patient gave a result of 3 points which indicated a problem with TMJ (2, 4). On clinical

examination, the mouth opening was 30 mm (Figure 2 A), which also referred to a problem with TMJ. The maximum mouth opening was 35 mm on assisted opening, and opening with mild pressure on the mandible was 40 mm and was painful. It is essential to emphasize that a slight deviation to the right was present during mouth opening. Lateral excursions noted a symmetrical pattern on both sides and were normal, 10 mm, on both sides (Figure 2 D, E). The amount of protrusion was 6 mm (Figure 2 C).

Dental status

Clinical examination and anamnesis of the patient revealed that the patient had undergone orthodontic treatment two years before. The upper alveolar arch (Figure 1 A, B, C) presented sufficient alignment on both sides. She reported that before starting with the orthodontic treatment she had a unilateral crossbite. Even though the crossbite was solved back then, it was obvious that the result was obtained only by dental inclination and the palatal vault remained high and narrow. The patient also reported the same painful sensations 2 years before starting with the orthodontic treatment but was given an explanation that after the treatment the pain would diminish. Clinical examination and panoramic radiographs did not reveal any pathology of the teeth, nor prosthodontic restoration was present. The overjet was 3 mm, and the overbite was measured at 2 mm. Panoramic radiography (Figure 1 D) did not reveal any serious joint pathology and since we were not able to make diagnostic conclusions only by panoramic radiography, we suggested a cone beam computed tomography (CBCT) of TMJ, which unfortunately, due to technical issues, was not performed. According to the overall functional, clinical examination, and available radiographic data, the preliminary diagnosis indicated intracapsular TMJ disorder which included disk displacement with reduction, TMJ arthralgia, and masseter myalgia. Regarding the patient's report of unsuccessful pain management in her previous medical history and diagnostic findings, a decision was made to administer stabilization occlusal splint therapy in combination with counseling and avoiding parafunctional activities, physiotherapy which would include wet moist wraps and self-massage of the masseter muscle, as well as exercises for joint distraction.

Treatment plan

The primary goal, of crucial and utmost importance, was the resolution of the pain problem. Treatment included stabilization splint fabrication under the guidance of Aqualizer™. The Aqualizer™ is a therapeutic and diagnostic tool for TMJ disorders. The Aqualizer™ was placed in the patient's mouth for 30 minutes while sitting on the dental chair. As a diagnostic tool, this phase also

exhibited problems with TMJ because while wearing it for a short amount of time, the patient reported mild pain relief (13, 14, 15).

The Aqualizer™ is primarily used as a prefabricated splint, for relieving acute TMD symptoms (16, 17). However, the always preferable solution is an individually fabricated splint of hard acrylic material. The splint was finished and placed on the lower arch, with mild impressions on the occlusal surface of the upper teeth. In addition to occlusal splint, the treatment protocol included self-administered exercises and passive muscle stretching which included exercises to open on a straight opening pathway while standing in front of the mirror. Also, exercises to open and close the mouth while keeping the tongue up on the palate. The stabilization splint was worn during the night.

As already noted, the patient mentioned bad habits like holding the phone on her shoulder while talking, and frequent chewing gums during the day, so we suggested immediate habit elimination. Also, we suggested very careful opening movements, especially to avoid sudden and excessive opening of the mouth.

Follow-up appointments were on the 1st, 2nd, and 3rd month, and after six months of wearing the splint. On the first appointment, the patient reported pain diminishing, but the opening was still not favorable. On the 2nd visit improvement in the symptoms was noted and after 6 months of wearing the splint, the amount of opening improved, from 30 mm to 45 mm, (Figure 2 B), and also correction of the deviation was noted.



Figure 1

- A. The intraoral photography shows a matching of the upper and lower interincisive line
- B. Class I canine relation and molar relation - left side
- C. Class I canine and molar relation - right side
- D. OPG did not reveal any severe pathology of the joints

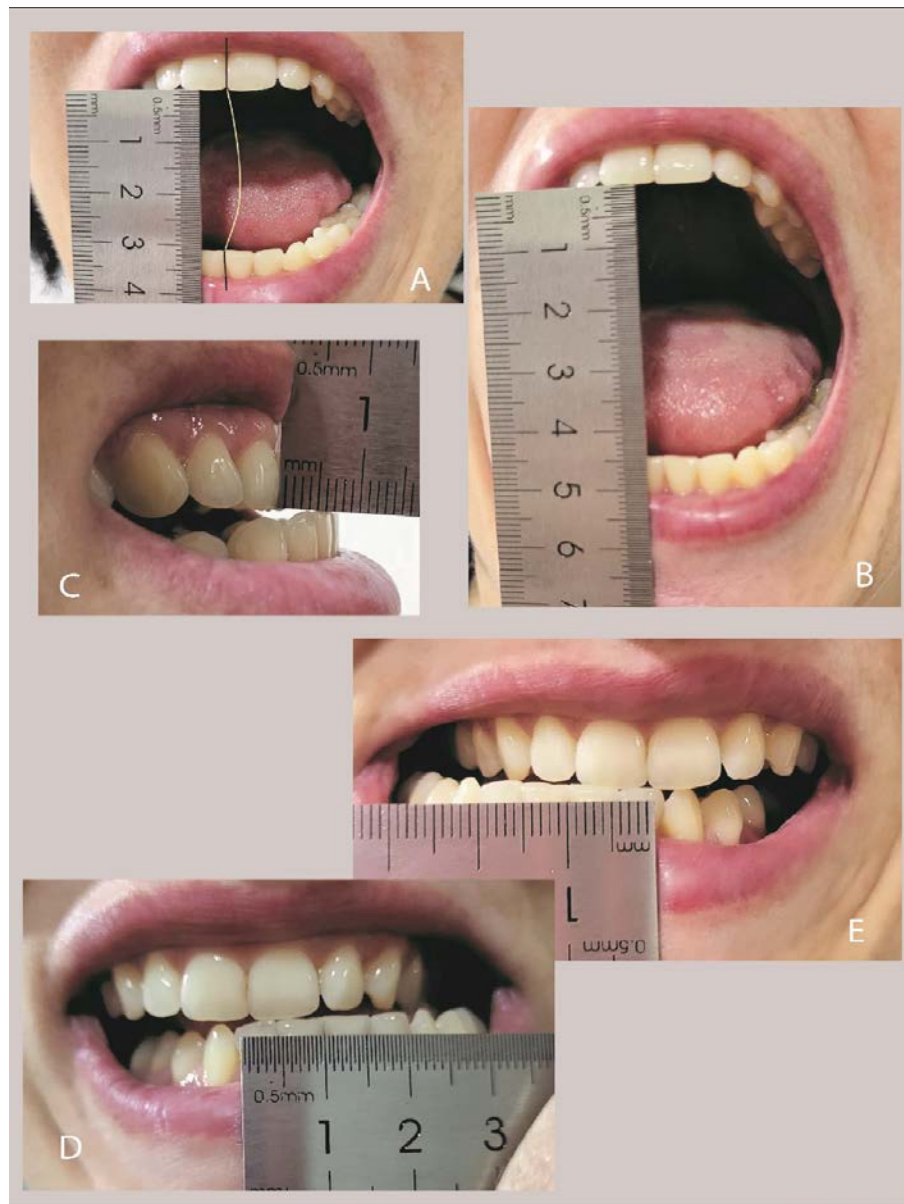


Figure 2

- A. Opening pattern with a slight deviation to the right before splint therapy measured 30mm,
 B. Opening pattern after 6 months of treatment with the splint measured 45 mm,
 C. Protrusive movement before the treatment and after the treatment remained the same at 6mm,
 D. Lateral movement to the left before treatment was 10 mm and remained the same after treatment,
 E. Lateral movement to the right was 10mm and remained the same after treatment

Discussion

Functional irregularities of the temporomandibular joints are undoubtedly the chief patient problem detected during examination for masticatory dysfunction. The cause for this is due to the high incidence of signs, and not necessarily symptoms (2, 3, 10). The focus of such an evaluation is to determine whether the patient has a TMD or not. Many of the signs such as joint sounds or deviated opening are not painful, and therefore the patient may not pursue treatment. These TMJ disorders generally fall into two broad

categories: Internal derangements and inflammatory joint disorders (2, 10, 11).

The mandibular condyle displays a different morphological variation in different groups and individuals due to developmental variations and constant condylar remodeling. The articular eminence inclination also varies in individuals, which determines the movement and degree of rotation of condyles in the glenoid fossa. The radiographic joint space is a radiolucent area between the mandibular condyle and the temporal bone (18). The condylar position is determined by this relative dimension of the radiographic joint

spaces between the glenoid fossa and the mandibular condyle (8, 18).

Clinicians should be vigilant in diagnosing TMD in patients who present with pain located in the TMJ area. A multidisciplinary approach is successful for the management of TMD. Initial treatment goals should focus on resolving pain and dysfunction. Occlusal splints are used to alleviate or prevent degenerative forces placed on the TMJ, articular disk, and dentition (5, 8, 9).

We presented an adult patient with severe pain in her left TMJ region. The patient did not report trauma in the TMJ region. She reported a unilateral crossbite problem which had been orthodontically treated 2 years before. The reason this woman had TMD problems was pretty much related to the unstable occlusion in this case. She also reported pain in the TMJ region before orthodontic treatment but was told that after the treatment she would feel pain relief. Unilateral crossbite might, but not necessarily be the reason for TMD. Usually, the problem that arises in unilateral crossbite cases is with the muscles that

need to be rehabilitated first for the prevention of condylar asymmetry (19).

Conclusion

We reported a 36-year-old female patient with TMJ problems with a 6-month follow-up. Based on the case of the presented patient, it can be concluded that TMJ disorders can be treated with conservative and occlusal splint therapy. The clinician aims to identify the symptoms, relieve the pain to help the patient have a normal life, and cease the progression of the TMJ problems. According to the case progression, it was concluded that conservative treatment in combination with counseling, exercises in combination with occlusal splint therapy, should be the first-choice therapy for TMD, due to their low risk of side effects. Regarding using occlusal splint therapy as a treatment modality, it should be highlighted that we can use it but not abuse it. However, further research should be exhibited for more accurate conclusions to be established

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**TRIDESETŠESTOGODIŠNJA BOLESNICA TRETIRANA
OKLUZALNIM SPLINTOM: PRIKAZ SLUČAJA**

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Ovaj članak opisuje slučaj tridesetšestogodišnje bolesnice koja je zatražila pomoć zbog dugotrajnog bola u predelu temporomandibularnog zgloba (TMZ). Kliničkim pregledom otkriven je bol na levoj strani u predelu TMZ i bol pri otvaranju, žvakanju i palpaciji; primećena je devijacija udesno, kao i klikćući zvuk pri otvaranju.

Palpacijom je otkriven bolni osećaj na levom mastikatornom mišiću. Dijagnoza je bila utvrđena na osnovu funkcionalne analize, kliničkog pregleda i anamnestičkih podataka. Doneta je odluka da se tretman započne ordiniranjem stabilizacionog okluzalnog splinta u kombinaciji sa vežbama, fizioterapijom i analgetičkim tretmanom za ublažavanje simptoma, po potrebi.

Ovaj protokol imao je za cilj da ublaži bolnu simptomatologiju i omogući opuštanje mastikatornih mišića u određenoj meri, pre ortodontskog lečenja, kako bi se dobila solidna osnova za stabilni ishod ortodontskog lečenja.

Na osnovu slučaja prikazanog bolesnika može se zaključiti da se poremećaji temporomandibularnog zgloba (TMZ) mogu lečiti terapijom okluzalnim splintom u kombinaciji sa vežbama i fizioterapijom. *Acta Medica Medianae 2023;62(3):81-87.*

Ključne reči: temporomandibularni zglob, temporomandibularni poremećaji, stabilizacioni splint, ortodoncija

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RISK OF COVID-19 INFECTION IN PATIENTS WITH MULTIPLE SCLEROSIS TREATED WITH OCRELIZUMAB – A SINGLE CENTER EXPERIENCE

Stefan Todorović

Ocrelizumab is a disease-modifying therapy (DMT) for active relapsing and early primary progressive multiple sclerosis (MS). During the COVID-19 pandemic, it was speculated that ocrelizumab might increase the risk of COVID-19 in patients with MS. The aim was to assess the risk of COVID-19 infection in MS patients treated with ocrelizumab. Our study included patients who met revised McDonald criteria and who were treated with ocrelizumab at the University Clinical Centre Niš. The diagnosis of COVID-19 was made by positive PCR (polymerase chain reaction) or antigen test. The severity of the disease was estimated based on the Australian guidelines for the clinical care of people with COVID-19. Out of 103 patients treated with ocrelizumab, 33 (32%) were found to be infected with COVID-19. Out of these, there were 10 (30.3 %) COVID-positive men and 23 (69.7%) women. The average age of affected patients was 43.9 ± 9.1 . Most of them had mild clinical presentation of COVID-19 infection (81.8%), 12.1% had moderate clinical presentation, 3% with severe clinical manifestation and one patient died. There was no significant impact of ocrelizumab administration in patients with MS on the increased risk of COVID-19 infection and the development of severe clinical manifestations of the disease. In our cohort, patients with moderate and severe COVID-19 disease were usually older than 50 (66.7%), although there were not many of those patients. *Acta Medica Medianae 2023;62(3):88-94.*

Key words: COVID-19, multiple sclerosis, ocrelizumab

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Introduction

Multiple sclerosis is a chronic inflammatory autoimmune demyelinating disease of the central nervous system and is one of the leading causes of disability in the world. MS predominantly affects females aged between 20 and 50 (1). This inflammation can affect different parts of the brain and spinal cord and it can cause a wide range of neurological symptoms and signs. There are four recognized patterns of MS: 1) relapsing-remitting (RRMS), 2) secondary progressive (SPMS), 3)

primary progressive (PPMS), and 4) progressive relapsing (PRMS). Relapsing-remitting disease is the most common form (70–80%) (2).

Ocrelizumab is an effective, humanized anti-CD 20, B cell-depleting, monoclonal antibody approved for treating relapse remitting multiple sclerosis (RRMS) and primary progressive multiple sclerosis (PPMS). It achieves its effect by binding to the CD20 receptor on B lymphocytes and thus leads to their depletion, with spontaneous recovery afterward (3).

SARS-CoV-2 virus was first identified in Wuhan, China (4). The disease was first discovered in our country in March 2020, and restrictive measures were put in place by the Serbian government. This period brought a lot of difficulties in the organization of medical systems, including prescribing therapies for patients with MS. The period of the COVID-19 pandemic overlapped with the commencement of the treatment with ocrelizumab at the University Clinical Center Niš.

It is estimated that patients with MS could be at increased risk of severe COVID-19 infection. Patients with comorbidities, including those on immunosuppressive therapy might be more susceptible to COVID-19 infection (5).

Materials and methods

We conducted a cross-sectional cohort study in the period from March 2020 to November 2022. Our study included patients selected according to the revised McDonald criteria and who were treated with ocrelizumab at the University Clinical Centre Niš.

All patients were receiving disease-modifying therapy (ocrelizumab) at the time of assessments.

We analyzed demographic data (sex and age), clinical patterns of MS (PPMS and RRMS), incidence and severity of the clinical picture of COVID-19 infection, and vaccination status of patients.

COVID-19 infection was confirmed by positive PCR or antigen test.

The severity of the disease was estimated based on the Australian guidelines for the clinical care of people with COVID-19:

- mild illness: no symptoms, or mild upper respiratory tract symptoms, or cough, new myalgia or asthenia without new shortness of breath or a reduction in oxygen saturation;

- moderate illness: prostration, severe asthenia, fever > 38 °C or persistent cough, clinical or radiological signs of lung involvement, no clinical or laboratory indicators of clinical severity or respiratory impairment; and

- severe illness: respiratory rate ≥ 30 breaths/min, oxygen saturation $\leq 92\%$ at a rest

state arterial partial pressure of oxygen (PaO_2)/inspired oxygen fraction (FiO_2) ≤ 300 (6).

All collected data were analyzed retrospectively.

Results

Out of 103 patient who were treated with ocrelizumab, 80 (77.7 %) patients had RRMS, and 23 (22.3%) patients had PPMS (Figure 1).

The investigated patients were aged between 23 and 64 (43.9 ± 9.1). The most frequent age group included individuals in the fifth decade, from 41 to 50 years of age.

Fifty-seven patients (55.3%) were vaccinated, while the other 46 patients (44.7%) were not vaccinated.

COVID-19 infection was confirmed in 33 patients. Out of these, there were 10 (30.3 %) positive men and 23 (69.7%) women. Among the vaccinated patients, there were 23 patients (40.3%) who were confirmed to have COVID-19 infection, 15 of them being women (65.2%) and 8 men (34.8%).

Among the unvaccinated patients, COVID-19 was confirmed in 8 women (80%) and 2 men (20%).

The main clinical and social epidemiological characteristics, as well as the status of the COVID-19 infection are presented in Table 1.

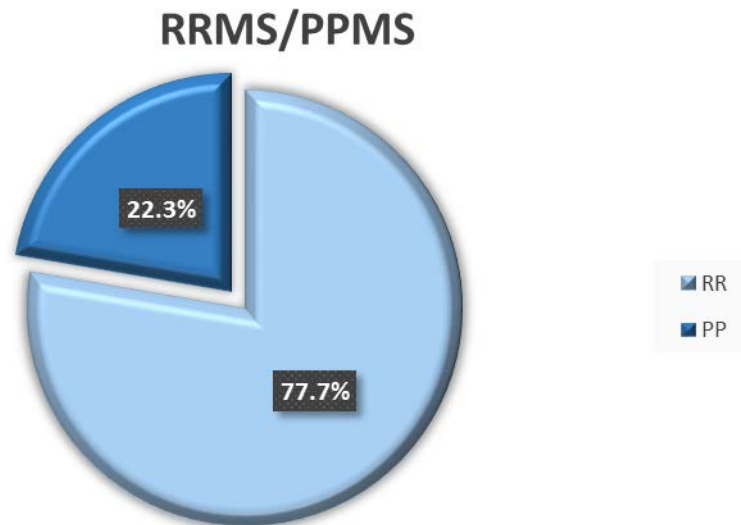


Figure 1. Number of patients with RRMS and PPMS treated with ocrelizumab

Table 1. The main characteristics of total patients treated with ocrelizumab

Characteristics	N° (%)
Gender	
Male	28 (27.2%)
Female	75 (72.8%)
Years	
23–30	8 (7.8%)
31–40	30 (29.1%)
41–50	43 (41.7%)
51–60	20 (19.4%)
>60	2 (1.9%)
Age, years, mean (SD)	43.9±9.1
The course of the disease	
RRMS	80 (77.7%)
PPMS	23 (22.3%)
COVID-19	33 (32%)
Vaccinated	57 (55.3%)
Confirmed COVID infection	23 (40.3%)
Male	8 (34.8%)
Female	15 (65.2%)
Unvaccinated	46 (44.7%)
Confirmed COVID infection	10 (21.7%)
Male	2 (20%)
Female	8 (80%)

We further compared patients with COVID-19 according to the severity of the disease. Most of them had mild clinical manifestation of COVID-19 infection (81.8%), 12.1% had a moderate clinical manifestation, and 3% had a severe clinical manifestation. One patient who had PPMS died (Figure 2).

Among those aged between 41 and 50, mild clinical manifestation was recorded in the largest number of COVID positive patients (11), while moderate and severe clinical manifestation was most common in middle-aged and elderly patients.

We had 2 patients with moderate clinical presentation in the third decade, 2 patients in the sixth decade, while one patient in the sixth decade had severe clinical presentation and one died. In our cohort, patients with moderate and severe COVID-19 disease were usually older than 50 (66.7%), although there were not many of those patients.

The main comparative characteristics of our patients with COVID-19 in relation to the severity of the disease are presented in Table 2.

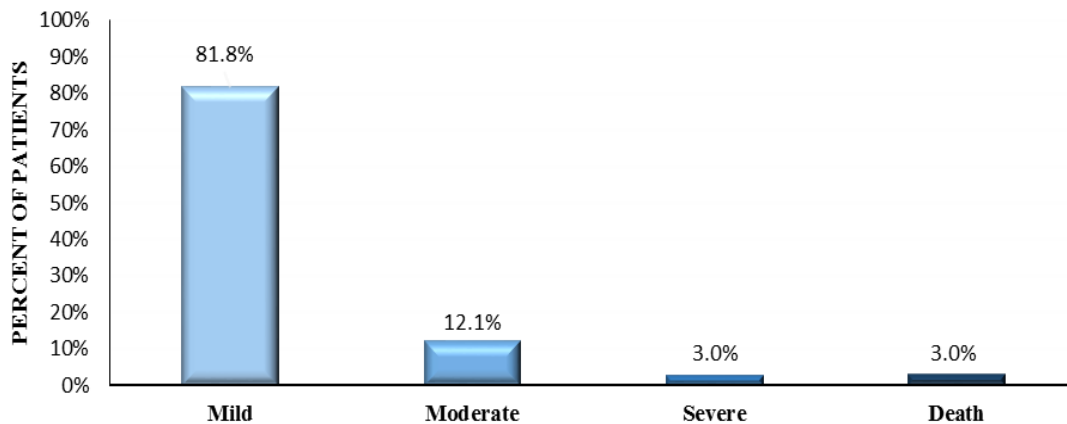


Figure 2. The severity of the COVID-19 disease

Table 2. Comparative characteristics of the patients with COVID-19 in relation to the severity of disease

Characteristics		Mild N° 27 (81.8%)	Moderate N° 4 (12.1%)	Severe N° 1 (3%)	Death N° 1 (3%)
Years	23–30	2 (7.4%)	0	0	0
	31–40	6 (22.2%)	2 (50%)	0	0
	41–50	11 (40.7%)	0	0	0
	51–60	6 (22.2%)	2 (50%)	1 (100%)	1 (100%)
	>60	2 (7.4%)	0	0	0

Discussion

There are now numerous disease-modifying therapies (DMT) that can slow down the progression of the disease successfully. These drugs have a different mechanism of action. Some of them act on B cells, some act on T cells, and some affect immunomodulation (7). Immunity to COVID-19 includes both cell immune responses. For this reason, the use of DMT during COVID-19 was controversial (8).

The beginning of the COVID-19 pandemic was a difficult period for organizing the administration of DMTs. Most of the patients were taking therapy in continuity. International data indicates that only 5% of the patients had significant reasons for therapy delays during the pandemic (9). All our patients started taking the therapy without delay, and the therapy was neither discontinued nor switched (except for one patient who died).

Current evidence does not indicate increased COVID-19 infection in MS patients (10). Prior to COVID-19 infection, it was considered that

patients with MS have 2–4 more risk of being hospitalized with a serious infection (11). Some studies showed a higher risk of infection with COVID-19 if patients were female, younger and have other comorbidities. A possible additional reason for this was the greater number of social interactions (12). On the other hand, some other studies did not find any significant risk of infection with COVID-19 between MS and the general population (13, 14).

Two large cohort studies in China and Italy also did not show an increased impact of DMT use on the incidence of COVID-19 (15, 16).

Anti-CD20 monoclonal antibodies are widely used in MS therapy. Those drugs can increase the risk of contracting serious infections compared to other DMTs (17). Ocrelizumab is one of the most effective DMTs. According to many studies, it was shown to have significantly reduced relapses in RRMS patients and slowed down disease progression in PPMS patients. Considering that ocrelizumab acts on B cells, and that cells play an important role in the immune response to SARS-CoV-2 virus, it was thought that its use would increase the frequency of COVID-19. However,

this was not confirmed by many studies and it was proven that there was no association between the duration of ocrelizumab exposure and rates of COVID-19 infections (18). Taking into account risk factors for severe COVID-19 infection (older age, disability status, comorbidities), patients treated with ocrelizumab were considered to be at a high risk of being infected by COVID-19. Patients treated with ocrelizumab were compared with other MS cohorts, and it was noticed that less than one-fourth of patients developed moderate infection. This study delayed the suspicion that depleting drugs increased the risk for severe COVID-19 infection. In addition, it showed that the mortality rate was not higher in patients treated with ocrelizumab. Fatal cases occurred only in patients with multiple risk factors (19, 20, 21).

A group of authors in Scotland estimated the risk of infection with COVID-19 in patients with inflammatory rheumatic diseases (IRD) treated with immunosuppressive therapy. The study included 433 patients treated with immunosuppressive therapy and infected with COVID-19. They concluded that the risk of COVID-19 infection in these patients is higher than in the general population. Some drugs (methotrexate, hydroxychloroquine and TNF inhibitors) had a lower risk of COVID-19 disease, and the highest risk was associated with prednisolone (22).

Accordingly, several studies have shown that COVID-19 infection is more common in women, as well as in patients with a longer duration of pulse corticosteroid therapy (23).

The availability of recent data indicates that the reduction of the humoral response is not associated with a high risk of COVID-19, although both cellular and humoral immune responses play an important role in the prevention of virus infection. This is supported by the report of two patients with X-linked agammaglobulinemia, who had COVID-19 pneumonia, but fully recovered despite the absence of B cells in peripheral blood (24).

Conclusion

In conclusion, bearing in mind that ocrelizumab is an immunosuppressive therapy, our study showed that ocrelizumab administration had no significant impact on the increased risk of COVID-19 infection and the development of severe clinical manifestations of the disease. During the pandemic, managing MS patients should be done optimally, and treatment decisions should be made according to an individual benefit-risk profile.

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RIZIK OD COVID-19 INFEKCIJE KOD BOLESNIKA SA MULTIPLIM SKLEROZOM LEČENIH OKRELIZUMABOM – ISKUSTVO JEDNOG CENTRA

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Okrelizumab je terapija koja modifikuje prirodni tok bolesti u multiploj sklerozi (MS) i koristi se za lečenje aktivne relapsno remitentne i/ili primarno progresivne forme bolesti. Tokom pandemije virusa COVID-19 spekulisalo se da bi okrelizumab mogao povećati rizik od infekcije izazvane virusom COVID-19 kod bolesnika sa MS. Cilj rada je procena rizika od COVID-19 infekcije kod obolelih od multiple skleroze i lečenih okrelizumabom. Našom studijom obuhvaćeni su bolesnici koji su ispunjavali revidirane McDonald kriterijume, a koji su lečeni okrelizumabom u Univerzitetskom kliničkom centru Niš. Dijagnoza COVID-19 infekcije postavljena je pozitivnim PCR (lančanom reakcijom polimeraze) ili antigenskim testom. Težina COVID-19 infekcije procenjena je na osnovu australijskih smernica za kliničku negu osoba zaraženih COVID-19 virusom. Od 103 pacijenta lečena okrelizumabom, kod njih 33 utvrđeno je da su zaraženi COVID-19. Od toga je bilo 10 (30,3 %) pozitivnih muškaraca i 23 (69,7 %) žene. Prosečna starost obolelih bila je 43,9 godina \pm 9,1 godina. Većina njih imala je blagu kliničku sliku COVID-19 infekcije (81,8%); 12,1% imao je umereno tešku kliničku sliku, 3% tešku kliničku manifestaciju, a jedan pacijent je preminuo. Nije bilo značajnog uticaja primene okrelizumaba kod bolesnika sa MS na povećan rizik od infekcije COVID-19 virusom i razvoj teške kliničke manifestacije bolesti. U našoj kohorti, bolesnici sa umerenom i teškom kliničkom slikom COVID-19 infekcije obično su bili stariji od 50 godina (66,7%), mada tih bolesnika nije bilo mnogo. *Acta Medica Medianae* 2023; 62(3):88-94.

Ključne reči: COVID-19, multipla skleroza, okrelizumab

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SURGICAL MANAGEMENT OF CUTANEOUS MELANOMA IN THE ERA OF COVID-19 PANDEMIC: A SINGLE CENTER EXPERIENCE

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Marija Andjelković Apostolović^{2,3}

Timely started surgical treatment represents the gold standard in melanoma therapy and keeps mortality at a low level. The pandemic caused by SARS-CoV-2 has affected the availability of health care both nationally and globally. The aim of this study was to examine the impact of the COVID-19 pandemic on newly operated cutaneous melanoma in patients and to contribute to identifying the impact of the pandemic on the healthcare system in Serbia. We conducted a single institution retrospective study including patients who consecutively underwent surgery for cutaneous melanoma at the Clinic for Plastic and reconstructive Surgery, University Clinical center of Niš, between January 1, 2018 and March 15, 2023. We compared the pre-pandemic (01/Jan/18 - 14/Mar/20) and pandemic (15/Mar/20 - 15/Mar/23) periods by evaluating patient age, sex, body distribution, Breslow thickness, pT staging, mitotic index rate and ulceration status. No differences were observed between age ($p = 0.666$), sex ($p = 0.720$), body distribution ($p = 0.109$), Breslow thickness ($p = 0.172$), pT staging ($p = 0.274$), mitotic index rate ($p = 0.257$), and ulceration status ($p = 0.787$) in the two examined groups. Statistically significant differences were observed in the melanoma subtype ($\chi^2 = 9.241$; $p = 0.026$). Distribution of lentigo maligna in patients diagnosed with melanoma during the pandemic was statistically lower. To date, the diagnostic delay caused by COVID-19 has generally not led to unfavorable characteristics of the primary cutaneous melanoma. Follow-up studies are needed in the coming years to identify the potential impact on stage distribution and long-term survival. *Acta Medica Medianae 2023;62(3):95-102.*

Key words: COVID-19, melanoma, surgical treatment, Breslow thickness, diagnostic delay

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Introduction

Melanoma is one of the most malignant tumors in human population and the most malignant skin tumor. The incidence of melanoma varies around the world and it has been steadily rising in recent decades. Timely started surgical treatment represents the gold standard in melanoma therapy and keeps mortality at a low level (1, 2, 3). Patients with a higher Breslow index and the presence of ulceration at the time of diagnosis have a worse prognosis and a more aggressive form of the disease (4). Melanomas detected in a screening program are usually

thinner than melanomas detected by self-examination (5).

The pandemic caused by SARS-CoV-2 has affected the availability of health care both nationally and globally (6). Elective surgical procedures were not performed during the lockdown while surgical treatment of cancer was postponed (7). Delaying the surgical treatment of melanoma is devastating for the patient and leads to increased health care costs (8).

As a consequence of the delay in screening programs and surgical treatment of melanoma, many studies have indicated a significant decrease in the number of newly diagnosed patients with melanoma (9-11), while on the other side the Breslow thickness was higher (11 - 19). An increase in the number of patients in stage II of the disease and higher was also noted (20). On the other hand, many studies indicate that the COVID-19 pandemic had no effect on melanoma thickness (19, 21-24).

According to our knowledge, there is only one study on the impact of the COVID-19 pandemic on patients with cutaneous melanoma in the Republic of Serbia, which includes only patients operated on at the Clinic for Burns, Plastic

and Reconstructive Surgery of the University Clinical Center of Serbia (25).

Aim

The aim of this study was to examine the impact of the COVID-19 pandemic on newly operated cutaneous melanoma in patients and to contribute to identifying the impact of the pandemic on the healthcare system in Serbia.

Material and methods

We conducted a single institution retrospective study including patients who consecutively underwent surgery for cutaneous melanoma at the Clinic for Plastic and Reconstructive Surgery, University Clinical Center of Niš, between January 1, 2018 and March 15, 2023. The clinic is also a tertiary national referral center for skin cancer patients for the region of southeastern Serbia. Medical history and a clinical records database of the Clinic for Plastic and Reconstructive Surgery were used as the sources of data for this study. Demographic characteristics (gender, age), localization of melanoma and TNM classification according to the American Joint Committee on Cancer (TNM classifications for cutaneous melanoma, eighth edition) were analyzed.

The study procedures were carried out in accordance with the Declaration of Helsinki.

The following statistical parameters are presented by descriptive statistical analysis: arithmetic mean, standard deviation, absolute frequency (N), and structure index (%). The comparison of the frequency of occurrence of individual modalities of attribute features between groups was performed by Pearson χ^2 test. The mean values of the numerical features between the two independent groups of respondents were compared by Student's T-test or Mann-Whitney U-test. Statistical analysis was performed using an Excel program from the Microsoft Office software package and an SPSS program (version 18.0). The threshold for statistical significance was the level of statistical error less than 5% ($p < 0.05$). The results of the statistical analysis are presented in tables and graphs.

Results

A total of 199 patients with cutaneous melanoma were included in the study. During

2018, 65 patients were registered, in 2019 there were 31, 16 in 2020, 30 in 2021, 47 in 2022, and 10 in the first 3 months of 2023 (Figure 1). In the period before the start of the pandemic, 104 cases (52.3%) were registered, while during the pandemic, there were 95 (47.7%). The average number of registered cases per year before the pandemic was 48 ± 24 , while during the pandemic, it was 31 ± 15 . Our sample contained almost equal numbers of males (99) and females (100). The most registered cases were in the age group of 61 to 80 years. The mean age of patients in the pre-pandemic group was 57.5 (SD 15.5), while in the pandemic group, it was 59.5 (SD 14.2). There were no significant differences in sex ($p = 0.720$) and age ($p = 0.666$) of the patients between the pre- and post-COVID groups (Table 1).

In both cohorts, the most common subtype of melanoma was superficial spreading melanoma (SSM), represented by 55.3%, followed by the nodular subtype 31.2%. Other subtypes of melanoma were represented by 13.5%. Statistically significant differences were observed in the melanoma subtype ($\chi^2 = 9.241$; $p = 0.026$). Distribution of lentigo maligna in patients diagnosed with melanoma during the pandemic was statistically lower.

Melanoma was most common on the trunk 49.2%, followed by the head and neck 20.1%, as well as the lower 16.1% and upper extremities 14.6%. There were no significant differences between the cohorts before and during the pandemic regarding the individual body distribution of melanoma and if there was an increase in the number of trunk melanomas and a decrease in the number of head and neck melanomas during the pandemic period ($p = 0.109$).

Melanomas operated on in the two periods did not show a significant difference in Breslow thickness (2.1 vs. 2.7; $p = 0.172$). Also there was no statistically significant difference in Breslow thickness during the examined years ($p = 0.549$) (Table 2). There was no statistically significant change in the mitotic rate in the pandemic cohort ($p = 0.257$), although the mitotic index doubled from 1 to 2. An increase in the number of melanomas with ulceration was also not observed. In both experimental groups, most patients were in the T1 stage of the disease. Also, there was no significant difference in the distribution of patients between stages in the groups ($p = 0.274$) (Figure 2).

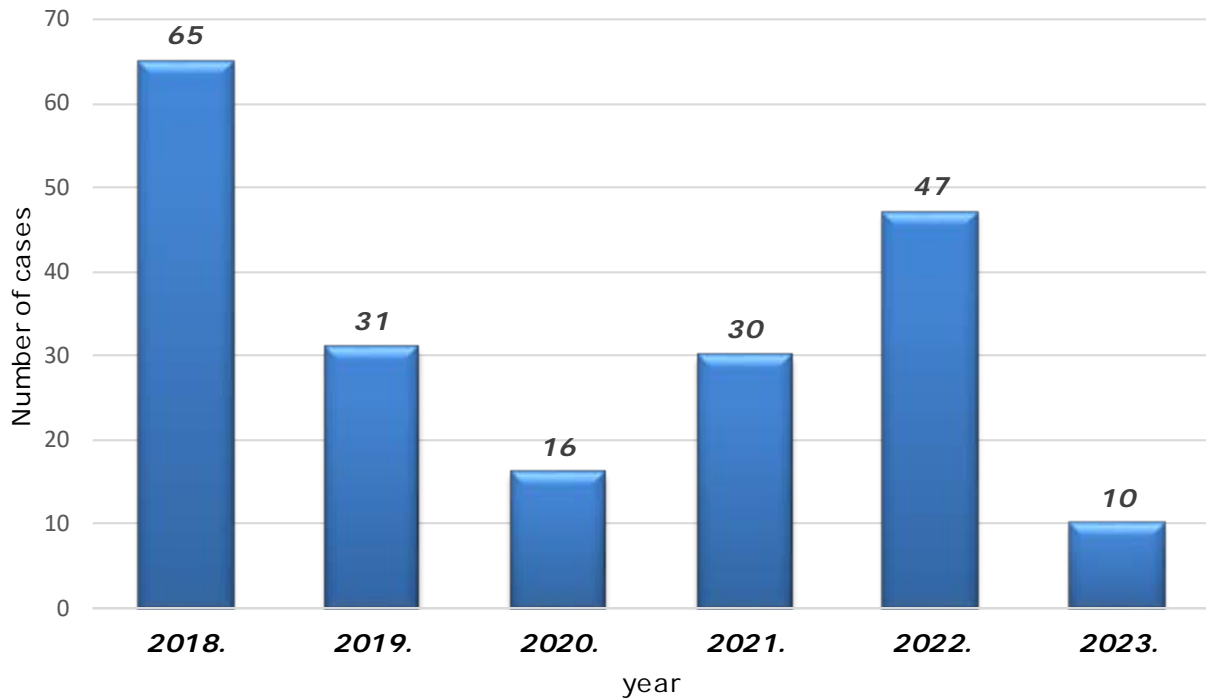


Figure 1. Number of diagnosed cases per year

Table 1. General characteristics of melanoma patients before and after the onset of the COVID-19 pandemic

	before COVID-19 n=104 (52.3%)	COVID-19 n=95 (47.7%)	p-value
Age (mean ± SD)	57.53±15.52	59.52±14.21	0.349 ¹
Age groups			
≤40 years	13(12.5)	9(9.5)	0.666 ²
41–60 years	41(39.4)	37(38.9)	
61–80 years	44(42.3)	46(48.4)	
>80 years	6(5.8)	3(3.2)	
Sex			
Male	53(51.0)	46(48.4)	0.720 ²
Female	51(49.0)	49(51.6)	
Body distribution			
Head and Neck	27(26.0)	13(13.7)	0.109 ²
Trunk	44(42.3)	54(56.9)	
Upper extremities	15(14.4)	14(14.7)	
Lower extremities	18(17.3)	14(14.7)	
Melanoma subtype			
Superficial spreading	61(58.7)	49(51.6)	0.026 ²
Lentigo maligna	13(12.5)	4(4.2)	
Nodular	28(26.9)	35(36.8)	
Others	2(1.9)	7(7.4)	
Breslow thickness (median, 25th–75th percentile)	0.75 (0.40-2.69)	1.0 (0.40-3.70)	0.172 ³
Breslow thickness			
<1 mm	72(69.2)	53(55.8)	0.132 ²
1.01–2 mm	6(5.8)	13(13.7)	

2.01–4 mm	12(11.5)	11(11.6)	
>4 mm	14(13.5)	18(18.9)	
pT staging			
In situ	23(22.1)	20(21.1)	0.274 ²
T1	46(44.2)	30(31.6)	
T2	6(5.8)	11(11.6)	
T3	13(12.5)	14(14.7)	
T4	16(15.4)	20(21.1)	
Clark level			
I	23(22.1)	20(21.1)	0.682 ²
II	45(43.3)	33(34.7)	
III	18(17.3)	19(20.0)	
IV	15(14.4)	19(20.0)	
V	3(2.9)	4(4.2)	
Mitotic index rate (median, 25th–75th percentile)	1.0 (1.0-3.0)	2.0 (0.0-4.0)	0.257 ³
Ulcerations			
Yes	31(29.8)	30(31.6)	0.787 ²
No	73(70.2)	65(68.4)	

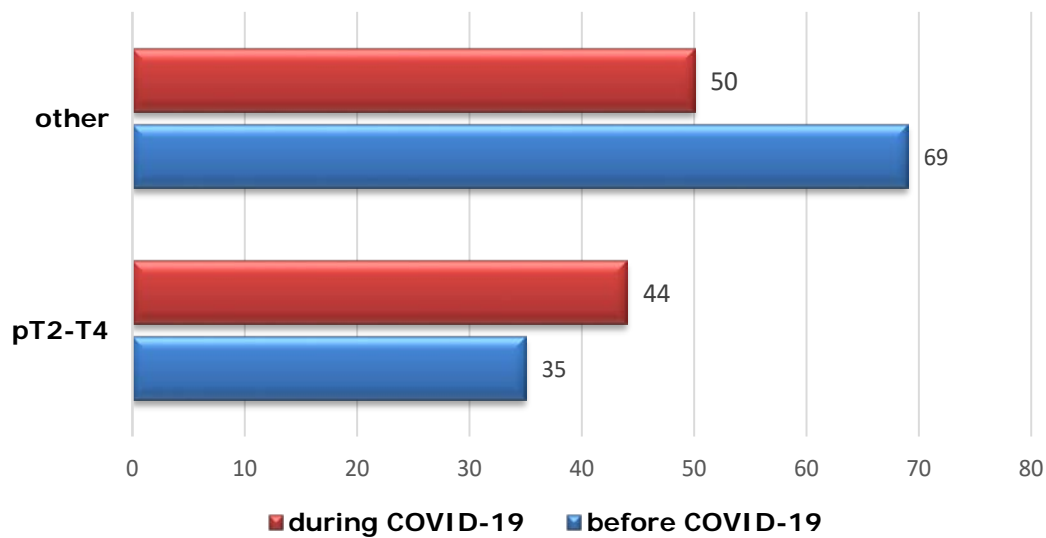


Figure 2. Combined pT staging of melanoma patients before and during the pandemic

Table 2. Breslow thickness during years

Year	mean	SD	p-value
2018.	2.32	3.97	
2019	2.23	4.04	
2020.	3.44	4.80	
2021	3.43	4.38	
2022.	1.89	2.70	
2023.	1.80	1.55	0.549

Discussion

The Coronavirus, formally known as SARS-Cov-2, detected in Wuhan (China) at the end of December 2019, affected the availability of health care around the world (26). Many studies across different countries have shown a decrease in the number of patients with skin tumors during the pandemic, as the research has shown in Serbia also (25). In some cases, this drop was up to 60% (12, 20, 27). The biggest drop in the number of newly diagnosed patients with skin tumors was in 2020, which corresponds with our results (28). The biggest reduction in the number of newly diagnosed cases was in patients over 60 years old, which is also the case in our study. Our results are partially in line with the results of other authors who indicate the impact of covid-19 on the overall decline in the number of melanoma diagnoses. While the decline in the number of melanoma diagnoses in other authors is present in all years since the beginning of the pandemic, in our study this decline is present only in 2020. (12, 15, 29). Studies have shown that early detection and adequate surgical treatment of thin lesions decrease the mortality rate in the short term. In the long term however, prevention could play a huge role (30).

The results of our study showed an increase in Breslow thickness with a higher mitotic index during the pandemic, which is in agreement with the results of other studies (11-19). In relation to this, we observed an increase in the number of patients in the pT-2 stage and above, which was also discussed by Shannon et al. who observed an

increased proportion in pT3-4 stage in patients operated on during the pandemic (13). A three month diagnostic delay according to Tejera et al. results in a significant drop off in the pT-1 stage (40% vs. 27%) and a doubled number of pT-4 cases (16% vs. 30%) as well as decreased five- and ten-year survival rate (31).

Because during the pandemic there is an evident delay in diagnosis and surgical treatment (32, 33), there are still no results on how this will affect five- and ten-year survival rate and how to improve screening programs, due to the lack of data and appropriate guidelines (34, 35).

The strength of our study is in the fact that it is based on the data of one of the largest institutions for the treatment of skin tumors in Serbia. Unfortunately, our study does not include patients with enlarged lymph nodes or distant metastases during this period, which represents a limitation of this study but leaves us space for further research. The sample of this study may contribute to further understanding of the impact of the Covid-19 pandemic and to promote the guidelines on how to improve the screening program and surgical treatment in these times.

Conclusion

To date, the diagnostic delay caused by COVID-19 has generally not led to unfavorable characteristics of the primary cutaneous melanoma. Follow-up studies are needed in the coming years to identify the potential impact on stage distribution and long-term survival.

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HIRURŠKO LEČENJE MELANOMA KOŽE U ERI PANDEMIJE KOVIDA 19: ISKUSTVO JEDNOG CENTRA

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Pravovremeno započeto hirurško lečenje predstavlja zlatni standard u terapiji melanoma i održava mortalitet na niskom nivou. Pandemija koju je izazvao virus kovid 19 uticala je na dostupnost zdravstvene zaštite, kako na nacionalnom, tako i na globalnom nivou. Cilj ove studije je da ispita uticaj pandemije kovid 19 na novooperisane bolesnike sa melanomom kože i da doprinese identifikovanju uticaja pandemije na zdravstveni sistem u Srbiji. Sproveli smo retrospektivnu studiju koja je uključivala bolesnike sa melanomom kože hirurški lečene na Klinici za plastičnu i rekonstruktivnu hirurgiju Univerzitetskog kliničkog centra Niš u periodu od 1. januara 2018. do 15. marta 2023. godine. Upoređivali smo periode pre pandemije (01/01/2018 – 14/03/2023) i tokom pandemije (15/03/2020 – 15/03/2023) procenom starosti bolesnika, pola, zastupljenosti melanoma na određenim delovima tela, debljine po Breslovu, pT stadijuma, stope mitotičkog indeksa, podtipa melanoma i prisustva ulceracije. Nisu uočene razlike između starosti ($p = 0,666$), pola ($p = 0,720$), zastupljenosti melanoma na određenim delovima tela ($p = 0,109$), debljini po Breslovu ($p = 0,172$), pT stadijumu ($p = 0,274$), stopi mitotičkog indeksa ($p = 0,257$) i prisustva ulceracije ($p = 0,787$) u dvema ispitivanim grupama. Statistički značajne razlike uočene su kod podtipova melanoma ($\chi^2 = 9,241$; $p = 0,026$). Distribucija lentigo maligna kod bolesnika sa dijagnozom melanoma tokom pandemije bila je statistički niža. Do danas, kašnjenje u postavljanju dijagnoze melanoma kože, usled pademije izazvane kovidom 19, generalno nije dovelo do nepovoljnih karakteristika primarnog kožnog melanoma. Potrebno je sprovesti dodatne studije u budućnosti kako bi se identifikovali potencijalni uticaj na distribuciju po fazama i dugoročna stopa preživljavanja. *Acta Medica Medianae* 2023;62(3):95-102.

Ključne reči: kovid 19, melanom, hirurško lečenje, debljina po Breslovu, dijagnostičko kašnjenje

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IMMUNOGLOBULIN G4 (IGG4) RELATED DISEASE AND GRANULOMATOSIS WITH POLYANGIITIS (GPA) IN CHILDHOOD: A CASE REPORT OF NEW OVERLAP SYNDROME

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Granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis (WG) is one of major variants of anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) and one of the most common vasculitis in children affecting small- to medium-sized blood vessels. Immunoglobulin G4 (IgG4)-related disease is characterised by inflammatory pseudotumours with elevated serum IgG4 concentrations. Orbital pseudotumour in children can be initial clinical presentation of both IgG4 related disease and GPA. Herein, we describe a young girl with a new overlap syndrome, initially presented with orbital pseudotumour. Furthermore, diagnostic challenges and applied therapy are described. *Acta Medica Medianae* 2023;62(3):103-108.

Key words: granulomatosis with polyangiitis (GPA), immunoglobulin G4 (IgG4)-related disease, orbital pseudotumour

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Introduction

Granulomatosis with polyangiitis (GPA), is one of major variants of anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV). It is a heterogeneous group of multisystem disorders characterised by pauci-immune necrotising vasculitis, that affects small- to medium-sized blood vessels, together with neutrophil or eosinophil-enriched granulomatous inflammation. AAV is related with the presence of circulating ANCA. GPA, formerly known as Wegener's granulomatosis (WG), is typically associated with granulomatous inflammation of the respiratory tract, necrotising glomerulonephritis and the presence of ANCA, usually directed against myeloperoxidase (MPO) (1, 2). Renal involvement plays a central role in the diagnosis, classification, prognosis and treatment of patients with this

disease. Ocular manifestations are seen in up to 60% of adult GPA patients and can occur either in the generalised or in the limited form of the disease. Although rare, GPA is the most common AAV in children, with a strong female predominance (61 to 80%) (3, 4). As opposed to adults with this disease, paediatric patients have ocular manifestations more commonly-up to 43% children with multisystem GPA, as noted in the largest paediatric study to date, conducted on 183 Canadian paediatric patients with GPA (5).

Immunoglobulin G4 (IgG4)-related disease is characterised by elevated serum IgG4 concentration together with multiorgan involvement and inflammatory pseudotumours with dense lymphoplasmacytic infiltrates rich in IgG4-positive plasma cells (6, 7). It has not been recognized as a systemic condition since 20 years ago, when Kiyosawa et al. published several reports of the systemic background of this process (8, 9, 10). Many medical conditions that may include single organ systems can be part of the spectrum of IgG4-related diseases (8). IgG4-related disease with orbital pseudotumour, has been reported only in adult systemic conditions until recently. In the last few years, it is considered to be more common in paediatric population than anticipated previously. According to a recent systematic literature review, ocular manifestations are reaching 44% among children with this disease (11). Herein, we describe a young girl with orbital pseudotumour as an initial

clinical presentation of IgG4 related disease and GPA.

Case report

This previously healthy girl started to have recurrent fever episodes at the age of 3. Five years later a tonsillectomy was performed due to recurrent rhinitis and sore throat. Few days after that, she showed up with ptosis of the right eyelid, together with swelling and redness (Figure 1 A and B). Magnetic resonance imaging (MRI) was performed and it confirmed an orbital pseudotumour. Thus, steroid therapy was initiated (20 mg/day prednisolone course with slow tapering), showing steroid dependent clinical response. However, in the next month she had deterioration presented with fever episodes for two months and septic appearance, nasal obstruction with mucopurulent secretion and recurrent nosebleeds. Triple antibiotics (ceftriaxone, vancomycin, ciprofloxacin) and antimycotics were administered due to persistently elevated ESR and CRP levels. Meanwhile serology tests for infection were negative and infectious diseases were ruled out. Due to worsening of ocular symptoms, orbital mass extirpation was performed and biopsy has shown a granulomatous lesion and vasculitic infiltration of the blood vessels, with the lack of eosinophilia in both blood and biopsy samples. In addition, a fibrosing chronic inflammatory process rich in plasma cells was observed, that appeared to have considerable IgG4 immuno-staining. Laboratory examination revealed markedly elevated serum IgG4 concentration (1400 mg/dl, cut off 135 mg/dl). This led to the diagnosis of IgG4 related disease, according to ACR/EULAR classification criteria for IgG4-related disease (12). After the pseudotumour extirpation, she was treated with vitamin supplements only and did well for one year. Unfortunately, the girl met a recurrence of the orbital mass in the same eye, along with left ankle arthritis and some granulomatous skin lesions with central induration and spider nevus (Figure 1 C and D). Thus she was referred to paediatric rheumatology clinic for the first time. Chest X-ray showed bilateral hilar adenopathy, without increase of angiotensin-converting enzyme (ACE) levels, to rule out sarcoidosis. MRI showed a relapse of pseudotumour in the right orbit and pan sinusitis. Laboratory findings revealed elevated perinuclear ANCA specific for MPO (p-ANCA=7.8 U/ml, norm <5), while antinuclear and anti-double stranded DNA were both negative, whereas lupus anticoagulant was moderately positive (LAC 1.41) and ESR and CRP highly elevated. Glomerulonephritis (urinalysis revealed proteinuria 1.5 g/24 hours, microalbuminuria and microscopic haematuria) without impairment of renal function (serum BUN 14mg/dl, serum creatinine 58 umol/l, GFR 1.23 ml/1.73 m²/24h) and elevated serum levels of β 2-microglobuline (2.15 mg/L), led to the renal biopsy, which confirmed necrotising pauci-

immune glomerulonephritis. GPA was diagnosed according to EULAR/PRINTO/PRES criteria (13) based on recurrent ocular inflammatory pseudotumour, chronic sinusitis, vasculitic rash, necrotising glomerulonephritis and ANCA positivity. Consequently, high doses of methylprednisolone and cyclophosphamide were commenced monthly for six months with good clinical response (fully resolved nephrological symptoms), followed by slow tapering of corticosteroids for 2 years. At this time, stable remission was maintained with mycophenolate mofetil (MMF, 2 g daily) and hydroxychloroquine (HCQ, 200 mg daily), along with antihypertensives and other symptomatic therapy and vitamin supplements. This was announced previously by our group on European Alliance of Associations for Rheumatology (EULAR) meeting in 2012, however monitoring continued in the next ten years (14). In the following years, exacerbation of chronic sinusitis was seen on MRI and renal involvement with microproteinuria. At that time rituximab was considered, but due to persistent hypogammaglobulinemia, biologic treatment was withdrawn. Thus, methotrexate (MTX, 15 mg weekly per os) was induced. Nevertheless, the girl was in stable remission of her disease under immunosuppressives and fully vaccinated with the Pfizer COVID-19 vaccine (BNT162b2, without any adverse events), she was tested PCR positive for SARS-CoV2 twice. Firstly, in spring 2021, infection has successfully resolved, without any complications but with deterioration of chronic sinusitis, not requiring therapy change. Secondly, in May of the current year, with similar symptoms. Currently, stable remission (Birmingham Vasculitis Activity Score, BVAS=0) is maintained along with MTX 10 mg weekly, MMF 1 g and HCQ 200 mg daily, together with antihypertensives and alphacalcidol.

Discussion

GPA is one of the most severe and necrotising vasculitis affecting mainly small vessels, characterised by relapses. Paediatric patients have a similar clinical presentation compared to adults with this disease, but have a different frequency of organ involvement. It appears that ocular manifestations are more common in children than in adults. The largest up-to-date paediatric cohort reported ophthalmic complications (including nonspecific red eye, retro-orbital mass lesion, proptosis, episcleritis, conjunctivitis, retinal exudates, haemorrhage and vascular thrombosis or aneurysm) in up to 43% of children with multi system disease (5). In other studies, 35% of paediatric patients had ocular involvement and more than 50% had necrotising glomerulonephritis. Secondly, gender disproportion was found (females predominate in children with GPA) (15). The ocular manifestation may be the only symptom in limited disease or it may be the first feature of GPA before progression

to multi system involvement (16). Long term follow up is therefore necessary to differentiate generalized from limited forms of GPA. There are no definitive diagnostic criteria for the localized disease, as opposed to systemic forms, and there are only a few reports presenting limited orbital GPA in children (17, 18, 19, 20).

On the other hand, IgG4 related disease is extremely rare in children and occurs mainly with orbital manifestations (21, 22). Due to its multifaceted presentation, it is considered substantial mimicker of many inflammatory, neoplastic and infectious diseases. Increased IgG4 levels in serum are often used to confirm IgG4-RD; but, they are neither sensitive nor specific to diagnose. Reliable biomarkers are lacking, so histopathology remains the key to diagnosis (23, 12). However, even orbital biopsy with considerable number of IgG4-positive plasma cells alone is not enough to differentiate between limited form of GPA and IgG4-RD in the orbit (24).

Recently, a new overlap syndrome between IgG4 related disease and AAV has been proposed in adults (25, 26), pointing to IgG4 subclass of ANCA being implicated as a pathogenic factor in this associated condition (27, 28). This may have been translated to the paediatric population, where notable IgG4 production in IgG4-related disease may promote AAV development in genetically susceptible individuals (29). This hypothesis may be supported with the presence of pauci-immune necrotizing vasculitis seen in the renal biopsy, together with IgG4 positive plasma cells on ocular biopsy of this girl. As noted in European multicenter survey commissioned by the French Vasculitis Study Group, there is a considerable association between AAV, particularly GPA, and IgG4 related disease in adults. This suggests pathophysiological similarities between these two entities, possibly including follicular T helper cells, which are increased in both conditions and are polarised towards the Th-2 subtype, reinforcing the polarisation of IgG4-plasma cells. Furthermore, this study found similar pathogenesis and good sensitivity to rituximab treatment, especially in GPA patients (30).

Some authors noted that since ANCA were predominantly of the IgG isotype, possible pathogenic mechanism of IgG4 subclass of MPO-ANCA in the development of GPA can be explained (27, 30). Most ANCA detected in GPA are directed against proteinase 3, making c-ANCA far more specific than p-ANCA. To the best of our knowledge, there is only one published case of paediatric orbital IgG4 related disease associated with GPA and elevated serum p-ANCA antibodies (31), as seen in our patient.

However, Erden et al. in their recent literature review questioned whether these two entities overlap or mimic each other (32). Similarly, in a case report (33) of a 12-years old boy with GPA that mimicked IgG4 related disease due to unusual orbital manifestation and borderline ANCA. Indeed, orbital GPA lesions can have clinical, radiological and even histopathological features that mimic IgG4 related disease, what can be a major diagnostic pitfall, leading to delayed diagnosis and treatment. (28). However, our patient had clinical and histopathological manifestations, therefore all criteria for both diseases were met, suggesting this new overlap syndrome.

Conclusion

We have described the case of a girl with laboratory and biopsy-proven IgG4 related disease, in whom initial manifestation of GPA was orbital involvement and later developed necrotizing pauci-immune glomerulonephritis and cutaneous manifestations. Furthermore, the appearance of other symptoms confirmed the overlap of GPA and IgG4-related disease, and with immunosuppressive therapy stable remission occurred.

This entity, which is associated with orbital involvement, could be of importance and help to establish early recognition of atypical disease forms, but also to make an earlier diagnosis and initiate treatment in order to prevent potentially fatal consequences of this rheumatic disease.

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Prikaz bolesnika

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BOLEST DEPONOVANJA IMUNOGLOBULINA G4 (IgG4) I GRANULOMATOZA SA POLIANGITISOM KOD DECE: PRIKAZ BOLESNIKA SA NOVIM SINDROMOM PREKLAPANJA

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Granulomatoza sa poliangitisom, prethodno nazivana Wegenerova granulomatoza, jedan je od glavnih oblika vaskulitisa udruženih sa antitelima protiv citoplazme neutrofila (engl. *anti-neutrophil cytoplasm antibody* – ANCA) i najčešći vaskulitis malih i srednjih krvnih sudova kod dece. Bolest deponovanja imunoglobulina G4 (IgG4) karakteriše se inflamatornim pseudotumorima uz povišene vrednosti IgG4 u serumu. Pseudotumor orbite kod dece može biti inicijalna klinička prezentacija obaju pomenutih entiteta. U radu je prikazana devojčica sa neuobičajenom pojavom novog sindroma preklapanja ovih dvaju entiteta, koja se inicijalno prezentovala pseudotumorom orbite. Takođe, predstavljeni su izazovi dijagnostičkog postupka i primenjena terapija. *Acta Medica Medianae* 2023; 62(3): 103-108.

Ključne reči: granulomatoza sa poliangitisom, bolest deponovanja imunoglobulina G4 (IgG4), pseudotumor orbite

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