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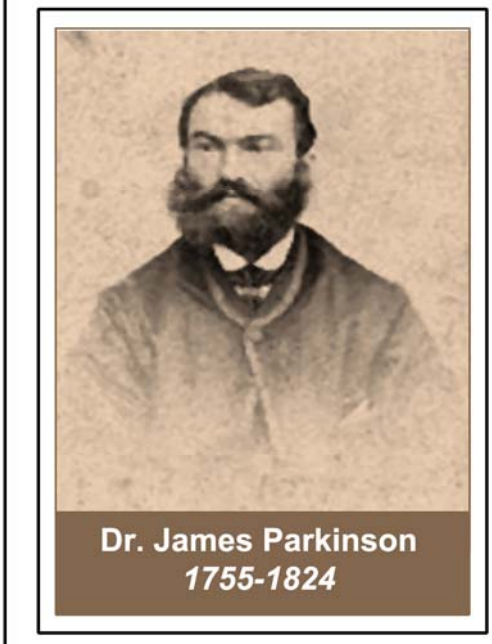

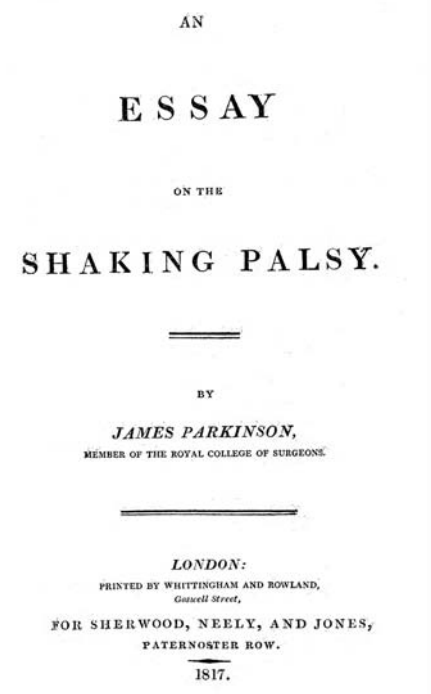

AN

ESSAY

ON THE

SHAKING PALSY.

BY

JAMES PARKINSON,

MEMBER OF THE ROYAL COLLEGE OF SURGEONS.

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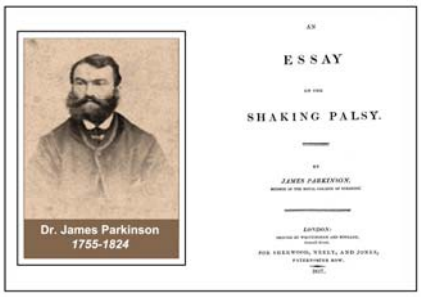

Dr. James Parkinson (11 April 1755 – 21 December 1824) was an English surgeon, apothecary, geologist, palaeontologist, and political activist. He is most famous for his 1817 work, *An Essay on the Shaking Palsy*, in which he was the first to describe "paralysis agitans", a condition that would later be renamed after him – Parkinson's disease. His birthday, 11 April, has been chosen for marking the World Parkinson's Day with the main goal to raise awareness of Parkinson's disease, promoting a greater understanding of this condition and supporting activities that help affected persons.
Today, enormous efforts are undertaking to reveal molecular basis of this and other neurodegenerative diseases, with the ultimate goal to find the right causal drugs that prevent their development and progression (see Editorial, p. 303–5).

Dr Džejms Parkinson (11. april 1755 – 21. decembar 1824), engleski hirurg, apotekar, geolog, paleontolog i politički aktivista, najpoznatiji je po svom delu "*An Essay on the Shaking Palsy*" u kome je prvi opisao *paralysis agitans*, stanje koje je kasnije nazvano po njemu Parkinsonova bolest. Na njegov rođendan, 11. april, obeležava se Svetski dan Parkinsonove bolesti sa ciljem podizanja svesti o toj bolesti, njenom boljem razumevanju i podupiranju svih aktivnosti koje pomažu obolelima.
Danas se ulažu ogromni napori za otkrivanje molekuske osnove ove, ali i drugih neurodegenerativnih bolesti, čiji je konačni cilj pronalaženje odgovarajućih kauzalnih lekova koji bi sprečili njihov nastanak i napredovanje (vidi Uvodnik, str. 303–5).

E D I T O R I A L / U V O D N I K

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Parkinson's disease – the hardship at old age

Parkinsonova bolest – tegoba poznih godina

Marija Mučibabić, Gerard W. Canters, Thijs J. Aartsma

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Within the recent millennia of turbulent human history frequent fatal diseases ravaged the world. It is estimated, for example, that severe pestilence in Athens in 430–426 BCE that occurred as the consequence of huge overcrowding in the city and insanitary conditions wiped out a third of its population. No less severe was the disease in Constantinople and throughout the Byzantine Empire in 541–544 AD. Medieval Europe also used to be devastated and decimated by frequent outbreaks of infections and contagious diseases, very often 'travelling' from the Far East along the old trade routes. The 'Black Death' hit Europe in 1347–1353 AD not only killing between a third to a half of the European population [1] but also causing economic rage unknown before. The rapid industrialization in the 19th century contributed to the spread of cholera and typhoid. It also emphasized the importance of public health and sanitary conditions. The First World War, 1918–1919, was followed by the 'Spanish flu', a pandemic that killed 50 million people, more than in the War itself.

In modern times human life style changed, hygiene improved tremendously and the development of antibiotics, as well as vaccines brought about breakthroughs in treating infectious diseases. Prolonged lifespans in modern society brought an increase in non-infectious diseases, where cancer, cardiovascular and neurodegenerative diseases took the lead. The number of people with neurodegenerative diseases increases steadily, and no proper therapeutical treatment is available yet. Parkinson's disease follows Alzheimer's disease on the list of most common neurodegenerative diseases [2] and affects approximately 6.3 million people worldwide.

James Parkinson was the first to define the manifestation of shaking palsy described in his, now historical, paper "An essay on the shaking palsy'' almost two centuries ago [3]. He examined three men and observed another three on the streets of London. They all shared symptoms as rest tremor, weakened muscular power, anomalous torso posture and dynamic gait. Parkinson clearly emphasized the difference between the early and the later stages of shaking palsy together with the need for repeated observation over an extended period of time to understand the nature of the disorder. In the 19th century Jean-Martin Charot observed non-tremulous forms of the disease and referred to it as Parkinson's disease [4]. In 1912 Fritz Heinrich Lewy investigated by light microscopy the brain sections of 25 Parkinson's disease patients and later on brain sections of 60 more patients. He identified the abnormal inclusions in nerve cell bodies to be a hallmark of the disease. They were named Lewy bodies after him by Konstantin Nikolaevich Tretiakoff who found similar deposits in the *substantia nigra* [5]. Thanks to Carlsson and his coworkers dopamine was discovered as a putative neurotransmitter [6]. Just a few years after it was discovered by Enringer and Hornykiewicz that Parkinson's disease patients shared a decreased concentration of dopamine in the striatum [5], and the first trials of levodopa, a precursor of dopamine, were started in Parkinson's disease patients [7] to increase dopamine levels in their brains. It resulted in the most potent drug so far for controlling the symptoms of Parkinson's disease, and the Nobel Prize in Medicine was awarded to Arvid Carlsson, Paul Greengard, and Eric R. Kandel in 2000 for their discoveries concerning the role of dopamine in signal transduction in the nervous system.

Due to the lack of laboratory test for Parkinson's disease, it is still diagnosed based on clinical observations. The acronym TRAP is used for the four major signs of Parkinson's disease and it stands for tremor at rest, rigidity, akinesia (bradykinesia) and postural instability [8]. These signs of the disease are associated with primary motor symptoms including dynamic gait, micrographia and others. Next to motor symptoms there are equally important non-motor symptoms such as cognitive impairment, apathy, sleep disorders, etc (Table 1). To monitor the impact of Parkinson's disease there was a need for a standardized scale for a wide

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Table 1

Symptoms in patients with Parkinson's disease

Motor symptoms	Non-motor symptoms
Tremor at rest, rigidity, bradykinesia, postural instability	Cognitive impairment, bradyphrenia, fatigue, anhedonia, paraesthesia
Dynamic gait, micrographia, trouble turning in bed, history of falls	Depression, apathy
Hypomimia, dysarthria, dysphagia, sialorrhoea	Sensory symptoms
Blepharospasm, dystonia, scoliosis, striatal deformity	Sleep disorders

range of parameters. Widely used nowadays, the Unified Parkinson's disease Rating Scale includes four parts. The first part of the scale comprises non-motor aspects, the second covers daily activities, the third one addresses motor symptoms and the fourth possible complications of prescribed therapy [9]. The disease develops not only in the central, but also in the peripheral and the enteric nervous system [10]. When the disease is diagnosed, approximately 70% striatum dopamine has already been depleted and it advances in time [11]. In the advanced stage of Parkinson's disease most of the dopaminergic neurons are lost, concomitant with significant cell death during the process [12]. Together with dopaminergic cells, choline neurons present in the dorsal vagal nucleus degenerate [13].

Medical treatment mostly involves levodopa, so far the most potent drug for controlling disease symptoms. Next to levodopa, catechol-o-methyl-transferase inhibitors, dopamine agonists and nondopaminergic therapy are applied [14]. Deep brain stimulation and the transplantation of nigral neurons [15] are alternatives in severe cases. All the above-listed approaches to the treatment of Parkinson's disease only control the symptoms, while targeting the underlying cause would be the most desirable solution to halt the development of the disease and to provide a cure. To be able to treat the cause of the disease, it is necessary to understand the molecular mechanism of the disease.

One of the first steps in genetic understanding of this complicated neurodegenerative disease began with the identification of a missense mutation in the gene coding for a small protein, α-synuclein that causes a rare form of Parkinson's disease [16]. Lewy bodies and Lewy neurites were both found to be immunoreactive for α-synuclein [17]. In fact, electron microscopy images showed that Lewy bodies and Lewy neurites are largely composed of 200–600 nm long α-synuclein filaments [18]. These findings brought α-synuclein in the spotlight of the scientific community. The small, 14.5 kDa protein, α-synuclein, is mostly present in the human brain, but is also found in the heart and muscles. It belongs to the group of intrinsically disordered proteins that are known to cling together and form aggregates. They are associated with a number of diseases, most of which are neurodegenerative [19] (Table 2).

Table 2

Intrinsically disordered proteins associated with human diseases

Protein/peptide	Disease(s)	Polypeptide length (number of amino acid residues)	Protein/peptide structure
α-synuclein	Parkinsons's disease Synucleopathies Dementia with Lewy body Multiple system atrophy Lewy body variant of Alzheimer's disease	140	Intrinsically disordered
Amyloid-β peptide	Alzheimer's disease	37–43	Intrinsically disordered
Huntingtin fragments	Huntington's disease	Variable	Mostly intrinsically disordered
Amylin	Type II diabetes	37	Intrinsically disordered
TDP43	Amyotrophic lateral sclerosis	414	Intrinsically disordered
Prion protein	Prion disease Creutzfeld-Jacob disease Bovine spongiform encephalopathy	231	Intrinsically disordered and α-helical

These diseases all correlate with the formation of inter- and intracellular inclusions made of insoluble amyloid fibrillar aggregates. The structures of these amyloids are very similar, although they may be composed of proteins with very different functionality. Amyloids are fibrillar aggregates with a length of up to a few microns, which are stabilized by a characteristic cross-β structure which plays a key role in the interaction between adjacent proteins within the fibrils [20].

A detailed characterization of the mechanism of α-synuclein aggregation, fibrillization and toxicity is crucial to

unravel the pathology of Parkinson's disease at the molecular level. So far the relevant processes during α-synuclein aggregation can be classified into three groups: processes that increase the number of aggregates such as nucleation and fragmentation, processes that increase the size of already formed aggregates, *ie,* the growth process, and the opposite processes that decrease the size and the number of aggregates like dissociation and/or degradation. Morphological heterogeneity of the species formed during the aggregation was studied by atomic force microscopy [21] and electron microscopy. In recent years, optical techniques [22] have been applied to study the aggregation of α-synuclein. On the other hand, many potential inhibitors were identified to inhibit the aggregation process [23, 24], but their precise mechanism remains to be unraveled.

The huge effort invested in the research of Parkinson's disease resulted in well-defined symptoms of the disease, potent therapeutics of these symptoms, and deeper understanding of the molecular mechanisms behind the disease. Nevertheless important questions remain to be answered in the coming years before a cure can be developed.

R E F E R E N C E S

1. *Haensch S, Bianucci R, Signoli M, Rajerison M, Schultz M, Kacki S,* et al. Distinct Clones of *Yersinia pestis* Caused the Black Death. PloS Pathog 2010; 6(10): e1001134. doi:10.1371/journal.ppat.1001134
2. *de Lau LM, Breteler MM.* Epidemiology of Parkinson's disease. Lancet Neurol 2006; 5(6): 525−35.
3. *Parkinson J.* An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci 2002; 14(2): 223−36; discussion 222.
4. *Kempster PA, Hurwitz B, Lees AJ.* A new look at James Parkinson's Essay on the Shaking Palsy. Neurology 2007; 69(5): 482−5.
5. *Goedert M, Spillantini MG, Del Tredici K, Braak H.* 100 years of Lewy pathology. Nat Rev Neurol 2013; 9(1): 13−24.
6. *Björklund A, Dunnett SB.* Dopamine neuron systems in the brain: an update. Trends Neurosci 2007; 30(5): 194−202.
7. *Birkmayer W, Hornykiewicz O.* The effect of l-3,4-dihydroxyphenylalanine (=DOPA) on akinesia in parkinsonism. Parkinsonism Relat Disord 1998; 4(2): 59−60.
8. *Jankovic J.* Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2008; 79(4): 368−76.
9. *Ebersbach G, Baas H, Csoti I, Müngersdorf M, Deuschl G.* Scales in Parkinson's disease. J Neurol 2006; 253 Suppl 4: IV32−5.
10. *Braak H, Del Tredici K.* Invited Article: Nervous system pathology in sporadic Parkinson disease. Neurology 2008; 70(20): 1916−25.
11. *Brooks DJ.* The early diagnosis of Parkinson's disease. Ann Neurol 1998; 44(3 Suppl 1): S10−8.
12. *Cookson MR.* alpha-Synuclein and neuronal cell death. Mol Neurodegener 2009; 4: 9.
13. *Gai WP, Blumbergs PC, Geffen LB, Blessing WW.* Age-related loss of dorsal vagal neurons in Parkinson's disease. Neurology 1992; 42(11): 2106−11.
14. *Jankovic J, Aguilar LG.* Current approaches to the treatment of Parkinson's disease. Neuropsychiatr Dis Treat 2008; 4(4): 743−57.
15. *Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW.* Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 2008; 14(5): 504−6.
16. *Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A,* et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997; 276(5321): 2045−7.
17. *Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M.* Alpha-synuclein in Lewy bodies. Nature 1997; 388(6645): 839−40.
18. *Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M.* alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 1998; 95(11): 6469−73.
19. *Uversky VN.* Intrinsically disordered proteins and their (disordered) proteomes in neurodegenerative disorders. Front Aging Neurosci 2015; 7: 18.
20. *Nelson R, Sawaya MR, Balbirnie M, Madsen AØ, Riekel C, Grothe R,* et al. Structure of the cross-beta spine of amyloid-like fibrils. Nature 2005; 435(7043): 773−8.
21. *Apetri MM, Maiti NC, Zagorski MG, Carey PR, Anderson VE.* Secondary structure of alpha-synuclein oligomers: characterization by raman and atomic force microscopy. J Mol Biol 2006; 355(1): 63−71.
22. *Pinotsi D, Buell AK, Galvagnion C, Dobson CM, Kaminski Schierle GS, Kaminski CF.* Direct observation of heterogeneous amyloid fibril growth kinetics via two-color super-resolution microscopy. Nano Lett 2014; 14(1): 339−45.
23. *Singh PK, Kotia V, Ghosh D, Mohite GM, Kumar A, Maji SK.* Curcumin modulates α-synuclein aggregation and toxicity. ACS Chem Neurosci 2013; 4(3): 393−407.
24. *El-Agnaf OM, Paleologou KE, Greer B, Abogrein AM, King JE, Salem SA,* et al. A strategy for designing inhibitors of alpha-synuclein aggregation and toxicity as a novel treatment for Parkinson's disease and related disorders. FASEB J 2004; 18(11): 1315−7.

ORIGINAL ARTICLES

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The structure of immunocompetent decidual cells in recurrent missed abortions

Struktura imunokompetentnih ćelija decidue kod ponavljanih spontanih pobačaja

Dragana Radović Janošević*, Jasmina Popović*, Miljan Krstić†, Aleksandra Tubić Pavlović*, Milan Stefanović*, Sonja Pop-Trajković*

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Abstract

Background/Aim. Recurrent or habitual missed abortions (RMA) are defined as three or more consecutive abortions. In the first trimester of pregnancy habitual missed abortions occur in about 1% of population. The aim of this immuno-histochemical study of decidua in RMA of unknown etiology was to identify subpopulations of decidual lymphocytes in recurrent miscarriages and compare the distribution of immunocompetent cells in artificial abortions and RMA. **Methods.** The study included 30 women with at least 2 consecutive miscarriages in the first trimester of pregnancy. Curettements of the third missed abortion were immunohistochemically analyzed. The control group consisted of 20 women without loaded reproductive anamnesis, with the abortion for social reasons. Criteria for exclusion from the study were diagnosed uterine anomalies, positive screening for thrombophilia and women who suffered from diabetes mellitus and disorders in the function of the thyroid gland. Immunophenotyping was performed by immuno-alkaline phosphatase (APAAP) using monoclonal antibodies: CD 30, CD 45 RO, CD 56 and CD 57, CD 68. **Results.** The number of missed abortions (1,223) was on the average 9.7% of all deliveriies during the test period. Among them RMA were registered in 52 (4.2%) patients and in 30 (57%) the exact etiology of abortions was not determined. RMA was most common in the 25–34 years of age group. The largest number of RMA showed the ultrasound characteristics of missed abortion in 60% of cases and was in nulliparous patients (76.7%). The number of natural killer (NK) CD56 positive cells did not differ significantly between the types of abortion. In the decidual tissue, a number of NK CD57 positive cells was significantly higher in missed abortions compared to artificial interruptions ($p < 0.01$). In artificial termination of pregnancy there was an absolute predominance of CD45RO lymphocyte subpopulations, whereas in the RMA group there was slightly greater predominance of CD30 positive cells. The completed analysis showed a significantly higher number of CD68 positive macrophages in a decidual tissue of RMA pregnancy ($p < 0.01$). **Conclusion.** The number and phenotypic structure of NK cells are significantly different in normal pregnancy decidua and in RMA. The NK cell dominance is present in the RMA group, in favor of CD56+ and CD 57 of subpopulations with increased CD30 of T lymphocyte subpopulations. Macrophages are more numerous in the decidua of pregnancies ended in abortion, so the cause of RMA of unknown etiology in a number of cases could be disregulation of immunocompetent cells.

Key words:
abortion, habitual; immunohistochemistry; uterus; killer cells, natural; abortion, missed; decidua.

Apstrakt

Uvod/Cilj. Ponovljeni, habitualni pobačaji (PSP) definišu se kao tri ili više uzastopnih pobačaja. Tokom prvog tromesečja trudnoće, habitualni pobačaj javlja se kod oko 1% trudnica. Cilj rada bio je da se imunohistohemijskom studijom decidue kod ponavljanih spontanih pobačaja nepoznate etiologije identifikuju subpopulacije decidualnih limfocita i uporedi distribucija imunokompetentnih ćelija decidue kod arteficijalnih abortusa i kod ponavljanih spontanih pobačaja. **Metode.** Ispitivanjem je obuhvaćeno 30 žena sa najmanje dva uzastopna spontana pobačaja u prvom trimestru trudnoće. Kiretman trećeg spontanog pobačaja bio je imnunohistohemijski analiziran. Kontrolu je činilo 20 žena bez opterećenja u reproduktivnoj anamnezi, sa prekidom trudnoće iz socijalnih razloga. Kriterijumi za isključivanje iz studije bili su dijagnostifikovane anomalije uterusa, pozitivan test na trombofilije, kao i dijabetes melitus i poremećaji u funkciji štitne žlezde. Imunofenotipizacija rađena je primenom metode imunoalkalne fosfataze, (APAAP) sa korišćenjem monoklonalnih antitela: CD 30, CD 45 RO, CD 56 i CD 57, CD 68. **Rezultati.** Ukupno je registrovano 1 223 spontanih pobačaja što predstavlja 9,7% svih spontanih pobačaja. PSP ustanovljen je kod 52 (4,2%) bolesnice. Od tog broja kod 30 (57%) bolesnica sa PSP nije utvrđena tačna etiologija pobačaja. PSP je bio najzastupljeniji u starosnoj grupi 25–34 godine života. Najveći broj PSP pokazivao je ultrazvučne karakteristike izostalog pobačaja (kod 60% bolesnica). Najveći broj PSP bio je kod nulipara (76,7%). Broj *natural killer* (NK) CD56 pozitiv-

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nih ćelija nije se značajnije razlikovao u zavisnosti od tipa prekida trudnoće. U tkivu decidue broj NK CD57 pozitivnih ćelija bio je značajno veći kod spontanih prekida trudnoće u odnosu na namerne prekide ($p < 0,01$). Kod namernih prekida trudnoće bila je prisutna apsolutna predominacija subpopulacije CD45RO limfocita, dok je u grupi sa PSP bila nešto veća predominacija CD30 pozitivnih ćelija. Urađena analiza pokazala je značajno veći broj CD68 pozitivnih makrofaga u tkivu decidue kod PSP trudnoće ($p < 0,01$). **Zaključak.** Broj i fenotipska struktura NK ćelija značajno se razlikuje u decidui normalne trudnoće i kod PSP. NK ćelijska dominacija u grupi PSP je u korist CD56+ i CD57 subpopulacija uz veću zastupljenost CD30 subpopulacije T-limfocita. Makrofagi su brojniji u decidui trudnoća završenih pobačajem, tako da bi uzrok PSP nepoznate etiologije u određenom broju slučajeva mogla biti disregulacija imunokompetentnih ćelija.

Ključne reči:
abortus, habitualni; imunohistohemija; materica; ćelije ubice, prirodne; abortus, izostali; decidua.

Introduction

Recurrent or habitual missed abortions (RMA) are defined as three or more consecutive abortions. In the first trimester of pregnancy habitual missed abortions occur in about 1% of population [1].

The causes of recurrent missed abortions are multifactorial but can be divided into: embryonic, mainly due to pathological embryonic karyotype, and maternal affecting the endometrium and placenta.

Known maternal causes are anatomic, endocrine and immunological – auto- and alo- immune. Recent works talk about sperm quality as another factor that influences the frequent occurrence of abortion [2].

Despite knowing all the above mentioned etiological factors of recurrent spontaneous abortions, 50% of them were classified as recurrent spontaneous abortions of unknown etiology [2]. It is believed that the largest number of them is the consequence of inadequate aloimmune response of a mother to pregnancy. Period from luteinising hormone (LH) jump to implantation to menstruation is critical for the transformation of the endometrium in terms of supporting implantation or the occurrence of menstruation. Endocrinological and the immunological systems are in a close interaction during the implantation and the maintenance of pregnancy. This communication is most striking on the endometrium of decidual pregnancy. Decidua makes a smaller part of the placenta and the only one that is of exclusively maternal origin. For this reason it does not show antigenic potential, but it is the place of significant process of establishing and maintaining gravid immunoregulation toward the fetal part of the placenta considering that it is a place of direct maternal fetal confrontation. It can be said that decidua is the place of selection, proliferation and maturation of unique population of uterine natural killer (NK) (uNK) cells [3, 4]. Apoptosis important for the selection and favorising certain populations of immunocompetent cells also occurs in the decidua. Reorganization of decidual immunocompetent cells provides its immunosuppressive effect by regulating the activity of NK cells, which themselves undergo subpopulation recomposition under the influence of hormones and cytokines. Decidual cell populations are most important in the implantation uNK cells, macrophages, dendritic cells (DCS) and T cells. B cells and neutrophils are insignificant in decidualization and implantation. Decidual composition of immunocompetent cells effectively establishes and maintains gravidity immunomodulation and probably control and guidance of placental growth [5, 6]. If fertilization and then implantation occurs on the day 20–24 of the cycle, granular NK cells rapidly proliferate and become dominant populations of lymphoid cells achieving close contact with invading trophoblast [7, 8].

The tissue-specific differentiation of uNK cells is related to their function and may be a critical determinant for decidualisation or desquamation of the endometrium [9, 10]. Despite the changes in the number of these cells, a fundamental change is subpopulation precomposition. A dominant subpopulation of NK cells of peripheral blood is CD56 dim, CD16+ and they constitute the basic population of uNK cells of the proliferative phase of the cycle. uNK cells have high-level of CD56 expression, so they belong to the CD56 bright subpopulation [7, 11] In addition to CD56 bright, uNK cells also express killer activating and inhibitory receptors but do not express any other typical NK cell markers, such as CD16 or CD57 [7, 12]. In pregnancy, progesterone causes the reduction in the number of NK cells, activity and cytotoxicity through direct influence on NK cells or promoting TH2 cytokine activities, progesterone-induced blocking factor (PIBF) production in T lymphocytes. Also, progesterone facilitates the regrouping of peripheral NK (pNK) cells in the endometrium by expression of receptors that induce vascular endothelial growth factor (VEGF) and macrophage inflammatory protein (MIP1β) in the endometrium. Endometrial stromal cells under the influence of progesterone produce IL15, prolactin and other factors that regulate uNK in their proliferation, differentiation and production of cytokines and other molecules that support placental trophoblastic development and promote local immunomodulation. uNK cell function is mainly related to the decision to launch the mechanism of menstruation or decidualization, control of maternal immune response to fetal allograft, degree of trophoblastic invasion and the formation of placenta – the modulation of cytokine expression during implantation.

It was long thought that T lymphocytes affect decidualisation and implantation exclusively through the TH1/TH2 balance, i.e. favourisation of TH2 cytokine immune response [3, 6, 13]. Pregnancy is considered a TH2 cytokine-mediated event. TH1 cytokines have been associated with miscarriage and infertility. These effects of TH1 cytokines in experiments were blocked by injection of TH2 cytokines IL-10 [3]. The conclusions of these experiments with mice were expanded to human population [6]. But the discovery of regulatory T lymphocytes with detailed elaboration of cytokine profiling of T cell subgroups showed that the

explanation is not so simple. It is evident that the TH1/TH2 hypothesis is not sufficient to explain the immune mechanisms during implantation. In human reproduction, TH1 cytokine activity is needed at several stages of pregnancy, especially during the early implantation period [14]. During this period, the cytokines IL1 and tumor necrosis factor alfa (TNFα) make pregnancy possible by stimulating the production of leukemia inhibitory factor (LIF) and increasing angiogenesis. It is also interesting that TH1 evironment stimulates the production of TH2 cytokine [14]. T regulatory (Tregs) lymphocytes play a role in implantation and are necessary for the establishment of peripheral immunomodulation. They suppress autoreactive T cells [15]. In the context of pregnancy, Tregs are characterized as essential for the establishment of alotolerance. Tregs have CD4 and CD25, these leukocytes are found in the decidua in various stages of pregnancy. The highest level in the peripheral blood is shown in the first trimester of pregnancy. Tregs cells are specific for Foxp3 marker required for their function, and in the experiments it was significantly lower in infetile women [16]. This supports the importance and fundamental role of Tregs from T cell population in implantation. Macrophages also play a role in implantation and decidualization. Macrophages in the endometrium in proliferative and secretory stage was fairly constant, but the number significantly increased in the decidua of early pregnancy [17]. Their concentration is about 45% higher in the preimplatation region of decidua which is largely achieved through redistribution of these cells from other decidual regions. Chemotaxis of macrophages and their accumulation at the site of implantation are favored by primarily trophoblast proinflammatory factors at the site of the endometrial erosion, increased levels of steroid hormones and increased concentrations of cytokines stimulated by steroid hormones [18]. Cytokine production of macrophages may help prepare the endometrium for pregnancy, cleaning apototic material of trophoblast cells which is very important because it prevents their presentation of immunocompetent cells and initiation of immune responses. Macrophages are likely to mediate the range of trophoblast invasion through TNFα and participate in the level of inflammatory reaction necessary in the early stages of pregnancy [18].

A number of systems are involved in creating a favorable environment for the acceptance of allogeneic blastocysts. The three most important ones involved in it are immune cells, cytokines and adhesion molecules in the place of decidua blastocyst contact [4].

The aim of this study was to identify subpopulations of decidual lymphocytes of recurrent spontaneous abortions by immuno-histochemical study of decidua in recurrent spontaneous abortions of unknown etiology and to compare the distribution of immunocompetent decidual cells in artificial abortion with those in recurrent spontaneous abortions.

Methods

The study included 30 women who had a history of 2 consecutive miscarriages in the first trimester of pregnancy, while the third miscarriage curettment was histopathologically immuno-histochemically analyzed with paraffin preparations. The control group consisted of 20 women without loaded reproductive anamnesis, where the abortion was done for social reasons. Criteria for exclusion from the study were diagnosed uterine anomalies, positive screening for thrombophilia and women who suffer from diabetes mellitus and disorders in the function of the thyroid gland.

Curettings were fixed in 10% formalin during 24 hours, and thereafter moulded in paraffin. Paraffin 5-micron sections were dyed using the hematoxylin-eosin method. Immunophenotyping was performed using immuno-alkaline phosphatase as a method (APAAP) with the following monoclonal antibodies (DAKO): CD30 (marker for activated cells); CD45 RO (marker for T lymphocytes); CD56 and CD57 (markers for NK cells); CD68 (marker for macrophages).

Visualization of the reaction products was performed using the new foschsina which resulted in red deposits in the places of positive reaction. The average number was determined at 10 high-power fields (hpf).

The results were statistically analyzed by forming a database. Statistical significance was tested by commercial software using appropriate tests-Student's *t*-test and *t*-test with corrective approximate method by Cohren and Cox for small samples when neccessary. The 5% level was taken as a borderline level of statistical significance.

Results

The number of missed abortions during the test period was 1,223. The percentage of missed abortions *per* year is shown in Table 1.

Out of 1,223 abortions during the test period, RMA was registered in 52 (4.2%) patients. Of that number, in 30 (57%) patients with RMA the exact etiology of abortion was not determined.

Table 1

Percentage of miscarriages by years

Year	Deliveries, n	Miscarriages, n (%)
2010	3,189	301 (9.7)
2011	3,167	305 (9.6)
2012	3,133	304 (9.7)
2013	3,109	313 (10.1)
Total	12,598	1,223 (9.7)

Ultrasound features of RMA of unknown etiology are shown in Table 2.

Table 2

Ultrasonic (US) characteristics of recurrent or habitual missed abortions

US finding	n	%
Missed abortion	18	60
Abortion spontaneus incipiens	6	20
Blighted ovulum	6	20
Total	30	100

The largest number of RMA showed the ultrasound characteristics of missed abortions (in 60% of cases).

Frequency distribution of RMA by age groups is shown in Table 3.

RMA is most common in the 25–34 years of age group.

Parity in patients with RMA is shown in Table 4.

The largest number of RMA was in nulliparous patients (76.7%).

Immunohistochemical studies of decidua in artificial abortions and recurrent miscarriages (the presence of cell subpopulations of NK lymphocytes) are given in Table 5.

Table 3

Distribution by the age groups

Age (years)	n	%
25–29	10	33.3
30–34	11	36.7
35–39	7	23.3
> 40	2	6.7
Total	30	100.0

Table 4

Parity of the patients with recurrent or habitual missed abortions (RMA)

Previous deliveries	n	%
0	23	76.7
1	6	20.0
2	1	3.3

Table 5

Characteristics of cellular subpopulations of natural killer (NK) cells in decidual tissue

Type of abortion	NK CD56 ($\bar{x} \pm SD$)	NK CD57 ($\bar{x} \pm SD$)
Spontenous	85.03 ± 23.3	95.3 ± 26.1**
Artificial	77.6 ± 13.27	15.2 ± 2.6

****p < 0.01.**

The number of NK CD56 positive cells did not differ significantly depending on the types of abortion (Table 5). In the decidual tissue, the number of NK CD57 positive cells was significantly higher in missed abortions compared to artificial interruptions ($p < 0.01$).

The average number of certain subpopulations of T lymphocytes in the decidual tissue (determined at 10 hpf) in RMA and artificial abortions is shown in Figure 1.

In artificial termination of pregnancy there was an absolute predominance of CD45RO lymphocyte subpopulations, whereas in the RMA group there was slightly greater predominance of CD30 positive cells (Figure 1).

The number of CD68 positive macrophages in decidual tissue in RMA and artificial abortions is shown in Figure 2.

The completed analysis showed a significantly higher number of CD68 positive macrophages in a decidual tissue of RMA pregnancy ($p < 0.01$)

Frequency percentage of leukocyte subpopulations in decidual tissue in RMA and the control group of artificial abortions is given in Figure 3.

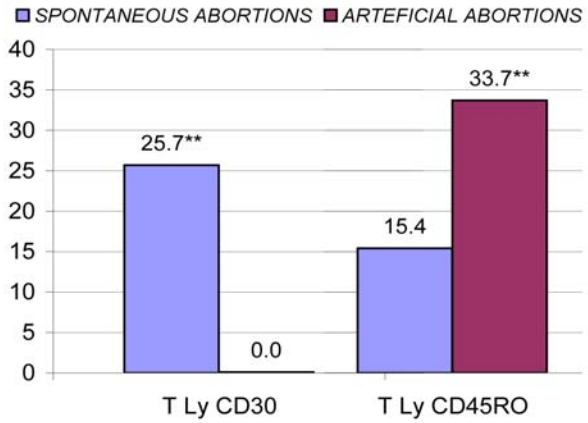

Fig. 1 – Average number of T-lymphocytes depending on the type of abortion.
Data are presented as $\bar{x} \pm SD$ (average number on 10 high-power fields – hpf); ****p < 0.01.

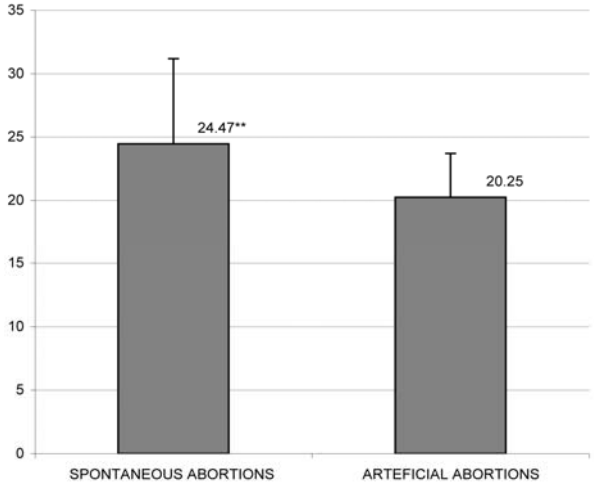

Fig. 2 – CD68 number of positive macrophages by the type of abortion.
Data are presented as $\bar{x} \pm SD$ (average number on 10 high-power fields – hpf); ****p < 0.01.

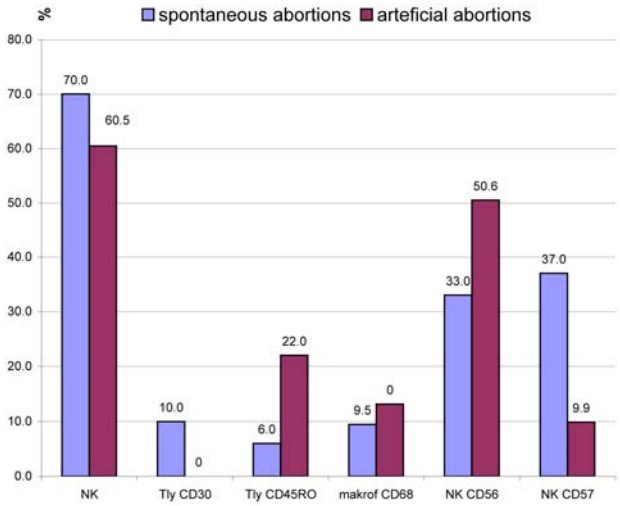

Fig. 3 – Percentage of present decidual leukocyte subpopulation, depending on the type of abortion.

Discussion

Stroma of the gravid endometrium, decidua, is an immunologically privileged position of the reproductive system of a woman with a dominant population of NK cells. Cell microenvironment, the environment in early pregnancy decidua of normal or completed by miscarriage is very important, especially in terms of intercellular contact and the success of establishing immune tolerance.

According to our results the total number of leukocytes in the decidua is 30% in artificial termination of pregnancy compared to 35% in RMA, which is a statistically significant difference. In addition to the increasing number of leukocytes, the most important results of our study show a significant difference in terms of subpopulation regrouping immunocompetent cells in the decidua of these two groups of patients. The total number of NK cells in the group of recurrent missed abortions comparing to artificial is significantly higher. If we look at the phenotypic structure in NK cells, our results show a significantly higher number of CD 56+ and CD 57+ of NK cells in the RMA group.

Our results show that CD 57 populations of NK cells occur in RMA decidua which is otherwise characteristic phenotype of NK cells of the peripheral circulation. We can say that a lot is known about the immunocellular population of decidua in normal pregnancy, but still not enough about the differences of the same population in RMA. There are several studies with the results similar to the results of this study in relation to the significant increase in the number of CD 57 cells in the decidua of pregnancies ended in miscarriage [18, 19]. The possible mechanism of increasing the number of CD57 NK cells in the endometrium of RMA would be the activating of cytokines attacking trophoblast. In a study from 2006, Ordi et al. [20] investigated the presence of different subpopulations of immune cells in the decidua and endometrium in four groups of women: (I) women treated with progesterone disregarding pregnancy; (II) in extrauterine pregnancy; (III) in intrauterine pregnancy of women with RMA; (IV) the group of women with inflammatory reaction at the level of endometrium.

The results of this study show the presence of CD 56 and CD 57 cells in all the groups; especially CD 57 subpopulation was present in the group with inflammation of the endometrium in 100% of cases, and in the RMA group in 55% of cases. CD 56 is present in the group of women with progesterone therapy and RMA, but much less in the decidua of ectopic pregnancy. We can say that NK cells, producing various cytokines, directly influence trophoblastic growth and hormone production allowing decidual vascularization and implantation, and by creating immunomodulatory proteins take part in immunomodulation at the maternal-fetal site of contact.

There are several studies that have used the immunohistochemical method and flow cytometry in NK cells in women with RMA. Most of these studies speak in favor of the increasing number of CD 56 and CD 57 in NK cells. Lachapelle et al. [11] in a study found a similar percentage of the total number of NK cells with increased CD 16+, CD 56 dim subsets and reduced subset of CD 16, CD 56 bright NK cells. Quenby et al. [9], in their immuno-histochemical study from 1999, found an increased percentage of CD 16+ and CD 56+ cells. Clifford et al. [19] in 1999, using immuno-histochemical method, reported an increased number of CD56+ NK cells in women with RMA under 13 gestational weeks. Emmer et al. [21] in 2002, also immuno-histochemically, obtained results on the increasing number of NK cells expressing CD 56 and CD 16 in RMA. There are two studies which show different results in terms of the number and phenotypic features of decidual NK cells. Shimada et al. [22] in the 2004 study, using flow cytometry, reported no difference in the percentage of CD 56+ and CD 56, CD16 + compared to CD 56+, CD16 NK cells. Furthermore, 13. Michimata et al. [13] in 2002, immuno-histochemically found no difference in the number of CD16 + compared to CD 56+ NK cells. There are two studies which show different results in terms of the number and phenotypic features of decidual NK cells. However, 13. Michimata et al. [13] immuno-histochemically found no difference in the number of CD16 + compared to CD 56+ NK cells.

Our study included examination of the frequency of CD 30 and CD 45 RO T lymphocyte subpopulations in the group of intentional abortions for social reasons and in the group of habitual miscarriages. In artificial abortions we found a significantly higher number of CD45 RO T lymphocytes, and CD 30 in RMA. Studies that researched the distribution of CD45 RO T lymphocytes report that decidual lymphocytes are expressed in 90% of CD 45 antigen RO cases [23].

As far as the literature data on decidual CD 30 T lymphocytes are concerned, they show their increase in secretory stage of the cycle and possible hormonal influence on them [22]. In the case of T lymphocyte activation, immune response that leads to the loss of pregnancy is taking place in lymphoid aggregates in the vicinity of endometrial glands, where decidual T lymphocytes are grouped [24, 25]. Our results show a slightly higher presence of macrophages in RMA decidua. The number of endometrial macrophages is partly under control of ovarian steroids and they possess estrogen and progesterone receptors [18]. They are also the main cells that, beside trophoblasts, synthesize prostaglandins, whose immunosuppressive role in pregnancy is known [26]. Macrophages are the most numerous at the site of implantation, and there are 45% of them more than in other parts of decidua [18].

In addition, they play an important role in the maintenance of pro- and anti- imflammatory cytokine balance [3]. They are also essential in the response to infective agents and in the removal of apoptotic material [18]. A slightly higher percentage of macrophages in RMA in our sample would indicate the presence of infection in a number of RMA. The normal phenotype of uNK cells with receptor system on their surface and cytokyne production encourage the development of pregnancy by adequate angiogenesis and anti-inflammatory reaction of the decidua. This role of uNK cell phenotype CD56brightCD16- is disrupted by their lack of or exposure to inflammatory agents (viruses and bacteria). Their insufficiency recruit T cytotoxic lymphocyte subpopulations with the beginning of inflammatory pathogenesis. Also, exposure to inflammatory agents causes the conversion of cytokine NK cell production with dominant TNF-α. In such environment there is a loss of immunotolerance with inadequate angiogenesis resulting in pregnancy loss [15].

Conclusion

The number and phenotypic structure of uterine NK cells is significantly different in normal pregnancy decidua and in that of RMA. Our results demonstrate NK cell dominance in the RMA group, with phenotypic structure in favor of CD56+ and CD57 subpopulations. Also, the prevalence of CD30 subpopulation of T lymphocyte is significantly higher in the RMA group. Macrophages are more numerous in the decidua of pregnancies ended in missed abortion, so that the cause of RMA of unknown etiology in a number of cases could be dysregulation of immunocompetent cells.

R E F E R E N C E S

1. *Yang CJ, Stone P, Stewart AW*. The epidemiology of recurrent miscarriage: a descriptive study of 1214 prepregnant women with recurrent miscarriage. Aust N Z J Obstet Gynaecol 2006; 46(4): 316−22.
2. *Harumi K*. Epidemiology of infertility and recurrent pregnancy loss in society with fewer children. JMAJ 2009; 52(1): 23−8.
3. *Erlebacher A*. Immunology of the maternal-fetal interface. Annu Rev Immunol 2013; 31: 387−411.
4. *Vassiliadou N, Bulmer JN*. Immunohistochemical evidence for increased numbers of 'classic' CD57+ natural killer cells in the endometrium of women suffering spontaneous early pregnancy loss. Hum Reprod 1996; 11(7): 1569−74.
5. *González IT, Barrientos G, Freitag N, Otto T, Thijssen VLJ, Moschansky P*, et al. Uterine NK Cells Are Critical in Shaping DC Immunogenic Functions Compatible with Pregnancy Progression. PLoS ONE 2012; 7(10): 46755.
6. *van Mourik M, Macklon SN, Heijnen JC*. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment J Leukoc Biol 2009; 85(1): 4−19.
7. *Dosiou C, Giudice LC*. Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. Endocr Rev 2005; 26(1): 44−62.
8. *Bulmer JN, Lash GE*. Human uterine natural killer cells: a reappraisal. Mol Immunol 2005; 42(4): 511−21.
9. *Quenby S, Bates M, Doig T, Brewster J, Lewis-Jones DI, Johnson PM,* et al. Pre-implantation endometrial leukocytes in women with recurrent miscarriage. Hum Reprod 1999; 14(9): 2386−91.
10. *Ordi J, Casals G, Ferrer B, Creus M, Guix C, Palacín A,* et al. Uterine (CD56+) natural killer cells recruitment: association with decidual reaction rather than embryo implantation. Am J Reprod Immunol 2006; 55(5): 369−77.
11. *Lachapelle MH, Miron P, Hemmings R, Roy DC*. Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. J Immunol 1996; 156(10): 4027−34.
12. *Bartmann C, Segerer SE, Rieger L, Kapp M, Sütterlin M, Kämmerer U*. Quantification of the predominant immune cell populations in decidua throughout human pregnancy. Am J Reprod Immunol 2014; 71(2): 109−19.
13. *Michimata T, Sakai M, Miyazaki S, Ogasawara MS, Suzumori K, Aoki K*, et al. Decrease of T-helper 2 and T-cytotoxic 2 cells at implantation sites occurs in unexplained recurrent spontaneous abortion with normal chromosomal content. Hum Reprod 2003; 18(7): 1523−8.
14. *Saito S, Nakashima A, Shima T*. Future directions of studies for recurrent miscarriage associated with immune etiologies. J Reprod Immunol 2011; 90(1): 91−5.
15. *Sharma S*. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol 2014; 58(2−4): 219−29.
16. *Nakashima A, Shima T, Inada K, Ito M, Saito S*. The Balance of the Immune System between T Cells and NK Cells in Miscarriage. Am J Reprod Immunol 2012; 67(4): 304−10.
17. *Dietl J, Hönig A, Kämmerer U, Rieger L*. Natural killer cells and dendritic cells at the human feto-maternal interface: an effective cooperation. Placenta 2006; 27(4−5): 341−7.
18. *Yang J, Zhang L, Yu C, Yang X, Wang H*. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res 2014; 2(1): 1.
19. *Clifford K, Flanagan AM, Regan L*. Endometrial CD56+ natural killer cells in women with recurrent miscarriage: a histomorphometric study. Hum Reprod 1999; 14(11): 2727−30.
20. *Ordi J, Casals G, Ferrer B, Creus M, Guix C, Palacin A,* et al. Uterine (CD56+) Natural Killer Cells Recruitment: Association with Decidual Reaction Rather than Embryo Implantation. Am J Reprod Immunol 2006; 55(5): 369−77.
21. *Emmer PM, Steegers EA, Kerstens HM, Bulten J, Nelen WL, Boer K,* et al. Altered phenotype of HLA-G expressing trophoblast and decidual natural killer cells in pathological pregnancies. Hum Reprod 2002; 17(4): 1072−80.
22. *Shimada S, Kato EH, Morikawa M, Iwabuchi K, Nishida R, Kishi R,* et al. No difference in natural killer or natural killer T-cell population, but aberrant T-helper cell population in the endometrium of women with repeated miscarriage. Hum Reprod 2004; 19(4): 1018−24.
23. *Gardner L, Moffett A*. Dendritic cells in the human decidua. Biol Reprod 2003; 69(4): 1438−46.
24. *Trovato M, Grosso M, Vitarelli E, Le DM, Barresi V, Trimarchi F,* et al. Immunoexpression of CD30 and CD30 ligand in deciduas from spontaneous abortions. Eur J Histochem 2005; 49(3): 285−90.
25. *Gharesi-Fard B, Zolghadri J, Haghbin H*. Soluble CD30 (sCD30) and effectiveness of leukocyte therapy in recurrent pregnancy loss patients. J Reprod Immunol 2013; 97(2): 240−4.
26. *Kitaya K, Nakayama T, Okubo T, Kuroboshi H, Fushiki S, Honjo H*. Expression of macrophage inflammatory protein-1beta in human endometrium: its role in endometrial recruitment of natural killer cells. J Clin Endocrinol Metab 2003; 88(4): 1809−14.

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Oxidative stress induced by chlorpromazine in patients treated and acutely poisoned with this drug

Oksidativni stres izazvan hlorpromazinom kod bolesnika lečenih i akutno otrovanih ovim lekom

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Abstract

Background/Aim. Although chlorpromazine (CPZ) is an antipsychotic drug widely used in clinical practice for a long time, its mechanism of action has not been entirely defined. An extremely difficult managing of patients acutely poisoned with CPZ is additional reason for detailed studying its toxicity mechanisms. In this clinical study, we investigated whether the oxidative stress (OS) mediates CPZ toxic effects in the exposed patients. **Methods.** The patients were organized into 3 groups: the T-group – hospitalized patients receiving therapeutic doses of 75–150 mg CPZ/day; the overdosed group, divided into two subgroups: the group M and the group S – mildly (CPZ serum concentration: 0.21 ± 0.05 mg/L) and severely (CPZ serum concentration: 2.66 ± 0.25 mg/L) poisoned patients, respectively, and the group C (control group of healthy volunteers). Oxidative stress parameters [total antioxidative status (TAS) and malondialdehyde (MDA) in plasma)] and superoxide dismutase (SOD) activity in erythrocytes were measured spectrophotometrically, and CPZ concentrations in serum were monitored chromatographically. One set of measurements was performed in the group C and T, whereas two sets of measurements (after 24 hours and 48 hours) were done in the poisoned patients, groups M and S. **Results.** A decrease of TAS and increase of SOD activity were obtained in both subgroups of the poisoned patients, compared to the controls and the group receiving therapeutic doses of CPZ. A significant increase of MDA was achieved in severely poisoned patients, compared to all other groups. **Conclusion.** Changed oxidative stress parameters in patients poisoned with chlorpromazine indicate involvement of oxidative stress in the toxicity mechanism(s) of chlorpromazine.

Key words:
chlorpromazine; poisoning; oxidative stress; malondialdehyde; superoxide dismutase.

Apstrakt

Uvod/Cilj. Iako hlorpromazin (CPZ) pripada grupi antipsihotika i široko se primenjuje u kliničkoj praksi već duže vreme, njegov mehanizam dejstva još uvek nije potpuno definisan. Izuzetno teško stanje bolesnika nakon akutnog trovanja CPZ dodatni je razlog za detaljno proučavanje mehanizama toksičnosti leka. U ovoj kliničkoj studiji, ispitivali smo da li je oksidativni stres (OS) uključen u toksične efekte kod bolesnika nakon akutnog trovanja CPZ. **Metode.** Bolesnici su bili podeljeni u tri grupe: T-grupa – hospitalizovani bolesnici koji su dobijali terapijsku dozu leka (75–150 mg CPZ dnevno); grupa akutno otrovanih osoba, podeljena u dve podgrupe: grupa M (blago trovanje: koncentracija CPZ u serumu: 0.21 ± 0.05 mg/L) i grupa S (teško trovanje: koncentracija CPZ u serumu: 2.66 ± 0.25 mg/L) i grupa C (kontrolna grupa zdravih dobrovoljaca). Parametri OS [ukupni antioksidativni status (TAS) i malondialdehid (MDA) u plazmi)] i aktivnost superoksid dizmutaze (SOD) u hemolizatu eritrocita određivani su spektrofotometrijski, dok je koncentracija CPZ u serumu praćena hromatografski. Svi parametri određivani su jednokratno u grupi C i T, dok su u grupi M i S merenja izvršena u dva termina, posle 24 h i 48 h nakon trovanja. **Rezultati.** Smanjenje TAS i povećanje aktivnosti SOD dobijeno je u obe grupe bolesnika nakon trovanja, u poređenju sa kontrolnom grupom i grupom bolesnika koja je bila na terapijskim dozama CPZ. Značajno povećanje MDA dobijeno je u grupi bolesnika sa teškim trovanjem, u poređenju sa svim drugim grupama. **Zaključak.** Promene parametara oksidativnog stresa kod bolesnika nakon trovanja hlorpromazinona pokazuju uključenost oksidativnog stresa u mehanizme toksičnosti ovog leka.

Ključne reči:
hlorpromazin; trovanje; stres, oksidativni; malondialdehid; peroksid dismutaza.

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Introduction

Chlorpromazine (CPZ) is an antipsychotic drug widely used in clinical practice for a long time, but its mechanism of action is not yet fully defined. The drug is dopamine D2 receptor antagonist, but also it has an affinity for other receptors as well [1]. Also, by its binding to calmodulin (Ca^{2+}- binding messenger protein) it may influence on a variety of Ca^{2+}-calmodulin dependent enzymes [2]. It is known that an excessive stimulation of Ca^{2+}-activated processes, such as those catalyzed by nonlysosomal proteases, endonucleases and phospholipases, may be the predominant cytotoxic event for a variety of toxic insults [3].

It is also known that CPZ has been shown to lower peroxidative damage *in vivo* and to protect cells from lethal injury presumably by acting as an antioxidant [4,5]. However, potent prooxidative effect of CPZ was documented by the generation of reactive oxygen species (ROS) within 15 minutes of CPZ treatment in differentiated human hepatoma HepaRG cells [6]. Energy disruption by CPZ was blamed for exacerbation of its toxicity.

Recent studies showed that chronic administration of CPZ reduces the activity of manganese superoxide dismutase (MnSOD) and copper/zinc SOD (CuZnSOD) (after 21 days), and catalase and glutathione (GSH) (after 180 days) and increases lipid peroxidation (LPO) based on the concentration of malonylaldehyde (MDA) determination [7,8].

Oxidative stress (OS) is implicated in the pathophysiology of many diseases including neurological ones [8]. Reactive oxygen species, such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals (OH•) are known to damage various cellular components, including membrane lipids, proteins, DNA and thereby contribute to cellular dysfunction [9]. The products of LPO (carbonyl compounds) readily react with amino acids residues, such as cysteine or lysine, and disturb protein function. Under normal conditions, antioxidant enzymes (like SOD, glutathione peroxidase, catalase, etc.) provide adequate protection against free radicals (FRs) harmful effects on all kind of biomolecules, unlike in OS when extrem FRs production occurs, and endogenous antioxidative system is insufficient to prevent oxidative injury of cells. There is evidence that the mitochondrial pathology and OS (reduced activities of antioxidant enzymes in plasma, red blood cells, and cerebrospinal fluid in patients) may be the most critical concerns in the pathophysiology and outcome of schizophrenia [10]. Such changes in oxidative defense mechanisms in patients with schizophrenia may be exacerbated by the treatment with antipsychotics having pro-oxidant properties [11].

Having in mind these contradictory findings, the aim of this study was to test the hypothesis that CPZ provokes OS development in dose-dependent manner. Therapeutic (T), mild (M) and severe (S) toxic doses of CPZ were tested in exposed patients by assessing total antioxidative status (TAS) and MDA concentration in plasma and SOD activity in erythrocytes.

Methods

Participants

The following groups of participants were organized in this cross-sectional study: the group C – the control group of healthy individuals, nonsmokers, on no medication, excluding personal or family histories of psychiatric/neurological illness; the group T – the hospitalized patients on therapeutic doses of 75–150 mg CPZ/day divided in three single daily doses, for minimum 60 days; and two groups of poisoned patients, subdivided into two categories according to the poisoning severity score (PSS) and measured serum concentration of CPZ: the group M – mildly poisoned patients (PSS: 0–1; CPZ in serum: 0.21 ± 0.05 mg/L) and the group S – severely poisoned patients (PSS: 2–3; CPZ in serum: 2.66 ± 0.25 mg/L). Patients with PSS 4 were not included in this study. The study was conducted in the Military Medical Academy in Belgrade. It was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia.

Reagents

All chemicals used in this study were of analytical grade. Also, all drugs solutions were prepared on the day of the experiment.

Sample preparation

Considering the circadian rhythm, blood samples were taken between 08:00 and 09:00 a. m. just before the breakfast.

Immediately after the blood test, tubes with lithium-heparin was put on ice to preserve the activity of SOD in erythrocytes. Blood with EDTA was immediately analyzed and it was determined from the concentration of hemoglobin, which is expressed through the activity of the SOD isoenzymes.

The chromatographic quantification of serum CPZ

Samples were prepared as follows: 5 g of previously homogenized and thawed sample was transferred to the polypropylene centrifuge tubes, and 20 mL of 0.1 mM sulfuric acid in acetonitrile was added. After homogenization on ultra-turrax, samples were sonicated for 5 minutes and centrifuged at 2,400 g for 10 minutes. Supernatant was decanted into the clean centrifuge tubes and the extracts were purified. Columns were conditioned with 5 mL of methanol and 5 mL of water, followed by sample loading and washing with 0.01 M sulfuric acid. CPZ was eluted with 6 mL of 0.1 mM sulfuric acid in acetonitrile and methanol (50 : 50 v/v). Eluates were evaporated at 40°C in the gentle stream of nitrogen. Dry residue was dissolved in mobile phase and 20 uL was injected into the high pressure liquid chromatography (HPLC) system.

Concentrations of CPZ were made by diluting stock and working standard solutions to achieve calibration concentrations expected to meet the therapeutic levels in serum of patients. Stock solution was prepared by dissolving the standard in methanol to the concentration of 0.1 mg/mL. Working solution was also prepared in methanol by diluting stock solution to the concentration of 1 ug/mL. Concentrations of calibration solutions (25 ng/mL, 50 ng/mL, 75 ng/mL and 100 ng/mL) were prepared by dissolving working solution in acetonitrile. The mobile phase was a mixture of 0.03 M sodium acetate-acetonitrile (67 : 33 v/v). Flow rate was set to

1 mL/min isocratic elution through the HPLC column. Detection was achieved by measuring ultra-violet absorption at 250 nm.

Five-point calibration (including zero) was performed at the beginning of each batch of samples, followed by blank and fortified samples [12].

Oxidative stress parameters (TAS, SOD, MDA) were determined spectrophotometrically. One set of measurements was performed in the group C and T, whereas two sets of measurements (after 24 hours and 48 hours) were done in the poisoned patients (groups M and S).

TAS determination

Total antioxidant status was performed by commercial "Randox" test on automatic biochemical analyzer AXON Technicon. The principle of the reaction is based on *in vitro* reduction of blue 2,2-azino-di-(3-etilbenztiazonil sulfonate) (ABTS) cation radical (ABTS•+) into its colorless molecular form, ABTS, by endogenous antioxidants. The intesity of blue color diminishes with the increase of TAS. This TAS assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay and is expressed as mmol/L [13].

SOD activity

Superoxide dismutase activity (including all three SOD isoenzymes) was determined by commercial "Randox" test on an automatic biochemical analyzer AXON Technicon. The dismutation of $O_2^{\bullet -}$ into H_2O_2 is catalyzed by SOD. The principle of the method is based on the reaction of xanthine and xantine oxidase that provides $O_2^{\bullet -}$ which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) forming red-colored formazan. The higher intensity of red color means the less activity of SOD. The results were expressed as U of SOD *per* mg Hb.

MDA concentrations

The LPO was determined by MDA quantification, using thiobarbituric acid reactive substances (TBAR) method. Namely, TBAR (15% trichloroacetic acid + 0.375% TBA + 0.25% mol HCl) reacts with MDA, originating from polysaturated fatty acid peroxidation. Formed MDA was measured spectrophotometrically at 533 nm. The results were expressed as mmol/L.

Statistical analysis

Types of data distribution within the groups were analyzed by Kolmogorov-Smirnov test. All data were analyzed statistically by one way ANOVA using Dunnett`s C-test. The statistical program GraphPad Prism was used. Statistical significance was defined at $p < 0.05$. The data was presented as mean ± SD.

Results

The demographic characteritics of subjects in the study are given in Table 1.

Generally, there were more females than males in all the studied groups, but with no differences in age among them.

The serum CPZ concentrations in the group T were within the therapeutic range (data not shown). The serum CPZ concentrations in the patients from the group S (2.66 ± 0.25 mg/L) were much higher than in the patients from the group M (0.21 ± 0.05 mg/L).

After 24 hours of poisoning, TAS values were lower in both groups (Figure 1) than in the controls ($p < 0.05$), as well as in the patients from the group T ($p < 0.01$). On the contrary, the SOD activities were higher in both M and S group (Figure 2), than in the controls ($p < 0.05$), at the same time (24 hours), as well as in the patients from the group T ($p < 0.05$). Both T and M group had the same MDA concentrations (Figure 3) as the control group, but we registered the significant MDA increase in the patients from the group S ($p < 0.001$). A significant negative correlation (Figure 4) between the serum CPZ concentration and TAS level in plasma of the patients from the group S ($r = -0.54$, $p = 0.0161$) was obtained after 24 hours of the poisoning.

After 48 hours of poisonings the results were similar for all the examined parameters: significantly decreased TAS level ($p < 0.05$) and increased SOD activity (M patients, $p < 0.05$ and the patients from the group S, $p < 0.01$) in the groups M and S, compared to the controls, as well as in the group T (Figures 1, 2). Also, in the patients from the group S, we registered a significant MDA increase ($p < 0.001$) compared to all the other groups (Figure 3).

Discussion

Our results showed that CPZ induces OS development in the dose-dependent manner in exposed humans, based on

Table 1

Characteristics of healthy volunteers and patients exposed to chlorpromazine (CPZ) enroled in the study

Study groups	Participants (n)	Females (n)	Average age ($\bar{x}$ ± SD)	Males (n)	Average age ($\bar{x}$ ± SD)
Control	30	10	43.5 ± 10.91	20	41.8 ± 17.72
T	39	14	39.7 ± 13.23	25	31.9 ± 10.70
M	13	7	46.7 ± 11.33	6	35.7 ± 8.83
S	19	10	38.9 ± 9.45	9	32.1 ± 5.55

The control group of healthy individuals, nonsmokers, on no medication, with no personal or family histories of psychiatric/neurological illness; the group T – hospitalized patients on therapeutic doses of CPZ; the group M – patients with mild CPZ poisoning; the group S – patients with severe CPZ poisoning.

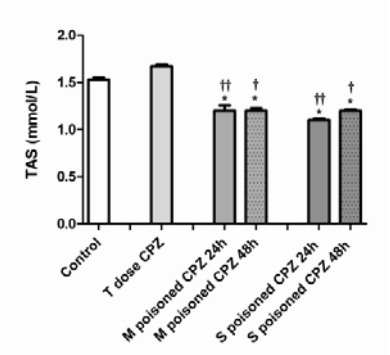

Fig. 1 – Total antioxidant status (TAS) level (mmol/L) in the control group and in the groups of patients with therapeutic doses (T-dose) of chlorpromazine (CPZ), as well as in the groups of CPZ mild (M) and severe (S) poisoned patients. Bars in the graph represent the mean ± SD. Labels of statistical significance: * – compared to the control group; † – compared to the group T. A statistical significance was considered at: *, †$p < 0.05$, ††$p < 0.01$, One Way ANOVA, Dunnett`s C-test.

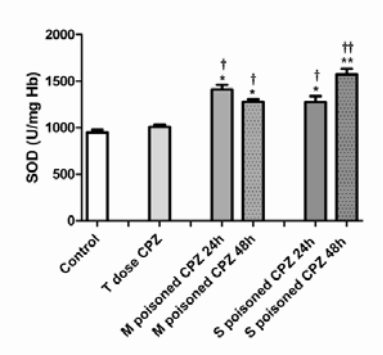

Fig. 2 – Superoxide dismutase (SOD) activity (U/mg Hb) in the control group and in the groups of patients with therapeutic doses (T-dose) of chlorpromazine (CPZ) as well as in the groups of CPZ mild (M) and severe (S) poisoned patient. Bars in the graph represent the mean ± SD; Labels of statistical significance: * – compared to the control group; † – compared to T group, a statistical significance was considered at: *, †$p < 0.05$, **, ††$p < 0.01$, One-Way ANOVA, Dunnett`s C-test.

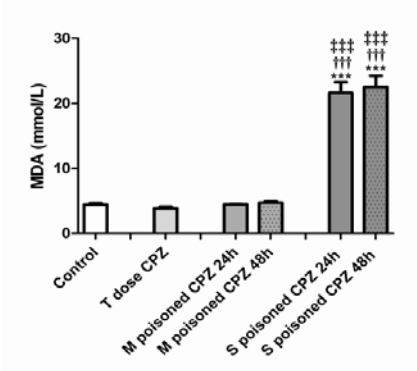

Fig. 3 – Malondialdehyde (MDA) concentration (mmol/L) in the control group and in the groups of patients with therapeutic doses (T dose) of chlorpromazine (CPZ) as well as in groups with mild (M) and severe (S) CPZ poisoning. Bars in the graph represent the mean ± SD; labels of statistical significance: * – compared to the control group; † – compared to the group T; ‡ – compared to the group M. A statistical significance was considered at: *, †††, ‡‡‡$p < 0.001$, One-Way ANOVA, Dunnett`s C test.**

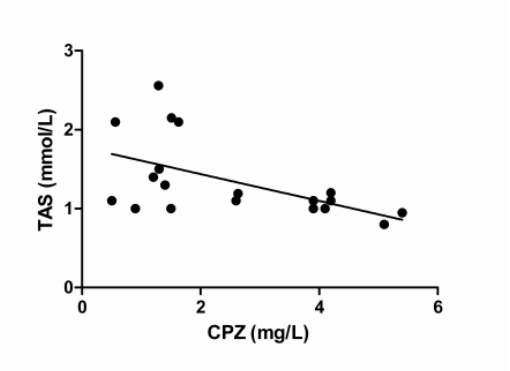

Fig. 4 – The correlation of the serum concentration of chlorpromazine (CPZ) (mg/L) in the patients with severe poisoning and total antioxidative status (TAS) level (mmol/L) in plasma. In the first 24 hours after poisoning, parallel with the increase of CPZ serum concentration, TAS level in plasma decreases, Pearson`s correlation, r = -0.54. There is a negative linear relationship between these variables ($p < 0.05$).

the measured OS parameters. Namely, LPO as the major indicator of lipid oxidative injury was highly elevated in the patients poisoned with higher toxic doses of CPZ (PSS: 2–3). Additionally, TAS levels were lower and SOD activities were higher in both the groups, M and S, of the patients than in the controls, as well as in the group T. The obtained results were in an agreement with recent clinical trials that even therapeutic doses of CPZ disrupt antioxidative defense system [14]. In addition to the reduced energy production, the products of anaerobic glucose metabolism lead to the formation of ROS, which affects the deterioration of the course and outcome of poisoning. However, we found that TAS levels in plasma of the patients from the group T did not significantly differ from the control group, indicating that the total antioxidant capacity of the organism in the patients from the group T was not diminished (Figure 1).

The study of Hu and Kulkarni [15] showed that CPZ is oxidized into CPZ cation radical in the presence of H_2O_2 and/or other FRs such as $O_2^{\bullet -}$ and hydroxyl radical (HO•). Also, auto-oxidation of dopamine is the potential source of undergoes, $O_2^{\bullet -}$. However, dopamine is primarily metabolized through oxidation by monoamine oxidase (MAO) to 3,4-dihydroxyphenyl-acetic acid (DOPAC), followed by the formation of H_2O_2, which can further react with iron or copper ions to produce OH• (the most toxic of FRs). Thus, the biochemical pathway of dopamine supports the oxidation process in the brain leading to neurological disorders and oxidation of administered neuroleptics such as CPZ, as well.

It has also been shown that different peroxidases and methemoglobin catalyze single electron oxidation of CPZ into biologically active CPZ cation radical. This radical is highly reactive and can interact with a number of endogenous substances and xenobiotics, as well. Neuroleptics block dopamine receptors, which may affect the increased dopamine turnover and, in turn, could eventually lead to the increased production of H_2O_2, resulting in OS [9]. It has also been shown that neuroleptics can induce cellular alterations that lead to production of ROS and cell death. In addition, CPZ increases in the level of brain manganese, which in turn may potentiate the damage caused by FRs.

Reacting with GSH, CPZ cation radical leads to the formation of GSH thiol radical cation of CPZ, which is responsible for both pharmacological efficacy and toxic effects of this drug. Inevitably, depleted GSH storage makes antioxidative defense system more fragile, what means that this drug can initiate OS in the brain [16].

A TAS value as the ratio of total antioxidative status in the groups of patients 24 and 48 hours after poisoning with CPZ, was lower than in the control group, as well as in the group T, indicating the consumption of antioxidant potential in the cases of both M and S poisoning (Figure 1). Neuroleptics may also have direct cytotoxic effect *via* the production of toxic metabolites [17].

In this study we obtained a negative linear relationship (Figure 4) between TAS level in plasma and serum CPZ level in the patients from the group S ($p < 0.05$, r = -0.54), confirming the antioxidative defense damage.

Changes in the activities of antioxidative enzymes may provoke an increase in ROS. Increased SOD activity in the poisoned patients from the group M and S and in the group T, indicate that CPZ may induce expression of gene for SOD synthesis (Figure 2). Antipsychotic drugs have been found to induce the expression of immediate early genes such as cfos and cjun, transcription factors, growth factors and peptides [4]. There is evidence that early genes and growth factors can then regulate the expression of antioxidant enzymes which provide a part of neuroprotective mechanisms associated with growth factors [12]. As noted previously, SOD can protect living organisms from oxidative injury by $O_2^{\bullet -}$ sequestration [16]. Increased SOD activities that are registered in both M and in S group are probably induced by the increased production of ROS and CPZ cation radical, consequently.

Membrane fluidity is an important factor that determines inter- and intracellular communication, membrane elasticity and biological transport of proteins and lipids. Guo et al. [18] have proposed that oxidation of membrane lipids leads to the formation of peroxidation degradation products (MDA), which leads to cross-linking reaction of the lipid-lipid and lipid-protein type, thereby rendering the membrane more rigid and less fluid. In patients of the group S, we registered a significant increase in LPO at 24 hours and 48 hours of poisoning (Figure 3). The process of peroxidation is an important indicator of membrane damage, which serves a lot to promote irreversible impairment of essential cellular components and eventually leads to cell death or necrosis. There is considerable evidence that schizophrenic patients have increased levels of LPO. Pall et al. [19] found increased MDA levels in the cerebrospinal fluid of patients taking CPZ.

Increased SOD activity was accompanied with decreased MDA concentration in the group M, emphasizing SOD capability to prevent OS, reducing LPO. This is not the case in the group S, where OS is present in spite of increased SOD activity. These results confirm that the organism was protected from the increased production of FRs in the patients from the group M, while the resulting OS leads to the initiation of the process of LPO in the patients from the group S. In the first 24 hours, after mild CPZ poisoning, the increase of SOD activity is not followed by the MDA increase, indicating a still effective antioxidant potential preventing from LPO. However, FRs production was steadily increased at the same time (24 hours) in the patients from the group S. The increase of SOD in this period is followed by MDA increase, indicating the existence of biological membrane damage by FRs and LPO processes. All of these changes were still present 48 hours of CPZ poisoning in this group.

Conclusion

Our results showed that chlorpromazine dose-dependent changed of SOD activity and malondialdehyde concentration in all the investigated groups. Oxidative stress, lipid peroxidation and changes of the antioxidant enzyme activity may be responsible for one of the molecular mechanisms of chlorpromazine induced tissue damage. Based on the changes in oxido-reductive status in the patients exposed to chlorpromazine it can be concluded that red-ox toxicity pathway might contribute to the overall chlorpromazine toxic effects in humans. This finding emphases the significance of disturbed oxido-reductive status in patients overdosed by chlorpromazine.

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R E F E R E N C E S

1. *Suzuki H, Gen K, Inoue Y.* Comparison of the anti-dopamine D(2) and anti-serotonin 5-HT(2A) activities of chlorpromazine, bromperidol, haloperidol and second-generation antipsychotics parent compounds and metabolites thereof. J Psychopharmacol 2013; 27(4): 396−400.
2. *Xu B, Xu Z, Deng Y, Yang J.* Protective effects of Chlorpromazine and Verapamil against cadmium-induced kidney damage in vivo. Exp Toxicol Pathol 2010; 62(1): 27−34.
3. *Christensen MH, Lien EA, Hustad S, Almås B.* Seasonal and age-related differences in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone in patients from Western Norway. Scand J Clin Lab Invest 2010; 70(4): 281−6.
4. *Parikh V, Khan MM, Mahadik SP.* Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. J Psychiatry Res 2003; 37(1): 43−51.
5. *Gattaz WF, Schmitt A, Maras A.* Increased platelet phospholipase A2 activity in schizophrenia. Schizophr Res 1995;16(1): 6.
6. *Anthérieu S, Bachour-El AP, Dumont J, Abdel-Razzak Z, Guguen-Guillouzo C, Fromenty B,* et al. Oxidative stress plays a major role in chlorpromazine-induced cholestasis in human HepaRG cells. Hepatology 2013; 57(4): 1518−29.
7. *Pillai A, Parikh V, Terry AV, Mahadik SP.* Long-term antipsychotic treatments and crossover studies in rats: differential effects of typical and atypical agents on the expression of anti-

oxidant enzymes and membrane lipid peroxidation in rat brain. J Psychiatr Res 2007; 41(5): 372−86.

8. *Naidu PS, Singh A, Kulkarni SK.* Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible antioxidant mechanisms. Br J Pharmacol 2002; 136(2): 193−200.
9. *Taylor A, Robson A, Houghton BC, Jepson CA, Ford WC, Frayne J.* Epididymal specific, selenium-independent GPX5 protects cells from oxidative stress-induced lipid peroxidation and DNA mutation. Hum Reprod 2013; 28(9): 2332−42.
10. *Bubber P, Tang J, Haroutunian V, Xu H, Davis KL, Blass JP,* Gibson GE. Mitochondrial enzymes in schizophrenia. J Mol Neurosci 2004; 24(2): 315−21.
11. *Mahadik SP, Evans D, Lal H.* Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2001; 25(3): 463−93.
12. *Hall MA, Robinson CA, Brissie RM.* High-performance liquid chromatography of alprazolam in postmortem blood using solid-phase extraction. J Anal Toxicol 1995; 19(6): 511−3.
13. *Barclay LR, Locke SJ, MacNeil JM.* Autoxidation in micelles. Synergism of vitamin C with lipid-soluble vitamin E and water-soluble Trolox. Can J Chem 1985; 63(2): 366−74.
14. *Persoon-Rothert M, Egas-Kenniphaas JM, van der Valk-Kokshoorn EJ, Mauve I, van der Laarse A.* Prevention of cumene hydroperoxide induced oxidative stress in cultured neonatal rat myocytes by scavengers and enzyme inhibitors. J Mol Cell Cardiol 1990; 22(10): 1147−55.
15. *Hu J, Kulkarni AP.* Metabolic fate of chemical mixtures. I. "Shuttle Oxidant" effect of lipoxygenase-generated radical of chlorpromazine and related phenothiazines on the oxidation of benzidine and other xenobiotics. Teratog Carcinog Mutagen 2000; 20(4): 195−208.
16. *Gutiérrez-Correa J, Krauth-Siegel R, Stoppani AO.* Phenothiazine radicals inactivate Trypanosoma cruzi dihydrolipoamide dehydrogenase: enzyme protection by radical scavengers. Free Radic Res 2003; 37(3): 281−91.
17. *Wu JQ, Kosten TR, Zhang XY.* Free radicals, antioxidant defense systems, and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2013; 46: 200−6.
18. *Guo L, Chen Z, Amarnath V, Davies SS.* Identification of novel bioactive aldehyde-modified phosphatidylethanolamines formed by lipid peroxidation. Free Radic Biol Med 2012; 53(6): 1226−38.
19. *Pall HS, Williams AC, Blake DR, Lunec J.* Evidence of enhanced lipid peroxidation in the cerebrospinal fluid of patients taking phenothiazines. Lancet 1987; 2(8559): 596−9.

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Radiographic cephalometry analysis of condylar position after bimaxillary osteotomy in patients with mandibular prognathism

Rendgen-kefalometrijska analiza pozicije kondila nakon bimaksilarne osteotomije mandibularnog prognatizma

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Abstract

Background/Aim. Postoperative condylar position is a substantial concern in surgical correction of mandibular prognathism. Orthognathic surgery may change condylar position and this is considered a contributing factor for early skeletal relapse and the induction of temporomandibular disorders. The purpose of this study was to evaluate changes in condylar position, and to correlate angular skeletal measurements following bimaxillary surgery. **Methods.** On profile teleradiographs of 21 patients with mandibular angular and linear parametres, the changes in condylar position, were measured during preoperative orthodontic treatment and 6 months after the surgical treatment. **Results.** A statistically significant difference in values between the groups was found. The most distal point on the head of condyle point (DI) moved backward for 1.38 mm (p = 0.02), and the point of center of collum mandibulae point (DC) moved backward for 1.52 mm (p = 0.007). The amount of upward movement of the point DI was 1.62 mm (p = 0.04). **Conclusion.** In the patients with mandibular prognathism, the condyles tend to migrate upward and forward six months after bimaxillary surgery.

Key words:
prognathism; surgery, oral; postoperative period; cephalometry; temporomandibular joint; centric relation.

Apstrakt

Uvod/Cilj. Postoperativna pozicija kondila je značajna za hirurško korekciju mandibularnog prognatizma. Ortognatska hirurgija može da promeni poziciju kondila, a to može biti jedan od faktora koji doprinosi ranom skeletnom recidivu i pojavi temporomandibularnih disfunkcija. Zbog toga je cilj ove studije bio da proceni promene pozicije kondila kao i da ne korelišu promene pozicije kondila sa angularnim skeletnim promenama nakon bimaksilarne hirurgije. **Metode.** Na telerendgenskim snimcima 21 bolesnika sa mandibularnim prognatizmom mereni su angularni i linearni parametri koji opisuju promene u položaju kondila, pre ortodontske pripreme i šest meseci nakon hirurške korekcije. **Rezultati.** Ustanovljena je statistička značajnost razlika u vrednosti parametara između grupa. Tačka DI – najdistalnija tačka na glavi kondila, pomerila se unazad 1,38 mm (p = 0,02), a tačka DC – tačka koja označava centar *collum mandibulae*, pomerila se, takođe, unazad za 1,52 mm (p = 0,007). Vrednost pomeranja tačke DI naviše bila je 1,62 mm (p = 0,04). **Zaključak.** Kod bolesnika sa mandibularnim prognatizmom, kondili su težili da migriraju unapred i naviše šest meseci nakon bimaksilarne operacije.

Ključne reči:
prognatizam; hirurgija, maksilofacijalna; postoperativni period; kefalometrija; temporomandibularni zglob; centrički odnos.

Introduction

Mandibular prognathism (MP) or skeletal Class III malocclusion with a prognathic mandible has long been viewed as one of the most severe maxillofacial deformities [1]. The treatment of MP is complex and includes preoperative orthodontic treatment and orthognatic surgery. In some severe cases both mandibular and maxillary osteotomy are needed. One of the preferred surgical procedures for the correction of mandibular prognathism, since its introduction by Trauner and Obwegeser [2], is billateral

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sagittal split osteotomy (BSSO). Another popular technique, mostly used for maxillary reposition, is Le Fort I osteotomy. In some severe cases of MP both mandibular and maxillary osteotomy are needed, and that form of correction is commonly known as bimaxillary surgery.

One of the goals of bimaxillary surgery is maintaining skeletal and occlusal stability. Occlusal stability, which is one of the most important factors in the prevention of postoperative relapse in orthognathic surgery, results from good dental occlusion and a normal postoperative condylar position [3]. Condylar processus is a part of the mandibular ramus and a part of the temporomandibular joint (TMJ), specific to the human body in its morphology, position and function [4]. This makes it particularly important, both in functional and in anatomical terms, because of its shape and position depending on the position of the mandible, the function of the TMJ and facial appearance [5]. Good dental occlusion depends on normal temporomandibular joint; that is, dental malocclusion or abnormal interdigitation with normal condylar position can be controlled postoperatively by orthodontic treatment, but an abnormal condylar position can not be corrected postoperatively and eventually disrupts postoperative occlusal stability [3]. Therefore, postoperative condylar position is a substantial concern in the surgical correction of a mandibular prognathism. Orthognathic surgery may change condylar position and this is considered a contributing factor for early skeletal relapse [6–9] and the induction of temporomandibular disorders (TMDs) [10–13].

Positional changes in the condyle have been hard to recognize and accurately measure following orthognathic surgery [14, 15]. Displacement of the condyle can be expected as a result of four variables: anterior-posterior, vertical, medial-lateral, and along the long axis of the condyle [16].

The purpose of this study was to evaluate changes in condylar position, and to correlate angular skeletal measurements following bimaxillary surgery in patients with mandibular prognathism.

Methods

The study included 21 patients (13 males, 8 females; ages between 18–25 years). Clinical examinations and standardized lateral cephalometric radiographs were conducted at the Belgrade University Faculty of Dentistry. The study was approved by the Ethics Committee at the Faculty of Dentistry in Belgrade. Informed consent was obtained from each patient. All the patients were diagnosed with mandibular prognathism on the basis of the following criteria: the angle of mandibular prognathism (SNB) $\geq 80°$; the angle of sagittal intermaxillary relationship (ANB) $\leq 0°$; reverse overlap of the frontal teeth and relationship of the first permanent molars in Class III, and had ended the growth and development of orofacial system. The patients with mandibular prognathism as a result of severe facial asymmetry, deformity secondary to trauma, syndromes, patients with systemic disease, degenerative joint disease, and with signs and symptoms of temporomandibular dysfunction were not included in the study.

The presurgical protocol included preoperative orthodontic treatment, model surgery, cephalometric and photocephalometric analysis. The preoperative orthodontic treatment lasted from 18 to 24 months.

The surgery began with soft tissue incision and initial osteotomy of the ramus of the mandible as in BSSO, but with no definitive separation of bone fragments. The wound was filled with gauze soaked in saline and then the complete Le Fort I osteotomy was done. Using the interocclusal splint and maxillo-mandibular fixation, the maxilla was positioned in a certain position and fixed with monocortical screws (at least four) and L-shaped plates. After fixing the maxilla, the maxillo-mandibular fixation was removed, so the separation of mandibular bone fragments was completed. A separated central fragment of mandible was placed in the correct occlusion with the maxilla, the intermaxillary fixation was restored, and bone fragments of the mandible were fixed with monocortical screws and plates. Monocortical screws were located on the buccal surface of the mandible, three of them on each side of the osteotomy line. Rigid intermaxillary fixation was maintained for 6 to 8 weeks and after that period of time, the elastic fixation was maintained for 4 weeks. Postoperative orthodontic treatment started 6 to 8 weeks after the surgery.

Standardized lateral cephalometric radiographs were obtained at the following 2 stages in all the patients: before the preoperative orthodontic treatment (T1) and 6 months after the surgical treatment (T2).

The machine used to obtain lateral cephalometric radiographs was Ortoceph (Simens, Germany). The scanning settings of the machine were: 65–80 kVp tube voltage, 20 mA tube current, and 1–1.5 second scan time. All the patients sat in an upright position with the teeth in centric occlusion. The patients' Frankfort horizontal (FH) plane was parallel to the floor.

Cephalometric radiographs were scanned by a scanner EPSON 1600 PRO (Japan) into jpg format. In that way all the radiographs were converted into digital form. The software Ax Ceph version 2.3 (Audax, Slovenia) was used for computerized cephalometric analysis. Cephalometric analysis was carried out by one examiner and included the reference points and lines shown in Figures 1 and 2. Analyses were performed twice by the same examiner, on different days. Statistically significant differences did not appear between these two analyses.

Certain linear and angular parameters were used to define the position of the condyle pre- and postoperatively (Table 1). Angular parametres included: SNA – the angle of maxillary prognathism; SNB – the angle of mandibular prognathism; ANB – the angle of sagittal intermaxillary relationships; Cd-DC-Xi/FH – the angle formed by centerline of mandibular rami and X axis; ArGoMe – gonial angle (angle of the mandible); Sna-Snp/FH – the angle formed by the main plane of maxilla and X axis. Linear parametres were the distances between the points Go, Ar, DC, Cd, PI, CI, A, B and Y axis; and distances between the points Go, Ar, DC, Cd, PI, CI, A, B and X axis (see Abbreviations in addendum).

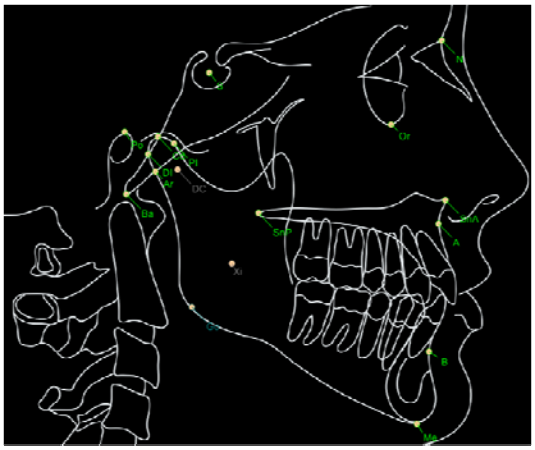

Fig. 1 – Reference points included in the analysis.

S (sella) – The point representing the geometric center of the *sella turcica*.; N (nasion) – The most anterior (midline) point of the frontonasal suture; A (*subspinale*) – The deepest point in the bony concavity in the midline below the anterior nasal spine; Or (*orbitalis*)- The point representing the lowest point on the inferior orbital rim; Po (porion) – The most superior point of the external auditory meatus; Sna (*spina nasalis anterior*) – The most prominent point of maxilla; Snp (*spina nasalis* posterior) – The most distal point of the conjunction of palatinal bone and pterygomaxillar fissure; B (*supramentalis*) – The innermost point on the contour of the mandible between the incisor tooth and the bony chin; Me (menton) – The lowest point of the mandibular symphysis; Go (gonion) – the midpoint of the mandibular angle between the ramus and mandibular body; Cd (condylion) – the most posterosuperior point on head of the condyle; Ar (articularis) – The point midway between the two posterior borders of the left and the right mandibular rami at the intersection with the basilar portion of the occipital bone; DI – The most distal point on the head of the condyle; PI – The most anterior point on the head of the condyle; DC – The center point of the collum mandibulae on the Ba-N line; Xi – The point located at the geographic center of the ramus; Ba (basion) – The point of the anterior margin of the foramen magnum – The midpoint of the curvature between upper and the lower surfaces of the basilar portion of the occipital bone.

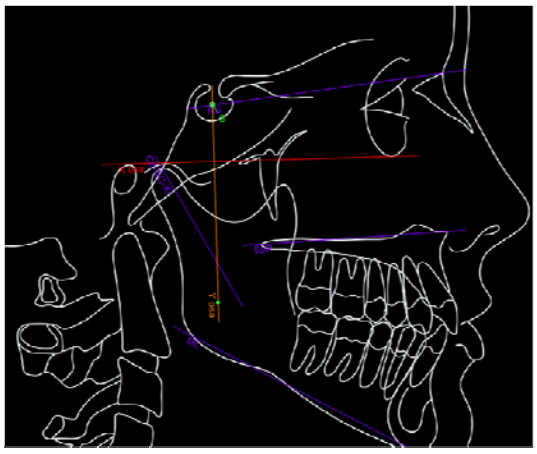

Fig. 2 – Reference planes included in the analysis.

N-S – The main plane of the anterior cranial base; Go-Me – The main plane of the mandible body; Sna-Snp – The main plane of the maxilla; Cd-DC-Xi – the centerline of the mandibular rami; X axis (Or-Po Frankfort horizontal (FH)) – The horizontal plane of the head; Y axis – The vertical plane which is normal to the X osis and goes from the point S.

Table 1

Linear and angular parameters included in the analysis

Linear parameters (millimeters)	Angular parameters (degrees)
Go-Y axis	Cd-DC-Xi / FH
Ar- Y axis	ArGoMe
DC- Y axis	Sna-Snp / FH
Cd- Y axis	ANB
PI- Y axis	SNB
CI- Y axis	SNA
A- Y axis	
B- Y axis	
Go- X axis	
Ar- X axis	
DC- X axis	
Cd- X axis	
PI- X axis	
CI- X axis	
A- X axis	
B- X axis	

SNB – Angle of mandibular prognatism; ANB – Angle of sagittal intermaxillary relationship; SNA – sella nasion, A point. See abbreviations in Addendum.

Using the software, after insertion the digital (jpg) format of lateral cephalogram, calibration was set up. The calibration is used to convert pixels of the inmages into milimetres. A metal ruler on a cephalostat which is visible on radiography was used for calibration (Figure 3).

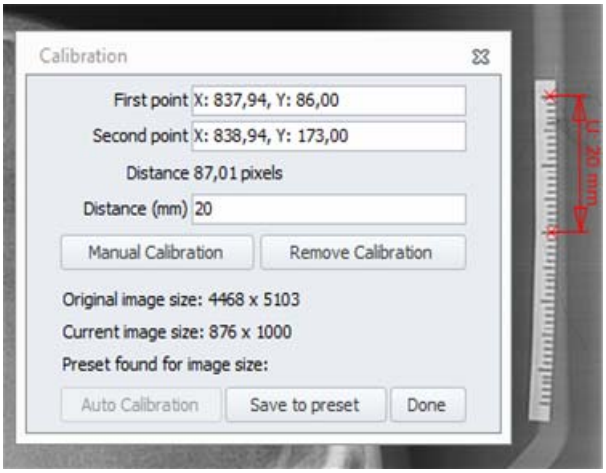

Fig. 3 – Calibration of the digital image usig the software "Ax Ceph".

Then, the location of reference points and lines were defined. To analyze linear (anterior-posterior and vertical) movement of the condyle, in every cephalometric radiograph the coordinate system with X and Y axis (as described in Figure 2) was inserted. After that, the distance between the points Go, Ar, DC, Cd, PI, CI, A, B and Y axis was measured to determine horizontal skeletal changes postoperatively. The distance between the points Go, Ar, DC, Cd, PI, CI, A, B and X axis was measured to determine vertical skeletal changes postoperatively. Angles SNA, SNB, and ANB were used to describe skeletal changes after the intervention. The angle Ar-Go-Me and angle Cd-DC-Xi/FH were used to analyze rotation of the condyle after the intervention. Angle Sna-Snp/FH was used to describe rotation of the maxilla after bimaxilary surgery (see Abbreviations in addendum).

Data analysis was not preformed until the last patient had been examined for the last time to prevent bias from the examiner's awareness of any trends in the basic data.

Statistical analyses were performed with SPSS version 15 (SPSS, Chicago, Ill). For the assessment of the differences between angular and linear parameters before (T1) and after (T2) the surgery, Students paired *t*-test was used. Pearsons correlation was used to correlate changes in condilar position with angular skeletal changes following bimaxillary surgery. The differences were considered significant at $p < 0,05$.

Results

Horizontal skeletal changes: the mean setback of the mandible 6 months postoperatively (T2-T1) was 0.91 mm at point B but the differences were not statistically significant ($p = 0.658$). Point Go showed tendency to go forward (1,19 mm) but also was not statistically significant ($p = 0.242$). On the other hand, maxilla was on average moved forward 3.29 mm at point A ($p = 0.0038$) (Table 2). *Horizontal changes in condylar position*: six months after the surgery the position of point DI and DC changed significantly. Point DI moved backward 1.38 mm ($p = 0.02$), likewise, point DC moved backward for 1.52 mm ($p = 0.007$). The movement of the points PI and Cd were not statistically significant, but it was noted that point PI showed the tendency to move forward by 0.1 mm (Table 2).

Vertical skeletal changes: the results showed the tendency of downward movement of the mandible at points B and Go (1.43 mm, $p = 0.644$; 3.85 mm $p = 0,058$, respectively). Maxilla was moved upward at point A for 3.18 mm but showed no statistical significance (Table 3).

Vertical changes in condylar position: the position of condyle changed only at point DI. The amount of upward movement of point DI was 1.62 mm ($p = 0.04$). Points PI and DC

Table 2

Distances between the reference points and Y axis

Distances (mm)		n	x̄	SD	Med	Min	Max	*p*
YB	T1	21	60.48	14. 84	65.00	39.00	100.00	0.658
	T2	21	59.57	10.82	60.00	37.00	85.00	
YA	T1	21	59.52	11.26	60.00	45.00	82.00	0.0038*
	T2	21	62.81	8.01	63.00	47.00	79.00	
YDI	T1	21	17.38	3.69	16.00	12.00	26.00	0.020*
	T2	21	18.76	4.21	19.00	8.00	24.00	
YPI	T1	21	6.48	3.31	6.00	1.00	13.00	0.653
	T2	21	6.38	3.15	6.00	0.00	11.00	
YCd	T1	21	14.38	3.97	14.00	8.00	24.00	0.446
	T2	21	14.86	3.62	16.00	6.00	20.00	
YDC	T1	21	9.19	3.70	10.00	2.00	16.00	0.007*
	T2	21	10.71	3.94	11.00	2.00	17.00	
YAr	T1	21	15.33	4.08	15.00	8.00	23.00	0.005*
	T2	21	16.86	3.92	16.00	7.00	23.00	
YGo	T1	21	7.00	6.32	6.00	0.00	24.00	0.242
	T2	21	8.19	5.95	6.00	0.00	20.00	

****p* < 0.05 (2-tailed); x̄ – mean; SD – standard deviation; Med – median;**
Min-Max – minimal-maximal value; T1 – Standardized lateral cephalometric radiographs obtained before preoperative orthodontic treatment; T2 – Standardized lateral cephalometric radiographs obtained 6 months after the surgical treatment.
See abbreviations in Addendum.

Table 3

Changes in distances between the reference points and X axis

Distances (mm)		n	x̄	SD	Med	Min	Max	*p*
XB	T1	21	71.86	13.28	71.00	45.00	95.00	0.644
	T2	21	73.29	9.03	71.00	61.00	96.00	
XA	T1	21	35.38	13.20	32.00	23.00	88.00	0.137
	T2	21	32.19	5.78	30.00	24.00	49.00	
XDI	T1	21	5.24	2.91	5.00	0.00	11.00	0.040*
	T2	21	6.86	3.41	7.00	0.00	14.00	
XPI	T1	21	3.62	1.99	4.00	0.00	8.00	0.887
	T2	21	3.81	3.17	3.00	0.00	12.00	
XCd	T1	21	4.33	3.79	4.00	0.00	16.00	0.263
	T2	21	3.57	2.73	3.00	0.00	8.00	
XDC	T1	21	8.48	5.47	11.00	0.00	16.00	0.335
	T2	21	9.10	6.20	10.00	0.00	17.00	
XAr	T1	21	12.86	4.05	12.00	6.00	20.00	0.007*
	T2	21	14.57	4.25	14.00	8.00	21.00	
XGo	T1	21	58.05	10.67	57.00	40.00	82.00	0.058
	T2	21	61.90	5.20	62.00	51.00	75.00	

****p* < 0,05 (2-tailed);); x̄ – mean; SD – standard deviation; Med – median; Min-Max – minimal-maximal value;**
T1 – Standardized lateral cephalometric radiographs obtained before preoperative orthodontic treatment;
T2 – Standardized lateral cephalometric radiographs obtained 6 months after the surgical treatment.
See abbrevations in Addendum.

showed the trend to move upward (0.19 mm and 0.62 mm, respectively). On the contrary, point Cd showed the tendency to move downward (0.76 mm; $p = 0.263$) (Table 3).

The results suggest that point Ar was moved significantly from both X and Y axis. Point Ar moved downward (mean difference T2-T1 was 1.71 mm; $p = 0.007$) and forward (T2-T1 was 1.53 mm; $p = 0.005$) (Tables 2 and 3).

SNA, SNB and ANB angle significantly changed postoperatively. SNA and ANB angle increased in dimensions (T2-T1) for 1.76° and 3.76° respectively ($p = 0.049$ and $p < 0.001$). On the other hand, SNB angle decreased for 1.95° ($p = 0.04$). Angles which predicted the rotation of the condyle – ArGoMe changed significantly ($p = 0.009$) for 5.1°, but Cd-DC-Xi/FH did not ($p = 0.277$). The rotation of maxilla (SnaSnp/FH angle) did not change significantly six months after the surgery ($p = 0.128$) (Table 4).

The study showed a positive correlation between the distance XB and the ArGoMe angle. The XA distance also correlated negatively with ArGoMe angle. The distance between the point DI and the X axis showed negative correlation with SNA angle (Table 5).

A positive correlation between the distance YB and the angle SNB, YA and SNB was noted. The distance between the Y axis and the point DI showed a positive correlation with the angle ArGoMe (Table 6).

Discussion

Condyle displacement from or in the glenoid fossa can be caused by abnormal mandibular movement, methods used for fixation, segment rigidity, or masticatory muscle tension [17]. Condylar displacement, especially after BSSO, can cause postoperative complications [18]. Rotational changes also contribute to idiopathic condylar resorption after BSSO [14–16]. The direction of movement of the jaw during surgery is of great importance because it is considered to be one of the factors that influence the postoperative position of the condyle [3]. This study shows a statistically significant movement of the mandible six months after the surgery, which significantly influenced the position of the condyle. Studies have shown that the method of fixation of fragments contributes significantly to the stability of the condyle [8, 16, 19], for

Table 4

Changes in angular parametres

Angle (°)		n	x̄	SD	Med	Min	Max	*p*
SNA	T1	21	81.86	5.51	82.00	72.00	96.00	0.049*
	T2	21	83.62	5.59	84.00	74.00	94.00	
SNB	T1	21	86.57	6.03	88.00	77.00	103.00	0.040*
	T2	21	84.62	5.04	84.00	75.00	92.00	
ANB	T1	21	-4.71	2.41	-4.00	-10.00	0.00	< 0.001**
	T2	21	-0.95	2.62	-1.0	-6.00	3.00	
ArGoMe	T1	21	139.48	7.94	137.00	126.00	155.00	0.009*
	T2	21	134.38	8.55	134.00	119.00	154.00	
Cd-DC-Xi/FH	T1	21	61.90	6.20	62.00	43.00	70.00	0.277
	T2	21	63.76	6.36	63.00	52.00	77.00	
SnaSnpFH	T1	21	4.10	3.27	4.00	0.00	9.00	0.128
	T2	21	5.19	3.37	5.00	1.00	11.00	

****p* < 0.05 (2-tailed); ** *p* < 0.001 (2-tailed);); x̄ – mean; SD – standard deviation; Med – median;**
Min-Max – minimal-maximal value; T1 – Standardized lateral cephalometric radiographs obtained before the preoperative orthodontic treatment; T2 – Standardized lateral cephalometric radiographs obtained 6 months after the surgical treatment.
See abbrevations in Addendum.

Table 5

Correlation of angular and linear parametres (Xaxis)

(T1-T2) n = 21		Cd-DC-Xi / FH	SnaSnp/FH	ArGoMe	SNA	SNB	ANB
X B	r	0.009	-0.031	0.652**	-0.206	-0.303	0.194
	p	0.969	0.893	0.001	0.371	0.182	0.400
X A	r	0.069	-0.188	-0.619**	0.041	0.280	-0.347
	p	0.768	0.415	0.003	0.859	0.218	0.123
X DI	r	-0.063	-0.053	0.148	-0.509*	-0.360	-0.191
	p	0.787	0.818	0.523	0.018	0.109	0.406
X PI	r	-0.067	-0.053	0.037	-0.130	-0.340	0.237
	p	0.775	0.818	0.873	0.574	0.131	0.300
X Cd	r	0.193	0.374	0.232	-0.238	-0.394	0.177
	p	0.403	0.095	0.311	0.299	0.077	0.442
X DC	r	0.077	0.010	-0.089	-0.414	-0.245	-0.261
	p	0.740	0.966	0.702	0.062	0.284	0.252
X Ar	r	-0.234	-0.123	0.261	-0.207	-0.146	-0.041
	p	0.308	0.594	0.252	0.368	0.527	0.860
X Go	r	0.035	-0.171	-0.339	0.074	0.274	-0.252
	p	0.881	0.457	0.133	0.749	0.229	0.271

***Correlation is significant at the level $p < 0.05$ (2-tailed); **Correlation is significant at the level $p < 0.01$ (2-tailed); (T1-T2) – The difference in dimensions in angles/distances before the preoperative orthodontic treatment and six months after the correction of mandibular prognathism.**
See abbrevations in Addendum.

Table 6

Correlation of angular and linear parametres (Y axis)

(T1-T2) n = 21		Cd-DC-Xi / FH	SnaSnp/FH	ArGoMe	SNA	SNB	ANB
Y B	r	-0.303	-0.005	-0.027	0.379	0.680**	-0.360
	p	0.181	0.983	0.906	0.090	0.001	0.109
Y A	r	-0.174	-0.053	-0.218	0.422	0.499*	-0.112
	p	0.450	0.819	0.343	0.057	0.021	0.630
Y DI	r	-0.099	0.078	0.498*	-0.235	-0.061	-0.194
	p	0.668	0.738	0.022	0.306	0.793	0.398
Y PI	r	0.204	-0.393	-0.370	-0.308	-0.234	-0.090
	p	0.374	0.078	0.099	0.175	0.308	0.700
Y Cd	r	-0.328	-0.123	0.178	-0.067	0.071	-0.163
	p	0.147	0.595	0.440	0.774	0.759	0.481
Y DC	r	-0.294	-0.225	0.425	-0.179	-0.236	0.131
	p	0.195	0.327	0.055	0.437	0.303	0.571
Y Ar	r	0.112	0.206	0.422	-0.415	-0.276	-0.129
	p	0.629	0.370	0.057	0.061	0.226	0.577
Y Go	r	0.175	0.009	-0.275	0.193	0.189	0.081
	p	0.447	0.969	0.227	0.401	0.413	0.727

***Correlation is significant at level $p < 0.05$ (2-tailed); **Correlation is significant at level $p < 0.01$ (2-tailed); (T1-T2) – difference in dimensions in angles/distances before preoperative orthodontic treatment and six months after the correction of mandibular prognathism.**
See abbrevations in Addendum.

these reasons, in this study the patients' jaw fragments were connected with rigid fixation.

Many researchers, using various radiographic methods, studied the movement of the condyle in patients after orthognathic surgery [20–22]. However, there are still few studies that deal with bimaxillary orthognathic surgery mandibular prognathism [23, 24]. In this study, four points on the condyle – DI, PI, DC and Cd were used and based on the distance of these points with the X and Y axis the anteroposterior and vertical changes in position of the condyle before the preoperative orthodontic preparation and 6 months after the bimaxillary surgical correction were established. The results of this study indicate the condyle tend to move forward and upward. The anterior condyle movement is similar with the study which Ueki et al. [25] conducted. They also reported that there was anterior and inferior movement of the condyle after BSSO and intraoral vertical ramus osteotomy, but there was no statistically significant difference between these different techniques. The possible reason for moving the condyle forward and downward is anatomical feature of the front part of the glenoid fossa [3].

On the other hand, Hu et al. [26] investigated the effect of sagittal split ramus osteotomy of the mandible on the temporomandibular joint. By comparing images of temporomandibular joints, they noticed a posterior condyle movement in the group of patients who underwent BSSO. They also found the forward rotation of the condyle, which is similar to our results. These results can be explained by the pulling force of the anterior and posterior segments of *m. temporalis* and *m. masseter*. In this study there was a decrease in the value of the angle ArGoMe six months after the surgery which could partialy influence the

forward rotation of the condyle. Contrary to the results of Hu et al. [26], a study by Harris et. al. [27] showed medial, posterior and superior movement of the condyle after BSSO, and also medial rotation of the condyle.

The results showed that the amount of the mandibular and maxillar movement postoperatively did not correlate statistically with condylar displacement as did the results of Harris et al. [27] and Lee and Park [3]. Interestingly, only changes in ArGoMe angle correlated with the changes in the distance Y-DI, and changes in the angle SNA correlated with changes in the distance X-DI.

Conclusion

This study shows that the position of the condyle after bimaxillary orthognathic surgery is altered. In our group of patients, six months after surgery, the condyles tend to migrate upward and forward. Only the most distal point on the head of the condyle (point DI) correlated with the gonial and SNA angle. Although this study yielded significant results over a period of six months, it was performed in a limited number of patients due to strict inclusion criteria. Further research on changes in condylar position is needed with a longer observation period.

R E F E R E N C E S

1. *Graber LW.* Chin cup therapy for mandibular prognathism. Am J Orthod 1977; 72(1): 23−41.
2. *Trauner R, Obwegeser H.* The surgical correction of mandibular prognathism and retrognathia with consideration of genioplasty. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1957; 10(7): 677−89.
3. *Lee W, Park JU.* Three-dimensional evaluation of positional change of the condyle after mandibular setback by means of bilateral sagittal split ramus osteotomy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94(3): 305−9.
4. *Cutović T, Pavlović J, Kozomara R.* Radiographic cephalometry analysis of dimensions of condylar processus in persons with mandibular prognathism. Vojnosanit Pregl 2008; 65(7): 513−9. (Serbian)
5. *Ricketts RM, Roth RH, Chacones SJ, Schlhof RJ, Engel GA.* Orthodontic diagnosis and planing. Denver: Rocky Mountain Data Systems; 1982.
6. *Joss CU, Vassalli IM.* Stability after bilateral sagittal split osteotomy advancement surgery with rigid internal fixation: a systematic review. J Oral Maxillofac Surg 2009; 67(2): 301−13.
7. *Epker BN, Wessberg GA.* Mechanisms of early skeletal release following surgical advancement of the mandible. Br J Oral Surg 1982; 20(3): 175−82.
8. *Will LA, Joondeph DR, Hohl TH, West RA.* Condylar position following mandibular advancement: its relationship to relapse. J Oral Maxillofac Surg 1984; 42(9): 578−88.
9. *Van SJ, Tiner BD, Keeling SD, Clark GM, Bays R, Rugh J.* Condylar position with rigid fixation versus wire osteosynthesis of a sagittal split advancement. J Oral Maxillofac Surg 1999; 57(1): 31−4.
10. *Ellis E, Hinton RJ.* Histologic examination of the temporomandibular joint after mandibular advancement with and without rigid fixation: an experimental investigation in adult Macaca mulatta. J Oral Maxillofac Surg 1991; 49(12): 1316−27.
11. *Lisniewska-Machorowska B, Cannon J, Williams S, Bantleon H.* Evaluation of force systems from a "free-end" force system. Am J Orthod Dentofacial Orthop 2008; 133(6): 791−10.
12. *Mladenović I, Jović N, Čutović T, Mladenović G, Kozomara R.* Temporomandibular disorders after orthognathic surgery in patients with mandibular prognathism with depression as a risk factor. Acta Odontol Scand 2013; 71(1): 57−64.
13. *Mladenović I, Dodić S, Stošić S, Petrović D, Cutović T, Kozomara R.* TMD in class III patients referred for orthognathic surgery: psychological and dentition-related aspects. J Craniomaxillofac Surg 2014; 42(8): 1604−9.
14. *Kawamata A, Fujishita M, Nagahara K, Kanematu N, Niwa K, Langlais RP.* Three-dimensional computed tomography evaluation of postsurgical condylar displacement after mandibular osteotomy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85(4): 371−6.
15. *Bettega G, Cinquin P, Lebeau J, Raphaël B.* Computer-assisted orthognathic surgery: Clinical evaluation of a mandibular condyle repositioning system. J Oral Maxillofac Surg 2002; 60(1): 27−34.
16. *Kundert M, Hadjianghelou O.* Condylar displacement after sagittal splitting of the mandibular rami. A short-term radiographic study. J Maxillofac Surg 1980; 8(4): 278−87.
17. *Ueki K, Degerliyurt K, Hashiba Y, Marukawa K, Nakagawa K, Yamamoto E.* Horizontal changes in the condylar head after sagittal split ramus osteotomy with bent plate fixation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106(5): 656−61.
18. *Ueki K, Nakagawa K, Takatsuka S, Yamamoto E.* Plate fixation after mandibular osteotomy. Int J Oral Maxillofac Surg 2001; 30(6): 490−6.
19. *Nishimura A, Sakurada S, Iwase M, Nagumo M.* Positional changes in the mandibular condyle and amount of mouth opening after sagittal split ramus osteotomy with rigid or nonrigid osteosynthesis. J Oral Maxillofac Surg 1997; 55(7): 672−6.
20. *Fang B, Shen GF, Yang C, Wu Y, Feng YM, Mao LX,* et al. Changes in condylar and joint disc positions after bilateral sagittal split ramus osteotomy for correction of mandibular prognathism. Int J Oral Maxillofac Surg 2009; 38(7): 726−30.
21. *Kim YI, Jung YH, Cho BH, Kim JR, Kim SS, Son WS,* et al. The assessment of the short- and long-term changes in the condylar position following sagittal split ramus osteotomy (SSRO) with rigid fixation. J Oral Rehabil 2010; 37(4): 262−70.
22. *Chen S, Lei J, Wang X, Fu K, Farzad P, Yi B.* Short- and long-term changes of condylar position after bilateral sagittal split ramus osteotomy for mandibular advancement in combination with Le Fort I osteotomy evaluated by cone-beam computed tomography. J Oral Maxillofac Surg 2013; 71(11): 1956−66.
23. *Draenert FG, Erbe C, Zenglein V, Kämmerer PW, Wriedt S, Al Nawas B.* 3D analysis of condylar position after sagittal split osteotomy of the mandible in mono- and bimaxillary orthognathic surgery - a methodology study in 18 patients. J Orofac Orthop 2010; 71(6): 421−9.
24. *Kim Y, Lee Y, Chun Y, Kang N, Kim S, Kim M.* Condylar positional changes up to 12 months after bimaxillary surgery for skeletal class III malocclusions. J Oral Maxillofac Surg 2014; 72(1): 145−56.
25. *Ueki K, Marukawa K, Nakagawa K, Yamamoto E.* Condylar and temporomandibular joint disc positions after mandibular osteotomy for prognathism. J Oral Maxillofac Surg 2002; 60(12): 1424−32.
26. *Hu J, Wang D, Zou S.* Effects of mandibular setback on the temporomandibular joint: a comparison of oblique and sagittal split ramus osteotomy. J Oral Maxillofac Surg 2000; 58(4): 375−80.
27. *Harris MD, Van SJ, Alder M.* Factors influencing condylar position after the bilateral sagittal split osteotomy fixed with bicortical screws. J Oral Maxillofac Surg 1999; 57(6): 650−4.

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Addendum

Abbreviations:

S (sella) – The point representing the geometric center of the *sella turcica*; N (nasion) – The most anterior (midline) point of the frontonasal suture; A (*subspinale*) – The deepest point in the bony concavity in the midline below the anterior nasal spine; Or (*orbitalis*) – The point representing the lowest point on the inferior orbital rim; Po (porion) – The most superior point of the external auditory meatus; Sna (*spina nasalis anterior*) – The most prominent point of maxilla; Snp (*spina nasalis* posterior) – The most distal point of the conjunction of palatinal bone and pterygomaxillar fissure; B (*supramentalis*) – The innermost point on the contour of the mandible between the incisor tooth and the bony chin; Me (menton) – The lowest point of the mandibular symphysis; Go (gonion) – the midpoint of the mandibular angle between the ramus and mandibular body; Cd (condylion) – the most posterosuperior point on head of the condyle; Ar (articularis) – The point midway between the two posterior borders of the left and the right mandibular rami at the intersection with the basilar portion of the occipital bone; DI – The most distal point on the head of the condyle; PI – The most anterior point on the head of the condyle; DC – The center point of the collum mandibulae on the Ba-N line; Xi – The point located at the geographic center of the ramus; Ba (basion) – The point of the anterior margin of the foramen magnum – The midpoint of the curvature between upper and the lower surfaces of the basilar portion of the occipital bone. N-S – The main plane of the anterior cranial base; Go-Me – The main plane of the mandible body; Sna-Snp – The main plane of the maxilla; Cd-DC-Xi – the centerline of the mandibular rami; X axis (Or-Po Frankfort horizontal (FH)) – The horizontal plane of the head; Y axis – The vertical plane which is normal to the X osis and goes from the point S.

ORIGINAL ARTICLE

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Respiratory diseases in preschool children in the city of Niš exposed to suspended particulates and carbon monoxide from ambient air

Respiratorne bolesti dece predškolskog uzrasta u Nišu izložene čestičastim materijama i ugljen-monoksidu iz vazduha okoline

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Abstract

Background/Aim. Analysis of air quality in Serbia indicates that the city of Niš belongs to a group of cities characterized by the third category of air quality (excessive air pollution). The aim of the study was to analyze the degree of causality between ambient air quality affected by particulate matter of 10 μm (PM_{10}) and carbon monoxide (CO) and the incidence of respiratory diseases in preschool children in the city of Niš. **Methods.** We quantified the influence of higher PM_{10} concentrations and carbon monoxide comprising motor vehicle exhausts in the city of Niš on the occurrence of unwanted health effects in preschool children by means of the hazard quotient (HQ), individual health risk (Ri), and the probability of cancer (ICR). The methodology used was according to the US Environmental Protection Agency (EPA), and it included basic scientific statistical methods, compilation methods, and the relevant mathematical methods for assessing air pollution health risk, based on the use of attribute equations. **Results.** Measurement of ambient air pollutant concentrations in the analyzed territory for the entire monitoring duration revealed that PM_{10} concentrations were significantly above the allowed limits during 80% of the days. The maximum measured PM_{10} concentration was 191.6 μg/m^3, and carbon monoxide 5.415 mg/m^3. The incidence of respiratory diseases in the experimental group, with a prominent impact of polluted air was 57.17%, whereas the incidence in the control group was considerably lower, 41.10 %. There were also significant differences in the distribution of certain respiratory diseases. **Conclusion.** In order to perform good causal analysis of air quality and health risk, it is very important to establish and develop a system for long-term monitoring, control, assessment, and prediction of air pollution. We identified the suspended PM_{10} and CO as ambient air pollutants causing negative health effects in the exposed preschool children population.

Key words:
air pollution; air pollutants; serbia; child, preschool; respiratory tract diseases.

Apstrakt

Uvod/Cilj. Analiza kvaliteta vazduha u Srbiji pokazuje da grad Niš spada u grupu gradova sa trećom kategorijom kvaliteta vazduha (značajno zagađenje). Cilj rada bila je analiza stepena uzročne povezanosti kvaliteta vazduha okoline iskazanog kroz sadržaj čestičastih materija (*particulate matter* prečnika 10 μm ili manje – PM_{10}) i ugljen-monoksida (CO) i učestalosti bolesti disajnih organa kod dece predškolskog uzrasta u Nišu. Prikazane su karakteristike vazduha okoline u Nišu i istaknut uticaj saobraćajnih tokova na aerozagađenje. **Metode.** Uticaj povišenih koncentracija PM_{10} i ugljen-monoksida koji se nalaze u sastavu izduvnih gasova motornih vozila na području grada Niša, na pojavu neželjenih zdravstvenih efekata kod dece predškolskog uzrasta, kvantifikovan je pomoću hazardnog koeficijenta (HQ), individualnog zdravstvenog rizika (Ri) i verovatnoće pojave kancera (ICR). Za procenu zdravstvenog rizika korišćena je metodologija Agencije za zaštitu životne sredine SAD (US EPA). **Rezultati.** Merenje koncentracija zagađujućih supstanci u ambijentalnom vazduhu na analiziranoj teriritoriji u celokupnom periodu praćenja pokazalo je da su koncentracije suspendovanih čestica PM_{10} bile znatno iznad dozvoljenih tokom 80% dana. Maksimalna izmerena koncentracija PM_{10} iznosila je 191,6 μg/m^3, a CO 5,415 mg/m^3. Učestalost respiratornih bolesti u eksperimentalnoj grupi, sa izraženim uticajem zagađenog vazduha, iznosila je 57,17%, dok je kod kontrolne grupe ta vrednost bila znatno niža i iznosila je 41,10%. Uočene su i značajne razlike u zastupljenosti pojedinih respiratornih bolesti. **Zaključak.** Za dobru analizu uzročne povezanosti kvaliteta vazduha i zdravstvenog rizika od posebnog je značaja uspostavljanje i razvoj sistema za dugoročno praćenje, kontrolu, procenu i prognozu stanja aerozagađenja. Suspendovane čestice PM_{10} i CO zagađuju vazduh okoline što dovodi do pojave negativnih zdravstvenih efekata izložene populacije dece predškolskog uzrasta.

Ključne reči:
vazduh, zagađenje; vazduh, zagađivači; srbija; deca, predškolska; respiratorni trakt, bolesti.

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Introduction

Analysis of air quality in Serbia conducted by the Serbian Environmental Protection Agency (SEPA) revealed that the city of Niš belongs to a group of cities characterized by the third category of air quality (excessive air pollution) [1]. This air quality category implies that during annual monitoring of the presence of ambient air pollutants one or more pollutants exceed not only the limit values, but also the tolerable concentration levels [2]. Degradation of ambient air quality in the city of Niš is primarily caused by high particulate matter of 10 μm (PM_{10}) concentrations, which are monitored at two measuring points in the city within the state network for automatic air quality monitoring. According to the "Annual Report on the Environment in the Republic of Serbia" by the Ministry of Environment, Mining, and Spatial Planning, the mean annual ambient air PM_{10} concentration in 2010 was 51 μg/m^3 (maximum allowed level: 50 μg/m^3), whereby limit values were exceeded during 123 days [1].

There is a qualitative and quantitative correlation between the emission of pollutants produced by fuel combustion and the air quality in the city of Niš.

Studies of road traffic flows in the city of Niš on the primary road networks showed a particularly high vehicle flow on main city roads. Access roads and highways are dominated by passenger vehicles, which comprise 80% of the total traffic participants. According to the annual reports on air quality in the city of Niš and Niška Banja Spa by the Public Health Institute in the city of Niš, during the latest monitored years, 2008–2011, carbon monoxide (CO) concentration on busy roads exceeded the allowed limits [3, 4].

In order to harmonize the criteria for qualitative-quantitative assessment of urban air quality in EU countries, the CITEAIR project was carried out from 2004 to 2007 with the purpose of determining pollutant concentration levels that would be represented as index values to be used in all EU countries (common air quality index – CAQI). The common air quality index was recommended for use in 2006, and in 2010 it was developed into a system for online comparison of air quality in EU cities. The index was created for easy comparison of air quality and health risk in European cities in real time and it was defined within the Ambient Air Quality Directive 2008/50/EC. The highest index value is determined in relation to the highest concentration of a specific pollutant in ambient air and it is taken as the authoritative factor for the final assessment of air quality and health risk. In order to harmonize domestic legislation with the Directive 2008/50/EC, Serbian official bodies adopted the Regulation on Monitoring Conditions and Air Quality Requirements ("Official Gazette of the Republic of Serbia, No. 11/2010, 75/2010, and 63/2013). The need to proceed with the research that was initiated with the CITEAIR project spurred a couple of domestic projects financed by the Serbian Ministry of Education, Science, and Technological Development as of 2011. The first of these is the project No III-43014, entitled "Improving the System for Monitoring and Assessment of Long-term Population Exposure to Environmental Pollutants By Means of Neural Networks", which was launched by the Faculty of Occupational Safety in the city of Niš. The second one is the project No III-42008, entitled "Assessment of Energy Properties and Quality of Building Interiors in Serbian Educational Institutions with Their Health Effects" and carried out by the Vinča Nuclear Institute in Belgrade.

There are multiple relationships between air quality and health, but their interaction has not yet been sufficiently established and their nature is very difficult to quantify. Certain pollutant concentration levels in ambient air need to be updated regularly and monitored for their health effects as far as current scientific development will allow it [5]. The impact of pollutants on the health of exposed population can be highly complex, which is why air quality assessment is typically determined based on exposure to a single pollutant with the highest concentration [6].

Since no detailed analysis of suspended particulate presence in the immediate vicinity of roads had not been conducted and bearing in mind that PM_{10} concentrations at the measuring points belonging to the state network for automatic air quality monitoring were above the allowed limits, the aim of this paper was to determine a causality between suspended particulates and CO from motor vehicle exhausts and the health of the exposed preschool children aged six or under.

In 2012, as part of the abovementioned projects by the Ministry of Education, Science, and Technological Development, we conducted a research on the causality between the level of ambient air pollution by suspended PM2.5 in and around "Bambi" kindergarten in the city of Niš and the occurrence of respiratory diseases in the exposed preschool children. The available Serbian literature does not cover this type of research, which examines the causality between spatial and temporal relation of measured pollutant concentrations at kindergarten locations and the occurrence of respiratory diseases. The research we conducted showed justifiable to proceed with further research and analysis of the cause-and-effect relationship between increased suspended particulate concentrations and the occurrence of respiratory diseases in preschool children. There is a valid probability that the trend of children suffering from respiratory diseases and the trend of ambient air pollutant concentrations are correlated, which would confirmn a direct causality between air pollution and health risk. The results of this research were published at the Fourth International WeBIOPATRE Workshop & Conference Particulate Matter: Research and Management [7].

The relationship between air quality and the occurrence of health issues in the exposed population is highly complex and this interaction is still in the domain of ongoing scientific research. Health risk has not yet been quantified with complete precision and accuracy, as it cannot be fully ascertained what levels of exposure to an air pollutant would lead to acute or chronic respiratory diseases or to carcinogenic diseases. The complexity of interaction between exposure to pollutants and unwanted health effects depends on whether pollutants are synergistic or additive. Adaptation to new conditions of environmental pollution is fundamentally non-existent; rather, we may speak of tolerance that is qualitatively, quantitatively, and temporally limited. Exhausting the adap-

tation mechanisms, crossing the tolerance threshold, and exceeding the capacity of protective and defence mechanisms causes pathophysiological reactions that manifest, to a greater or lesser extent, through various forms of pathological conditions or diseases of modern civilization, often referred to as environmental diseases.

Methods

The United States Environmental Protection Agency (USEPA) has developed a methodology to assess health risk from air pollution in which all other factors affecting the development of environmental diseases are eliminated. For the assessment of health risk, this paper utilized the US EPA methodology, beginning with hazard identification, risk characterization, and risk quantification [8–10].

Since PM_{10} and CO concentrations monitored in the city of Niš up to 2011 exceeded the allowed limits at most measuring points, they can be considered as ambient air pollutants detrimental to the health of the exposed population. In order to quantify substance hazard in the risk assessment stage, it is necessary to analyze the relationship between different doses and the occurrence of unwanted health effects – risk characterization (Figure 1) [9].

the representative individual in the observed subgroup, represented as y (kg); EDi – exposure duration for the representative individual in environment i (year); ATx – average time of effect duration of pollutant x (days); and EFi – exposure frequency (day/year).

Respiratory rate and distribution and resorption of the inhaled air pollutant vary according to the features of individuals in a subgroup. The average uptake of air pollutants was assessed through parameters for a representative individual in a subgroup. Exposure in relation to average uptake of airborne pollutant x and in relation to the representative individual with average anatomical and physiological features in their subgroup, in environment i, was calculated with the following physical equation [6]:

$$E_{i,x,y} = 0{,}001 \cdot C_{i,x} \left(\frac{IR_y}{BW_y} \right) \left(\frac{ED_i \cdot ET_i \cdot EF_i}{AT_x} \right) \text{mg/kg/day} \quad (2)$$

Ei,x,y – exposure, or the average uptake of pollutant *x* as a function of time, for the representative individual *y* in the observed subgroup in environment *i*.

For air pollutant exposure, the EPA established a reference concentration (R*f*C), which represents the exposure

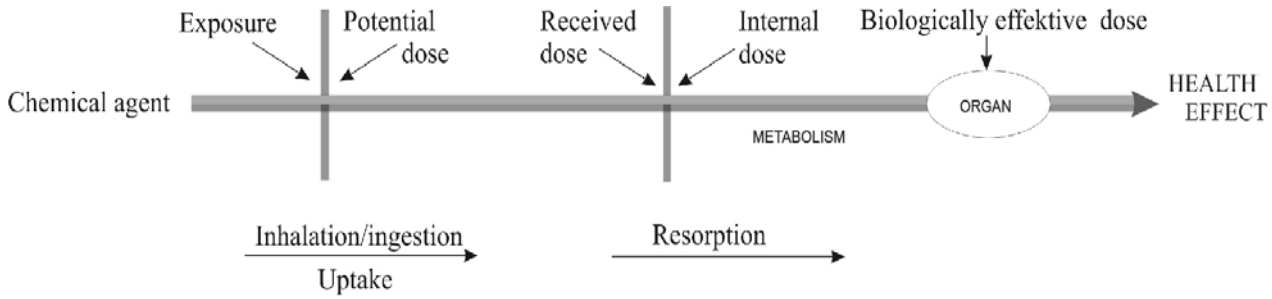

Fig. 1 – Exposure-dose risk characterization.

It is difficult to determine with complete certainty the relationship between a received dose and the response it causes (response or internal dose) because there are certain absorption barriers inside the exposed individual's body that are inaccessible for direct determination of dose level.

According to the methodology for health risk assessment provided by the US EPA [8], the quantity of the exposing chemical agent is related with the uptake dose and its contact with the exposed person's body *per* unit body weight *per* unit time (expressed as mg/kg/day), according to the defined uptake pathway. The uptake dose can be expressed with the equation [5].

$$\text{Uptakedose} = C_{i,x} \left(\frac{IR_y}{BW_y} \right) \left(\frac{ED_i \cdot ET_i \cdot EF_i}{AT_x} \right) [\text{mg/kg/day}] \quad (1)$$

Ci,x – concentration of pollutant x in environment i (mg/m^3); IRy – individual respiratory rate at rest per unit time for a representative individual in the subgroup y in environment i (m^3/day); ETi – exposure time of the representative individual in environment i (days/years); BWy – body weight of

concentration threshold below which, even with continuous inhalation, there are no detrimental health effects, including the highly-sensitive population. R*f*C can be equated with the reference dose (*RfD*) by multiplying the R*f*C with the individual respiratory rate of the individual y (*IRy*) and dividing it with the individual's body weight (*BWy*). When assessing the probability of non-carcinogenic diseases, the risk of non-carcinogenic diseases can be assessed as the ratio of exposure and the corresponding R*f*D for a given pollutant. The increased probability of health risk in the individual y exposed to non-carcinogenic pollutant x in a given subgroup in environment i can be obtained by calculating the health risk hazard quotient (*HQ*) [11–13].

$$HQ_{i,x,y} = \frac{E_{i,x,y}}{RfD} \quad (3)$$

$HQ_{i,x,y}$ – health risk hazard quotient for non-carcinogenic substances (dimensionless quantity).

If the exposure level exceeds this limit, i.e. if the *E/RfD* ratio exceeds 1, there is a possibility of negative non-carcinogenic effects. The bigger the *E/RfD* ratio, the bigger the possibility of detrimental effects.

According to the EPA methodology, health risk assessment for exposure to substances classified as carcinogenic involves quantifying individual health risk based on known exposure using the equation: [7, 10, 14].

$$R_i = Ei,x,y \cdot \text{Toxicity} \quad (4)$$

Individual health risk was expressed as one in a million (10^{-5}) of the probability of occurrence of health risk for asthma or lung cancer, for instance. Individual health risk Ri can be calculated through the potential dose and the SFI (Inhalation Slope Factor) [7, 8, 15]:

$$\text{SFI} = \text{unit risk } (\mu g/m^3)^{-1} \cdot \text{BW (kg)} \cdot \text{IR } (m^3/\text{day})^{-1}. \quad (5)$$

For the assessment of carcinogenic effects due to long-term exposure to pollutants, the potentially higher risk of carcinogenic diseases can be determined as the product of exposure and carcinogenic coefficient, established for every carcinogenic pollutant [8, 16]. The potentially higher risk of an individual in subgroup *y* developing cancer due to exposure to pollutant *x* is:

$$ICR_{i,x,y} = E_{i,x,y} \cdot SF_x \text{ (mg/kg/day)} \quad (6)$$

ICRi,x,y – probability of individual cancer risk for the individual y exposed to pollutant x in environment i; SFx – carcinogenic coefficient of pollutant x.

This paper presented the results of monitoring of suspended PM_{10} in one of the busiest intersections and the assessment of consequent health risk for the exposed group of children through calculation of individual cancer risk probability with the equation (6).

Since concentrations of suspended particulates near busy roads in the city of Niš were not officially monitored, for the 2012 and 2013 study we performed ambient air concentration measurements of particulates 2.5 μm ($PM_{2.5}$) and 10 μm (PM_{10}) in diameter with the help of the Air Pointer automatic measuring station and monitored respiratory diseases in 305 children at the kindergarten "Bambi", subsidiary of the Preschool Institution "Pčelica". The kindergarten "Bambi" is located in a densely populated part of the city with prominently heavy road traffic due to the vicinity of the intersection of Bulevar Nemanjića and Vojvode Mišića Street (Figure 2).

Results

The results of $PM_{2.5}$ monitoring in 2012 and the occurrence of respiratory diseases in the exposed children were published in the paper "Health Effects of Ambient Particulate Matter on Preschool Children in the City Center of Niš" [7].

Since high concentrations of $PM_{2.5}$ suspended particulates, which cause health risk, were registered at the kindergarten "Bambi" in 2012, in 2013 we measured PM_{10}, as well as CO, NO_2, and SO_2 concentrations. Measuring was performed from November 28 to December 27 (data from August are missing because kindergartens are closed during August). The results are given in diagrams (Figures 3–6) as mean 24-hour concentrations.

The results of measured concentrations shown in Figures 3–6 indicate that during the entire monitored month the PM_{10} concentrations significantly exceeded the allowed concentrations or were near the threshold limit values. The maximum registered concentration was 191.6153 μg/m^3, which is triple the allowed value.

Concentrations of CO were also high, occasionally exceeding or nearing the threshold limit values (Figure 4). NO_2 concentrations were below the limit values for the monitored period, with a note that on certain days they neared

Fig. 2 – Micro-location of the kindergarten "Bambi" in the city of Niš.

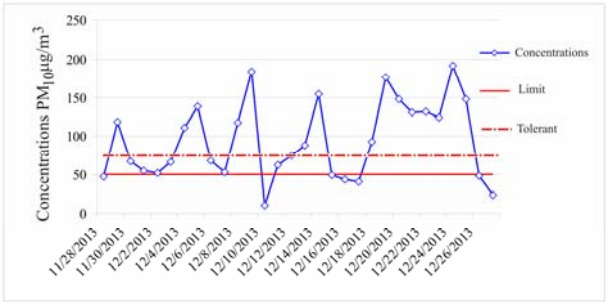

Fig. 3 – Trend of daily mean PM_{10} concentrations between November 28 and December 27, 2013.

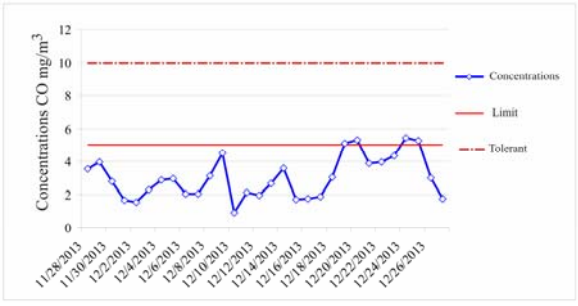

Fig. 4 – Trend of daily mean CO concentrations between November 28 and December 27, 2013.

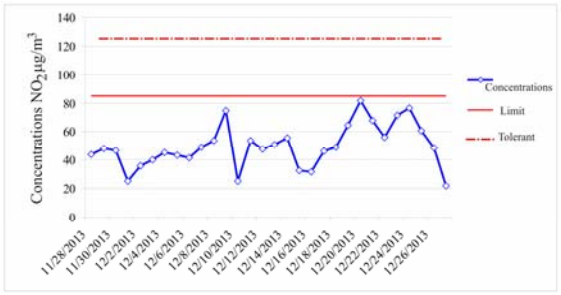

Fig. 5 – Trend of daily mean NO_2 concentrations between November 28 and December 27, 2013.

Fig. 6 – Trend of daily mean SO_2 concentrations between November 28 and December 27, 2013.

the threshold limit values (Figure 5). The SO_2 concentrations were well below the limit values (Figure 6). Such trend of pollutant concentrations can objectively be related to the activity of mobile pollution sources. This is evident in the graphic representation of the direction of pollutants in relation to "Bambi" kindergarten (Figure 2). Emitted PM_{10} concentrations from the east of the kindergarten, where the traffic roundabout is located, exceeded 100 µg/m^3, whereas concentrations emitted from the northwest, where bus stops are located, remained in the proximity of 100 µg/m^3 (Figures 2 and 7). The emission directions for other monitored pollutants were similar to the direction of suspended PM_{10}, which helps us draw a general conclusion that mobile pollution sources are the dominant pollutant emitters in the studied area (Figure 7).

In 2013 we also monitored the monthly incidence of the abovementioned respiratory diseases in children attending the kindergarten "Bambi". The percentage of sick children

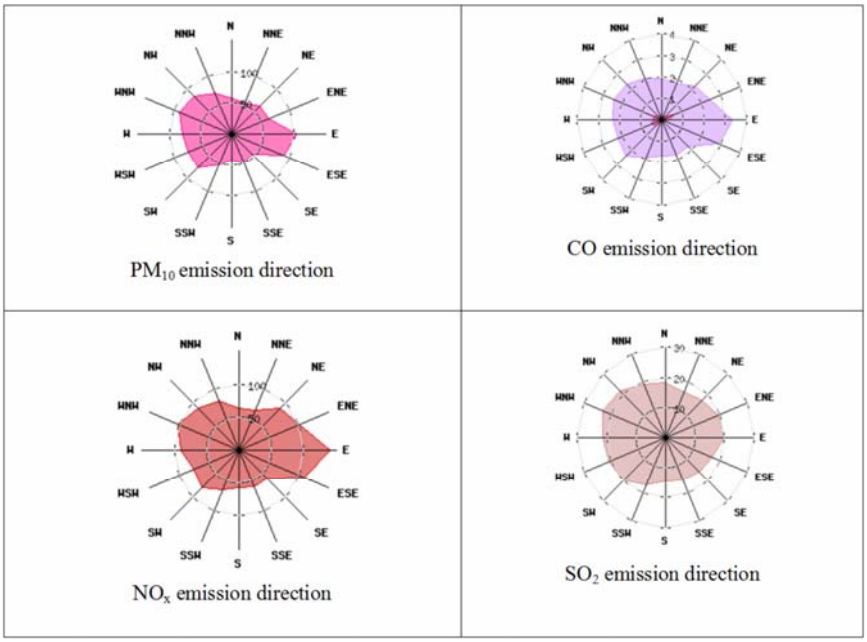

Fig. 7 – Emission directions of pollutants in relation to the kindergarten "Bambi".

was very high, exceeding 30% for the entire monitored period, with the exception of July and September. In February, March, and April, the number of children suffering from respiratory diseases was highest and ranged from 50% to 58% (Figure 8).

Fig. 8 – Percentage of respiratory diseases in the exposed preschool children between January and November 2013.

The incidence of respiratory diseases in preschool children at "Bambi" kindergarten in 2013 is shown in Table 1 by monthly and annual level.

Numerous foreign epidemiological and toxicological studies have proven that suspended PM2.5 and PM_{10} in ambient air contribute to the occurrence and development of carcinogenic diseases, as they contain substances with varying levels of toxicity, depending on their place of origin.

Table 2 shows the individual risk (Ri) calculated by the equations (4) and (5) in relation to measured PM_{10} concentrations at "Bambi" kindergarten (Figure 3) [8, 15, 17]. It is necessary to stress that, according to the US EPA methodology for individual risk assessment, calculated with the equations (4) and (5), risk is determined solely based on exposure to a given pollutant from ambient air, disregarding other risk factors. This allows for exclusive determination of the impact of air pollution on the development of specific respiratory diseases. The probability of individual cancer risk was also calculated by the equation (6) (Table 2). The individual risk and the probability of cancer were calculated for a two-hour exposure to PM10 concentrations for the mean daily concentration of 97.1 $\mu g/m^3$, under the assumption that the children were exposed to the given concentration twice a month during a year. The assumption is based on a long-standing analysis of monitoring the concentration of particulate matter PM10 in ambient air in the city of Niš, at the measuring point located 600 m from the examined kindergarten.

The calculated probability of individual cancer risk ranges from $5.060 \cdot 10^{-5}$ to $9.170 \cdot 10^{-5,}$ which means that 5–9 *per* million exposed preschool children can contract the disease at this level of exposure.

Considering that we registered high daily CO concentrations (for children) at the kindergarten "Bambi" during our monitoring period (November and December 2013) (Figure 4), it is necessary to determine the expected health risk for the

Table 1

Incidence of respiratory diseases in preschool children from the kindergarten "Bambi" in Niš in 2013, monthly and annual levels (%)

Respiratory disease name and ICD	Disease (%) Month										Annually
	I	II	III	IV	V	VI	VII	IX	X	XI	
Laryngitis acuta et Tonsillitis acuta – J02-J03	55.56	52.78	41.67	29.78	30.30	48.89	55.17	35.71	39.47	57.14	44.78
Bronchitis et Bronchiolitis acuta – J20-J21	22.21	5.56	12.50	19.14	27.27	13.32	20.69	42.85	11.76	28.57	20.00
Infectiones tractus respiratorii superioris – J00-J01, J05-J06	0	16.67	25	27.65	27.27	22.22	20.69	7.14	23.68	2.38	18.22
Laryngitis acuta and tracheitis acuta – J04	7 .41	5.56	8.34	3.36	3.03	11.11	0	14.28	15.78	4.76	7.47
Tracheitis and other obstructive lung diseases – J40-J44	7.41	2.78	8.34	3.36	3.03	2.22	3.45	0	0	4.76	3.88
Other nose and paranasal sinus diseases - J30-J31. J33-J34	7.41	0	0	3.36	9.09	2.22	0	0	0	2.38	2.98
Influenza – J10-J11	0	8.33	4.17	2.12	0	0	0	0	0	0	1.49
Pneumonia – J12-J18	0	5.56	0	2.12	0	0	0	0	0	0	0.89
Chronic tonsillar disease and vegetative syndrome – J35	0	2.78	0	0	0	0	0	0	0	0	0.29
Sinusitis chronica – J32	0	0	0	0	0	0	0	0	0	0	0
Bronchial asthma – J45-J46	0	0	0	0	0	0	0	0	0	0	0
Mean incidence:	50.00	66.67	41.38	79.66	58.92	72.58	36.25	28.00	69.09	72.41	57.17

ICD – International classification of diseases.

Table 2

Individual risk and probability of cancer in children exposed to PM_{10} particulates at the kindergarten "Bambi"

Children's age (years)	Individual risk R_i (%)	Probability of individual cancer risk (ICR)
Under 1	2.124	$5.060 \cdot 10^{-5}$
1 to 3	11.59	$7.880 \cdot 10^{-5}$
3 to 6	28.51	$9.170 \cdot 10^{-5}$

exposed children. To assess health risk from carbon monoxide, we used the mean annual concentrations monitored at 16 measuring points in 2011 (the last monitored year in the city of Niš), with the help of which we predicted the concentrations at the kindergarten "Bambi" using a radial basis function (RBF) network and subsequently mapped air quality. It is worth noting that no air pollutant was monitored in kindergarten locations in the monitoring network of the city of Niš. Accordingly, as the result of the project No III-43014, we implemented an original methodology of mapping air quality, which enabled us to predict CO diffusion in the analyzed territory by using an radial basic function (RBF) network. The map of air quality is shown in Figure 9, with the purple, red, and orange fields representing health risk zones in which CO concentrations range from 3 to 9.7 mg/m^3. Such high CO concentrations cause unwanted health effects, especially in highly sensitive population groups such as preschool children. The effects could be heavy breathing, chest "pressure", and fatigue and chest pain in people with respiratory diseases.

If we use the mean annual concentration for 2011, which is 7.769 mg/m^3, to assess health risk, we can calculate the health risk HQ for long-term exposure of preschool children at the kindergarten "Bambi". The health risk HQ calculated by the equation (3), equals 2.96 for children aged 3 to 6, assuming they were exposed for two hours during a day, which places the risk in the "high" category. If we assume that preschool children aged 3 to 6 are exposed to mean annual CO concentrations of 7.769 mg/m^3 for 4 hours *per* day, the HQ is 5.88 and the health risk is considered to be "very high" [10, 18, 19].

In order to obtain the best possible assessment of the causality between health risk from CO exposure and the occurrence of respiratory diseases in preschool children, we also monitored the number of children with respiratory disease at the kindergarten "Maslačak" in 2013. We selected the children from the kindergarten "Maslačak" to be the control group, as this kindergarten, similarly to "Bambi", is located in a central densely-populated part of the city and is surrounded with high-rise residential buildings (Figure 10).

The number of analyzed children at the kindergarten "Maslačak" was 310, which was very close to the number of analyzed children at the kindergarten "Bambi" (305). Hygienic-sanitary and socio-economic conditions in both kindergartens were almost identical, and the percentage of

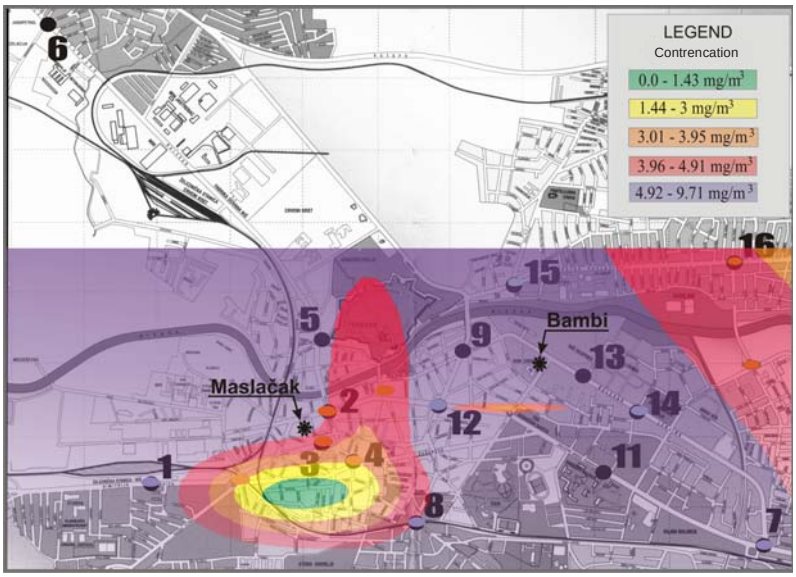

Fig. 9 – Air quality zone map with approximate CO concentrations at kindergartens "Bambi" and "Maslačak" in the city of Niš, 2011.

Fig. 10 – Micro-location of the kindergarten "Maslačak" in the city of Niš.

non-smoking family members of the exposed children was also approximately the same (Table 3).

The only significant difference between the two kindergartens was their distance from busy roads. The busiest road along the dominant wind direction in relation to the kindergarten "Maslačak" is 126 m away, whereas the busiest road near "Bambi" is only 15 m away. Table 4 shows the mean 15-minute CO emission concentrations as well as concentrations at distances of 15 and 100 metres from the nearest road to the kindergartens "Bambi" and "Maslačak". The calculation of concentrations was done by means of the Screening Air Dispersion Model (SCREEN) [11].

The mass of CO emission on the road near the kindergarten "Bambi" was larger than the mass near "Maslačak". Based on the above calculations, CO concentration at the kindergarten "Maslačak" was significantly lower compared to "Bambi", which is in keeping with the CO concentration prediction for "Maslačak" by means of the RBF network (Figure 9).

Using the RBF network at the kindergarten "Maslačak", we predicted the mean annual CO concentration to be 4.91 mg/m^3 (Figure 9). Based on mean annual concentrations, the health risk HQ, calculated by the equation (3), equals 1.87 for children aged 3–6, assuming they were exposed for 2 h per day (health risk is "moderately high"). For 4-hour daily exposure, the HQ is 3.72 (health risk is "high") [19].

In 2013 we also monitored the monthly occurrence of respiratory diseases in children attending the kindergarten "Maslačak", and the percentage is shown in Figure 11.

The flowchart of respiratory diseases shown in Figure 11 reveals that the number of affected children is higher during the winter months. The winter percentage of diseases ranges from 26.80% to 40.21%. Table 5 shows the incidence of respiratory diseases found in children at the kindergarten "Maslačak".

Discussion

According to the Regulation on Monitoring Conditions and Air Quality Requirements ("Official Gazette of the Re-

Fig. 11 – Percentage of respiratory diseases in the exposed preschool children at the kindergarten "Maslačak" between January and November 2013.

Table 3

Percentage of smokers in the families of children from the "Bambi" and "Maslačak" kindergartens in the city of Niš

Children's age (years)	Number of smoking family members							
	"Bambi" kindergarten				"Maslačak" kindergarten			
	One	Two	Three or more	No smokers	One	Two	Three or more	No smokers
3	26.09	3.35	4.38	66.18	16.20	2.70	-	81.10
4	20	6.15	3.07	70.78	32.65	16.32	2.05	48.98
5	18.66	4	0	77.34	21.06	14.70	-	64.24
6	39.34	4.92	0	55.74	19.64	17.85	1.78	60.73
Average percentage	26.02	4.6	3.72	65.66	22.39	12.98	1.91	62.72

Table 4

Calculated 15-minute CO emission concentrations on busy roads in the city of Niš

Location	Distance from source [m]	Concentration [mg/m^3]	Emission [kg]
Roundabout near the kindergarten "Bambi"	15	35.66	3.935
	100	1.72	
Road near the kindergarten "Maslačak"	15	22.99	2.546
	100	0.691	

Table 5

Incidence of respiratory diseases in preschool children (control group) at the kindergarten "Maslačak" in the city of Niš in 2013, monthly and annual level (%)

Respiratory disease name and code	Disease (%) Month										Annually
	I	II	III	IV	V	VI	VII	IX	X	XI	
Laryngitis acuta and Tonsillitis acuta – J02-J03	0	0	0	83.34	60.00	51.87	61.11	50.00	53.85	56.53	46.63
Infectiones tractus respiratorii superioris - J00-J01, J05-J06	96.67	7.14	0	11.11	22.86	14.81	38.89	27.78	30.76	13.04	32.21
Bronchitis et Bronchiolitis acuta – J20-J21	0	57.15	0	5.55	8.57	14.81	0	11.11	15.39	21.74	12.50
Laryngitis acuta and tracheitis acuta – J04	3.33	35.71	0	0	5.71	14.81	0	0	0	8.69	6.73
Tracheitis and other obstructive lung diseases – J40-J44	0	0	0	0	0	0	0	11.11	0	0	0.96
Sinusitis chronica – J32	0	0	0	0	2.86	0	0	0	0	0	0.48
Bronchial asthma – J45-J46	0	0	0	0	0	0	0	0	0	0	0.48
Other nose and paranasal sinus diseases – J30-J31. J33-J34	0	0	0	0	0	0	0	0	0	0	0
Influenza – J10-J11	0	0	0	0	0	0	0	0	0	0	0
Pneumonia – J12-J18	0	0	0	0	0	0	0	0	0	0	0
Chronic tonsillar disease and vegetative syndrome – J35	0	0	0	0	0	0	0	0	0	0	0
Mean incidence:	57.70	26.92	0	34.61	67.30	51.92	34.61	33.33	74.28	53.49	41.10

public of Serbia, No. 11/2010, 75/2010, and 63/2013) [2], the PM_{10} concentration of 50 μg/m^3 must not exceede that value more than 35 times within a single calendar year. Suspended particulate concentrations at the kindergarten "Bambi" during the 29 monitored days exceeded 50 μg/m^3 on 23 days. The tolerable limit value of 75 μg/m^3 was exceeded on 16 days during the monitored period. Such high PM_{10} values can cause serious health risk in the exposed population, to an even greater extent. The calculated percentage of individual risk applies only to a 2-hour exposure to high concentrations twice a month.

Based on the results of earlier studies it is estimated that approximately 3% of cardiopulmonary and 5% of lung cancer deaths are attributable to PM globally. In the European Region, this proportion is 1–3% and 2–5%, respectively, in various subregions [20].

Results emerging from a recent study indicate that the burden of disease related to ambient air pollution may be even higher. This study estimates that in 2010, ambient air pollution, as annual $PM_{2.5}$, accounted for 3.1 million deaths and around 3.1% of global disability-adjusted life years.

Exposure to $PM_{2.5}$ reduces the life expectancy of the population of the Region by about 8.6 months on the average. Results from the scientific project Improving Knowledge and Communication for Decision-Making on Air Pollution and Health in Europe, which uses traditional health impact assessment methods, indicate that the average life expectancy in the most polluted cities could be increased by approximately 20 months if the long-term $PM_{2.5}$ concentration was reduced to the WHO (AQG) annual level [21].

Adverse health effects from other gaseous pollutants, such as SO_2, nitrogen dioxide (NO_2), and carbon monoxide (CO), should not be underappreciated. Recent epidemiologic studies conducted throughout the world have provided valuable insight into the associations between SO_2, NO_2, and CO exposure and increases in cardiopulmonary mortality, respiratory and cardiovascular hospital admissions, emergency admissions caused by stroke (NO_2), and myocardial infarction (NO_2 and CO) [22].

The study "Air pollution and sudden infant death syndrom" [23] nevertheless have not been able to identify the specific components of particulate matter and carbon monoxide, nor elucidate the mechanism by which these pollutants affect health in children and infants, which may be different from adults.

Our study shows that mean annual CO concentrations in ambient air for the analyzed location of the kindergarten "Bambi" (7.769 mg/m^3) are 63.20% higher than the same concentrations at the kindergarten "Maslačak" (4.91 mg/m^3). By comparing the difference of the hazard quotient and health risk in exposed children from the two kindergartens, we concluded that the health risk for children at the kindergarten

"Bambi" is 63.18% higher for a 2-hour exposure and 63.26% higher for a 4-hour exposure. If we compare the values of the average respiratory disease incidence monitored in the kindergartens, we can conclude that the average incidence at the kindergarten "Bambi" is 57.15%, as opposed to 41.10% at the kindergarten "Maslačak", which is a 16.05% difference. It is also significant that there is 3% higher percentage of non-smoking family members of the children attending the kindergarten "Bambi". According to the presented results, there is a causality between pollutants emitted from mobile sources and the occurrence and development of respiratory diseases in preschool children. A direct link between the percentage of occurrence and development of a specific respiratory disease and the concentration of a specific pollutant in ambient air is still the subject of ongoing scientific research.

The mean annual incidence of respiratory diseases in the experimental group, with prominent effects of polluted air, was 57.17%, whereas the value was considerably lower in the control group – 41.10%. We also noticed significant differences (up to four times higher incidence) in certain respiratory diseases – for instance, *Bronchitis et Bronchiolitis acuta*, 12.50% in the control group and 20.00% in the experimental group or *Tracheitis* and other obstructive lung diseases, 0.96% in the control group and 3.88% in the experimental group. Some respiratory diseases, such as Other nose and paranasal sinus diseases, code J30-J31 and J33-J34, and Influenza, code J10-J11, were not even registered in the control group. Monthly monitoring indicates considerable seasonal fluctuations of respiratory disease incidence.

The probability of individual cancer risk, caused by PM_{10} concentrations in the experimental area, ranges from $5.060 \cdot 10^{-5}$ to $9.170 \cdot 10^{-5}$ (5–9 *per* million exposed preschool children).

Conclusion

Based on the presented results and the discussion, we unequivocally conclude that there is a directly proportional relationship between ambient air quality and respiratory diseases in preschool children.

Development of a network and system for monitoring ambient air quality in urban areas can significantly contribute to respiratory disease prevention, primarily for preschool and school children.

R E F E R E N C E S

1. SEPA. Annual Report on the Environment in the Republic of Serbia for 2010. Available from: www.sepa.gov.rs/download/Izvestaj_o_stanju_zivotne_sredine_za_2010_godinu.pdf
2. Regulation on Monitoring and Air Quality Requirements. Official Gazette RS; 11/2010, 75/2010 and 63/2013.
3. Institute of Public Health Niš. Annual reports on air quality in the city of Niš. Available from: www.izjz-nis.org.rs
4. *Đorđević A, Milošević L., Rašić M.* Analysis of gaseous substance toxicity assessment. J Sci Eng Safety Eng 2013; 3(1): 17−21.
5. *Longhurst* J. 1 to 100: Creating an Air Quality Index in Pittsburgh. Environ Monit Assess 2005;106(1−3): 27−42.
6. *Cairncross EK, John J, Zunckel M.* A novel air pollution index based on the relative risk of daily mortality associated with short-term exposure to common air pollutants. Atmos Environ 2007; 41: 8442−54.
7. *Đorđević A, Živković N, Milošević L., Mijailović I, Mihajlović E.* Health effects of ambient particulate matter on preschool children in the City Center of Niš, Serbia. Proceedings from the 4th WeBIOPATR workshop & conference; Belgrade, Serbia; 2013 October 2−4; Belgrade: WeBIOPATR2013, Particulate Matter Research and Management; 2013. p. 209−16.
8. *United States Environmental Protection Agency.* Chemicals under the Toxic Substances Control Act (TSCA). Available from: http://www.epa.gov.
9. *Baldauf RW, Lane DD, Marote GA.* Ambient air quality monitoring network design for assessing human health impacts from exposures to airborne contaminants. Environ Monit Assess 2001; 66(1): 63−76.
10. Integrated Risk Information System (IRIS). U.S. EPA. Available from: https://www.gov.com
11. *Papić V, Vidović M.* Determining the Quantity of Gaseous Pollutants Emitted from Road Traffic Using the COPERT 4 Model of the European Environment Agency. Belgrade: University of Belgrade, Faculty of Transport and Traffic Engineering, Institute of Faculty of Transport and Traffic Engineering. 2010. (Serbian)
12. *Đorđević A.* Causal Analysis of Air Quality and Health Risk from Air Pollution [dissertation]. Niš: Faculty of Occupational Safety; 2010. p. 299.
13. Federal contaminated site risk assessment: Guidance on human health preliminary quantitative risk assessment (PQRA), Environmental Health Assessment Services Safe Environments Programme. Cat. H46-2/04-367E ISBN 0-662-38244-7. Ottawa, Canada: Minister of Health; 2004.
14. *Sills R, Hultin ML., Depa M.* General Approach for the Screening-Level Risk Assessment of Air Toxics Mixtures and Cumulative Risk Assessment. Lansing: MDEQ AQD; 2008.
15. *Sills R, Sadoff M.* Risk Assessment for Lead Emissions of the Wolverine Clean Energy Venture (WCEV). Lansing: MDEQ AQD; 2008.
16. *Baldauf RW, Lane DD, Marote GA.* Ambient air quality monitoring network design for assessing human health impacts from exposures to airborne contaminants. Environ Monit Assess 2001; 66(1): 63−76.
17. *Đorđević A, Todorović B, Živković N, Raos M, Milošević L.* Determination of Health Risk Zones of Air Pollution in the City of Niš caused by Presence of Soot by Use of RBF Neural Network. Facta Universitatis 2013; 4(2): 119−28.
18. *Đorđević A, Živković N, Mihajlović E, Radosavljević J, Raos M, Živković L.* The effect of pollutant emission from district heating systems on the correlation between air quality and health risk. Thermal Science 2011; 15(2): 293−310.
19. *Đorđević A, Milošević L., Krstić I. Rašić M.* Exposure Assessment for Characterization of Health Risk From Air Pollution. Proceedings, III International Conference „Ekology of Urban Areas 2013"; Zrenjanin; 2011 December 7; Zrenjanin: University of Novi Sad, Technical Faculty „Mihailo Pupin"; 2013. p. 105−11.
20. *Cohen AJ, Anderson RH, Ostro B, Pandey KD, Krzyzanowski M, Künzli N,* et al. Urban air pollution. In: *Ezzati M A, Lopez DA, Rodgers A, Murray C,* editors. Comparative quantification of health risks. Global and regional burden of disease attributable to selected major factors.Geneva: World Health Organization; 2004; p. 1353–433.

21. *Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H*, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380(9859): 2224−60.
22. *Koken PJ, Piver WT, Ye F, Elixhauser A, Olsen LM, Portier CJ.* Temperature, air pollution, and hospitalization for cardiovascular diseases among elderly people in Denver. Environ Health Perspect 2003; 111(10): 1312−7.
23. *Tong S, Colditz P.* Air pollution and sudden infant death syndrome: a literature review. Paediatr Perinat Epidemiol 2004; 18(5): 327−35.

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Differences in postural disturbances between female adolescents handball players and nontraining peers

Razlike u posturalnim poremećajima između rukometašica i netreniranih adolescentkinja

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Abstract

Background/Aim. Physical activity and sport can influence the extent of the presence of the postural disturbances in children. The aim of this study was to investigate the occurrence of differences in the postural disturbances in female adolescents in relation to team handball training. **Methods.** This investigation involved 150 female adolescents with the average age of 13.2 ± 1.34 years divided into two groups (50 adolescents trained handball and 100 did non train it). **Results.** The study determined a statistically significant difference in the total number of postural disturbances between the two groups of adolescents ($p < 0.001$). The presence of the flat foot was statistically significantly higher in untrained adolescents ($p < 0.001$), but the presence of the scoliosis, kyphosis, lordosis, and *pes varus* was not found ($p > 0.05$). **Conclusion.** Handball adolescents players have less postural disturbances than untrained adolescents. Flat foot is significantly less frequent in female adolescents handball players than in untrained ones. Findings obtained in this investigation can help us in planning continuous prevention, observation and care for untrained and trained team handball female adolescents with postural disturbances.

Key words:
adolescent; athletes; female; spinal curvatures; scoliosis; kyphosis; foot deformities.

Apstrakt

Uvod/Cilj. Fizička aktivnost i sport mogu uticati na pojavu posturalnih poremećaja kod dece. Cilj studije bio je da se istraži postojanje razlika u posturalnim poremećajima kod adolescentkinja koje treniraju i njihovih vršnjakinja koje ne treniraju rukomet. **Metode.** U ovo istraživanje bilo je uključeno 150 adolescentkinja prosečne starosti 13,2 ± 1,34 godina, podeljenih u dve grupe (50 adolescentkinja koje su trenirale rukomet i 100 njihovih vršnjakinja koje nisu trenirale ovaj sport). **Rezultati.** Istraživanjem je utvrđena statistički značajna razlika u ukupnom broju posturalnih poremećaja između dve grupe adolescentkinja ($p < 0,001$). Ravno stopalo bilo je statistički značajno češće prisutno kod adolescentkinja koje ne treniraju ($p < 0,001$), nego kod adolescentkinja koje treniraju rukomet. Za skoliozu, kifozu, lordozu i *pes varus* razlika nije bila statistički značajna ($p > 0,05$). **Zaključak.** Adolescentkinje koje treniraju rukomet imaju manje posturalnih poremećaja od onih koje ne treniraju. Ravno stopalo je značajno ređe zastupljeno kod rukometašica nego kod njihovih vršnjakinja koje ne treniraju. Rezultati ovog istraživanja mogli bi pomoći u planiranju stalne prevencije, praćenja i lečenja adolescentkinja sa posturalnim poremećajima, bilo da one treniraju ili ne treniraju rukomet.

Ključne reči:
adolescenti; sportisti; žene; kičma, krivine; skolioza; kifoza; stopalo, deformacije.

Introduction

Postural disturbances can become more pronounced during the growth and development, especially in school children, under the influence of various internal and external factors.

Common postural disorders are postural disorders of the spine (scoliosis, kyphosis, lordosis) and postural disorders in the lower extremities. Principles for normal posture are optimal load on the skeletal system, balans between antagonistic muscle groups and optimal activity for internal body systems.

In postural disorders there is an imbalance in the loads imposed on different areas. Where loads exceed normal physiological limits consistently and over prolonged periods of time, structural changes occur in the skeletal bones. The damage of this type is usually irreversibile [1, 2]. Postural disorders can develop in variety of forms, but most types can be

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classified as either functional (flexible) [3] or structural (rigid) postural disorders [4]. Increase of the physiological thoracic kyphosis angle may be attributed to an alteration in the supporting anterior and posterior soft tissues and musculature. Lumbar lordosis is a key postural component. There is evidence that many individual factors, such as age, gender, muscular strength, activity, sport and flexibility of the spine and lower extremities [5] may affect the lumbar lordosis angle. It is worthy of consideration whether the degree of thoracic kyphosis and lumbar lordosis depends on one's style of life, for example, taking part in sports almost every day [6].

Several studies [7, 8] suggested that the pediatric flat foot as postural disorder in the lower extremities is a frequent presentation in clinical practice. Potential pain and disability are the reasons to discuss prevention and treatment of this problem. Although definition of flat foot varies under different classifications of this entity, it is widely accepted that the low medial arch, *valgus* heel position and the foot flexibility are consistent atributes [8].

Postural status is the result of earlier growth and development, but also of dominant physical activity or a sport and individual practices [9]. Systematic physical activity is a necessary element of normal development of a young human body [10]. From early childhood, people should participate in sports and recreational activities involving movement. However, organised and systematic learning of a given form of motor activity (physical training), particularly participation in sport competitions, may result in development of certain abnormalities connected with excessive physical load of a young body.

The guidelines of the International Scientific Society on Scoliosis Orthopaedic and Rehabilitation Treatment (SOSORT) recommend that sports are not prescribed as a treatment for idiopathic scoliosis. It is recommended that general sport activities are performed because of the specific benefits they offer to patients in terms of psychological, neuromotor and general organic well-being and that during all treatment phases physical education at school is continued. Based on the severity of the curve and progression of the deformity and the opinion of a clinician specialized in conservative treatment of spinal deformities, restrictions may be placed on practicing certain types of sport activities. It is recommended that sport activities are continued also during brace treatment because of the physical (aerobic capacity) and psychological benefits these activities provide. The SOSORT recommends that during brace treatment, contact or highly dynamic sport activities are performed with caution and that competitive activities that greatly mobilize the spine are avoided in patients with scoliosis at high risk of progression [11].

There are different results in the literature about the effect that various sports can have on the prevalence of postural disturbance in children. Sports associated with jumping and marked stress for the spine must be warned against in the Scheuermann's disease [12]. A high frequency of thoracic hyperkyphosis in the standing position was observed, whereas predominately neutral values were found in the lumbar spine [13]. Adolescent dancers are at significantly higher risk of developing scoliosis than nondancers of the same age (dancers were 12.4 times more likely to have scoliosis than nondancers of the same age) [14].

Different types of competitive sports exert different effects on the various subsystems of posture control [15]. Postural deformities of the locomotor apparatus among athletes from different sports were the subject matter of many studies [12–14, 16–19]. Postural disorders and asymmetry of the musculoskeletal system occur both in the general population and among athletes [20]. The studies were mostly conducted with the aim of comparatively analyzing posture in athletes and their non-athlete peers, or analyzing some of the postural disturbance among athletes (adolescents) from different sports.

The scientific knowledge regarding female adolescent handball team demands is limited. There are few data in the literature about the effect of the handball training on the occurrence of postural disturbance in female adolescents [16, 17]. In these investigations various samples, methods and parameters for assessment of postural disturbances were used, as well as for assessment of postural disturbances in athletes that trained various sport.

But, there are no investigations that analyzed the shape of the spine in the sagittal and frontal plane, together with assymetry of shoulder, scapulas, pelvic position, asymmetry of waist (Lorenz's triangle) and asymmetry of line of the Achilles tendon, or assessments of feet disturbance.

That's why the aim of this study was to investigate the occurrence of differences in the presence of postural disturbances, specifically in the spine and feet, in trained and untrained team handball female adolescents.

The findings obtained in this investigation could give us information about potential disturbances related to training handball and for planning continuous care for adolescents with postural disturbances, trained and untrained female adolescent team handball players. These findings could also show a total number of postural disturbances and which postural disturbances are less frequently seen in female handball players.

Methods

Subjects and design

In this investigation we included 150 female adolescents, average age 13.2 ± 1.34 years (range 12–13 years), divided into two groups. The group I included 50 female adolescents with the average age of 13.4 ± 1.5 years that participated in team handball training in local clubs for at least 2 years. The group II included 100 female school adolescents, average age 13.1 ± 1.7 years, that did not train team handball or any other sports, but were occupied with usual physical activities for that age. The groups were similar on all demographic characteristics.

Exclusion criteria were female adolescents with congenital musculoskeletal deformities, other deseases or injuries that resulted in deformities, disturbance of function of the musculoskeletal system and subjects with shoulder pain. All the participants underwent physical examination of the spine and feet. Estimate of the child conditions with spinal

and feet deformity included medical history, clinical physical, orthopaedic, pediatric, neurology examination and diagnostic tests.

The posture was examined visually and with special tests. Standardized physical examination of the spine to diagnose scoliosis included evaluation of patient posture in a standing position (inspection from behind, from the side, from the front and palpation). Physical assymmetry is examined in the following areas: shoulder height, scapulas position, chest area, pelvic and hip position, asymmetry of waist (Lorenz's triangle) and lateral deviation of the spinal column. Physical examination included examination of vertebral rotation (rib hump) with the Adam's forward bending test. The patients were in standing position, bending torso forward, legs are together, knees extended and shoulder relaxed. Practitioner made evaluation of imbalance or protuberance (hump) in the upper back area or prominence in the lumbar area.

Physical examination of the spine to diagnose kyphosis and lordosis included evaluation of patients posture in a standing position. We asked the patients to stand and looked at the the spine from the side. Although normal posture is difficult to define, we made assessment of the thoracic and lumbar curvatures, noting whether the curve is quite regular or apparently increased.

Physical examination of the feet was performed visually and with special tests. We estimated medial arch, *valgus* heel position and the foot flexibility. We asked patients to try to arch the foot. In mobile flat foot the arch can often be restored voluntarily. In estimate of posture we looked at the foot from behind, paying particular attention to the slope of the heels. Valgus heels are associated with *pes planus* [7].

Testing procedures

Modified original physical activity and postural disturbance test which was developed from the classification of musculoskeletal disturbance was used. The original physical activity and postural disturbance test contains also the part with questions about physical activity, which we did not study in this work.

Postural disturbance test contains information about age, gender and 10 sections. Five sections are for assessment of the body asymmetry and degree of disappearance when child actively corrected: asymmetry of the scapulae, shoulder girdle, waistline (Lorenz's triangles), pelvic alignment in the frontal plane-anterior superior iliac spine (ASIS) and asymmetry of line of the Achilles tendon. Three sections are intended for assessment of spine postural disturbances and flexibility (scoliosis, kyphosis and lordosis) and 2 sections for assessment of of feet disturbance and flexibility (flat feet and *pes varus*) [5–7] using a rating on the scale from 0 to 2. "0" estimate normal findings, "1" mild degree and flexible postural disturbance an "2" clear, nonflexible deformity.

In a spontaneous standing position obvious asymmetries were noted along the contour of the back: elevation of a shoulder, prominence of a scapula, uneven waistline, levels of the anterior superior iliac spines or a rib hump, asymmetry of the line of the Achilles tendon and the degree of disappearance when a child actively corrected [3, 21]. Visual (scaled) observations [8] and the test of contraction of plantar flexor muscles were performed for physical examination of the feet (if an arch is reconstituted on toe standing, then it is termed a flexible flat foot) [21]. A pedoscope was used to see the pattern of weight distribution in the foot. We registred an increase in the area of central part of sole taking part in weight-bearing.

For assessment of spine postural disturbances and flexibility we performed tests for assessment of spine flexibility: test of lying in a prone position, test of hanging position [22], the Adam's forward bending test [3, 4, 11]. Children were classified as having normal findings (estimated „0"), flexible postural disturbance or mild asymmetry (grade „1") or structural, clear, nonflexible deformity or asymmetry (grade „2").

Informed consent was obtained from all the subjects. All parameters that were collected, age, clinical examination and testing procedures are the part of the regular clinical and ethical procedures in medical practice.

Statistical analyses

To assess the occurrence of differences in postural disturbances and symmetries between team handball trained and nontrained female adolescents, χ^2 test and Student *t*-test were used. Statistical significance of differences was on the level of $p < 0.05$.

Results

The characteristics of the study participants are shown in Table 1. The female handball players had 64 (27.8%) and untrained 166 (72.2%) of the total number of disturbances in the total sample of participants. As we can see, there was a statistically significant diference in the total number of postural disturbances in trained *vs* nontrained female athletes ($p < 0.001$). The presence of the flat foot was statistically significantly higher in untrained adolescents ($p < 0.001$). Difference between groups regarding scoliosis, kyphosis, lordosis, and *pes varus* was not statistically significant ($p > 0.05$).

Table 2 presents the results obtained by Student *t*-test. As we can see, there is a significantly higher asymmetry of the prominence of the scapula in the handball female adolescent players ($p < 0.05$), but not in the shoulder girdle ($p > 0.05$). Compared to the untrained peers, there is a statistically significantly higher asymmetry of the pelvic alignment in the frontal plane ($p < 0.05$) and the line of the Achilles tendon in the control group of adolescents compared to the handball players ($p < 0.05$).

Discussion

Modern life style has reduced physical activity in children. The various sports have an influence on the musculoskeletal system. There is evidence that school children who are not actively involved in sport activities have significantly higher probability of poor posture than children performing sports [23], which is in accordance with our findings.

Table 1

Characteristics of the study participants (n = 150; 50 female handball players and 100 female untrained adolescents)

Parameter	Group	Score			Total	Statistical analysis χ^2 test
		0	1	2		
Age (years), $\bar{x} \pm SD$	handball		13.4 ± 1.5		13.2 ± 1.3	
	control		13.1 ± 1.7			
Scoliosis, n (%)	handball	34 (68)	12 (24)	4 (8)	50 (100)	$p > 0.05$
	control	70 (70)	29 (29)	1 (1)	100 (100)	
Kyphosis, n (%)	handball	40 (80)	8 (16)	2 (4)	50 (100)	$p > 0.05$
	control	80 (80)	19 (19)	1 (1)	100 (100)	
Lordosis, n (%)	handball	40 (80)	8 (16)	2 (4)	50 (100)	$p > 0.05$
	control	72 (72)	25 (25)	3 (3)	100 (100)	
Flat foot, n (%)	handball	24 (48)	16 (32)	10 (20)	50 (100)	$p < 0.001$
	control	16 (16)	67 (67)	17 (17)	100 (100)	
Pes varus, n (%)	handball	48 (96)	2 (4)	0 (0)	50 (100)	$p > 0.05$
	control	96 (96)	4 (4)	0 (0)	100 (100)	
Total of postural disturbances, n (%)	handball		46 (71.9)	18 (28.1)	64 (27.8)	$p < 0.001$
	control		144 (86.7)	22 (13.3)	166 (72.2)	
Total, n (%)			190 (82.6)	40 (17.4)	230 (100)	

$\bar{x}$ ± mean value; SD – standard deviation.

Table 2

Assessment of symmetry of the scapulas, shoulders, pelvis and Achilles tendon of the handball players and untraining peers (score from 0–2, where 0 is a normal finding)

Symetries	No trained female adolescents	Adolescent handball players	*t* (Student's *t*-test)	*p* value
	Average score (± SD)	Average score (± SD)		
Scapula	0.24 (0.52)	0.56 (0.77)	-1.89	< 0.05
Shoulder girdle	0.22 (0.51)	0.12 (0.33)	0.39	> 0.05
Waistline (Lorenz's triangle)	0.08 (0.28)	0.08 (0.27)	0	> 0.05
Pelvic alignment in the frontal plane-ASIS	0.06 (0.24)	0 (0)	1.77	< 0.05
Line of the Achilles tendon	0.48 (0.77)	0.12 (0.44)	2.38	< 0.05

ASIS – anterior superior iliac spine; SD – standard deviation.

A total number of postural disturbances in female handball adolescents was significantly lower than in the control group in our research. This could be explained by involvement of various muscle groups, as well as by higher spine mobility during team handball training. The total sample contained a lower percentage of lordosis and kyphosis in our investigation compared to previous study [23]. These differences could be explained by differences in the samples.

Scoliosis has been found in up to 80% of athletes with an asymmetric load on the trunk and shoulders, such as javelin throwers and tennis players [24]. A 10-fold higher incidence of scoliosis was found in rhythmic gymnastic trainees [25]. But, research that investigated the prevalence of kyphosis, hyperlordosis and back asymmetry in children playing and not playing sports reported that the incidence of back asymmetry was higher in girls playing basketball. Tennis was found not to be a suitable sport for either male or female prepuberal children. In females the incidence of hyperlordosis did not correlate with any of the sports [26].

Scoliosis was found in 32% of the female handball players in our research (24% flexible and 8% nonflexible) and in 30% of adolescents in the control group (29% flexible and 1% nonflexible). Nonflexible scoliosis was found in a higher percentage (8%) of female handball players in our research than in non-training peers (1%). An overall prevalence of adolescent idiopathic scoliosis (AIS) is 0.47–5.2% in the current literature [27]. These results may be because of the size of the samples, other factors or potentially related to the overheadthrowing motion that is a highly repetitive skilled motion performed at high velocities.

We did not find a statistically significant diference in the number of scoliosis in female adolescent players in relation to nontraining peers that is in accordance with other report, showing that systematic exercising is probably not associated with the development of AIS [28].

Young athletes may have a spinal deformity that might have been incidental or potentially related to their sport [29]. Excessive mechanical loading and the frequency of training

could lead to an increase in kyphosis in the immature athlete [30]. There is the lack of prospective, controlled trials addressing this issue.

To assess the shape of anteroposterior vertebral curvatures in adolescents who practice team sports, recently reported investigation analyzed 57 females and 104 males aged 14–17 years, playing volleyball, basketball or handball, and 63 females and 99 males as a control group [16]. The author found that female athletes had lower thoracic kyphosis than the control group ($p < 0.01$). The percentage of functional (flexible) kyphosis and lordosis in our research was lower in the trained handball (16% and 16% respectively) than in female untrained adolescents (19% and 25%, respectively). The presence of the unflexible kyphosis was higher for 3% in handball trained. But, there was no statistically significant difference in the presence of kyphosis and lordosis in the two groups of adolescents. These differences could be the result of selection of the sample and of the applied method.

Poor posture and loss of muscle tone were contributory to lumbar lordosis in untrained adolescents. It is also reasonable to postulate that lower physical activity in female in relationship to male adolescents [31] may decrease the tone of the spinal ligaments and muscles in untrained female. There is a report that the relationship between kyphosis and lordosis decreased in girls but not in boys [32, 33].

Karski et al. [34] reported favorable influence of some sports on the development of children skeleton. In the classification of sports, team handball presents sports activity with modest statics and high dynamics [35]. Several recent studies in the prevalence of postural disturbances in children suggest problems with foot being one of the most common [7, 8]. There is no standardized framework from which to evaluate the pediatric flat foot [7]. The prevalence of flat foot estimates are vastly variable and range from 0.6–77.9% [7]. We find that 73.3% of female adolescents have flat feet. Of course, these differences could be the result of selection of the sample, sex and age of participants, diagnostics and cultural habits. Our results showed that asymmetry of the line of the Achilles tendon were statistical significantly lower in trained than in untrained female.

The results of our study show significantly lower number of flat feet and asymmetry of the line of the Achilles tendon in team handball players. These results could be explained by the fact that handball training requires endurance and maintenance of the foot arch which needs involvement of sole arch muscles. There are reports that the formation of foot arches probably end at late school age [36].

There is a significantly higher asymmetry of the prominence of scapula in the handball female adolescent players in our research, that is in accordance with the results of a recently report [17]. Asymmetric scapular posture is often associated with abnormalities of the shoulder complex joint. We did not find a difference in the shoulder girdle between female handball adolescent players and untraining peers. However, shoulder asymmetries may also be related to adaptations to sports practice [18]. It was noted in previous studies that in handball athletes, the dominant scapula was more anteriorly tilted than in non-athletes groups. The authors concluded that a certain degree of scapular asymmetry may be normal in some athletes. It should not be considered automatically as a pathological sign but rather an adaptation to sports practice and extensive use of upper limb [18].

We did not find a statistically significant difference in the presence of waistline (Lorenz's triangle) between trained and untrained females. This result may be explained by low percentage of non-flexible scoliosis in both groups of adolescents.

There is a report that states that correct pelvic alignment in the frontal plane was more common in handball players than in untraining young females [17]. It is in accordance with our results showing no asymmetry of the pelvic alignment in the frontal plane in female adolescents handball players in any case. We found a significantly higher asymmetry of the pelvic alignment in the frontal plane in the control group of adolescents. This result can be explained by the fact that handball training contributes to and maintains elasticity of hip muscles.

Conclusion

The results of this investigation show that postural disturbances are less frequent in 13-year female team handball players than in untrained adolescents of the same age. Flat foot is significantly less frequent in female adolescents handball players than in untrained ones.

We did not find a statistically significant difference in the presence of scoliosis, kyphosis, lordosis and *pes varus* between young female handball players and untraining peers.

The obtained results show that asymmetry of the line of the Achilles tendon and asymmetry of pelvic alignment in the frontal plane-anterior superior iliac spine were statistically significantly lower in female handball trained than in untrained adolescents, but asymmetry of the waistline and the shoulder girdle were not. There is a significantly higher asymmetry of the prominence of the scapula in the handball female adolescent players than in the untrained adolescents.

The findings obtained in this investigation can help in planning continuous prevention, observation and care for trained and untrained team handball female adolescents with postural disturbances.

Understanding of what we know (and do not know) about team handball trained differences in female adolescents is important for improving quality of care for musculoskeletal development of children. The findings of this study could be useful in practice and further investigation. These may be the strengths of this study. The primary limitation of this study was that we could not include emotional and mental health domains and pretraining physical shape. Further studies are required to confirm these results in other sets of adolescents.

R E F E R E N C E S

1. *Górecki A, Kiwerski J, Kowalski IM, Marczyński W, Nowotny J, Rybicka M,* et al. Prophylactics of postural deformities in children and youth carried out within the teaching environment- experts recommendations. Pol Ann Med 2009; 16(1): 168−77.
2. *Łabaziewicz L.* Faulty postures. In: *Marciniak W, Szulc A*, editors. Orthopaedics and Rehabilitation of Wiktor Dega. Warsaw: PZWL; 2008. p. 63−7.
3. *Taft E, Francis R.* Evaluation and management of scoliosis. J Pediatr Health Care 2003; 17(1): 42−4.
4. *Jandrić S*. Idiopathic scoliosis. Med Pregl 2012; 65(1−2): 35−40. (Serbian)
5. *Been E, Kalichman L.* Lumbar lordosis. Spine J 2014; 14(1): 87−97.
6. *Bernhardt M, Bridwell KH*. Segmental analysis of the sagittal plane alignment of the normal thoracic and lumbar spines and thoracolumbar junction. Spine 1989; 14(7): 717−21.
7. *Evans AM, Rome K.* A Cochrane review of the evidence for non-surgical interventions for flexible pediatric flat feet. Eur J Phys Rehabil Med 2011; 47(1): 69−89.
8. *Jordan KP, Kadam UT, Hayward R, Porcheret M, Young C, Croft P.* Annual consultation prevalence of regional musculoskeletal problems in primary care: an observational study. BMC Musculoskelet Disord 2010; 11(1): 144.
9. *Shumway C.* Motor control: theory and practical applications, 2nd. Baltimore: Lippincott, Williams and Wilkins; 2004.
10. *FIMS/WHO ad Hoc Committee on Sports and Children.*Sports and children: consensus statement on organized sports for children. Bull World Health Organ 1998; 76: 445−7.
11. *Negrini S, Aulisa AG, Aulisa L, Circo AB, de Mauroy JC, Durmala J,* et al. 2011 SOSORT guidelines: Orthopaedic and Rehabilitation treatment of idiopathic scoliosis during growth. Scoliosis 2012; 7(1): 3.
12. *Fürgaard B, Agertoft A.* Scheuermann's disease. Ugeskr Laeg 1990; 152(39): 2843−6. (Danish)
13. *Muyor JM, López-Miñarro PA, Alacid F.* Spinal posture of thoracic and lumbar spine and pelvic tilt in highly trained cyclists. J Sports Sci Med 2011; 10(2): 355−61.
14. *Longworth B, Fary R, Hopper D.* Prevalence and predictors of adolescent idiopathic scoliosis in adolescent ballet dancers. Arch Phys Med Rehabil 2014; 95(9): 1725−30.
15. *Schwesig R, Kluttig A, Leuchte S, Becker S, Schmidt H, Esperer HD.* The impact of different sports on posture regulation. Sportverletzung Sportschaden 2009; 23(3): 148−54.
16. *Grabara M.* Anteroposterior curvatures of the spine in adolescent athletes. J Back Musculoskelet Rehabil 2014; 27(4): 513−9.
17. *Grabara M.* A comparison of the posture between young female handball players and non-training peers. J Back Musculoskelet Rehabil 2014; 27(1): 85−92.
18. *Ribeiro A, Pascoal AG.* Resting scapular posture in healthy overhead throwing athletes. Man Ther 2013; 18(6): 547−50.
19. *Aydog ST, Tetik O, Demirel HA, Doral MN.* Differences in sole arch indices in various sports. Br J Sports Med 2005; 39(2): e5.
20. *Vařeková R, Vařeka I, Janura M, Svoboda Z, Elfmark M.* Evaluation of postural asymmetry and gross joint mobility in elite female volleyball athletes. J Hum Kinet 2011; 29: 5−13.
21. *Sass P, Hassan G.* Lower extremity abnormalities in children. Am Fam Physician 2003; 68(3): 461−8.
22. *Kuroki H, Inomata N, Hamanaka H, Chosa E, Tajima N.* Significance of hanging total spine x-ray to estimate the indicative correction angle by brace wearing in idiopathic scoliosis patients. Scoliosis 2012; 7(1): 8.
23. *Kratenová J, Zejglicová K, Malý M, Filipová V.* Prevalence and risk factors of poor posture in school children in the Czech Republic. J Sch Health 2007; 77(3): 131−7.
24. *Swärd L.* The thoracolumbar spine in young elite athletes. Current concepts on the effects of physical training. Sports Med 1992; 13(5): 357−64.
25. *Tanchev PI, Dzherov AD, Parushev AD, Dikov DM, Todorov MB.* Scoliosis in rhythmic gymnasts. Spine 2000; 25(11): 1367−72.
26. *Boldori L, da Soldà M, Marelli A.* Anomalies of the trunk. An analysis of their prevalence in young athletes. Minerva Pediatr 1999; 51(7−8): 259−64.
27. *Konieczny MR, Senyurt H, Krauspe R.* Epidemiology of adolescent idiopathic scoliosis. J Child Orthop 2013; 7(1): 3−9.
28. *Kenanidis E, Potoupnis ME, Papavasiliou KA, Sayegh FE, Kapetanos GA.* Adolescent idiopathic scoliosis and exercising: is there truly a liaison. Spine 2008; 33(20): 2160−5.
29. *d'Hemecourt Pierre A, Hresko M.* Spinal deformity in young athletes. Clin Sports Med 2012; 31(3): 441−51.
30. *Ashton-Miller JA.* Thoracic hyperkyphosis in the young athlete: a review of the biomechanical issues. Curr Sports Med Rep 2004; 3(1): 47−52.
31. *Jandric S.* Differences between boys and girls in terms of physical activity. Facta Universitatis 2010; 8: 1−7. (Serbian)
32. *Widhe T.* Spine: posture, mobility and pain. A longitudinal study from childhood to adolescence. Eur Spine J 2001; 10(2): 118−23.
33. *Panjabi MM, Goel VK, Takata K.* Physiological strains in the lumbar spinal ligaments: an in vitro biomechanical study. Spine 1982; 7(3): 192−203.
34. *Karski T, Kalakucki J, Karski J.* Relationship of "syndrome of contractures", in newborns with the development of the so-called idiopathic scoliosis. World J Pediatr 2007; 3(4): 254−9.
35. *Póvoas SC, Seabra AF, Ascensão AA, Magalhães J, Soares JM, Rebelo AN.* Physical and physiological demands of elite team handball. J Strength Cond Res 2012; 26(12): 3365−75.
36. *Mihajlović I, Smajić M, Sente J.* Frequency of foot deformity in preschool girls. Vojnosanit Pregl 2010; 67(11): 928−32. (Serbian)

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Cerebral palsy in preterm infants

Cerebralna paraliza kod prevremeno rođene dece

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Abstract

Background/Aim. Cerebral palsy (CP) is one of the leading causes of neurological impairment in childhood. Preterm birth is a significant risk factor in the occurrence of CP. Clinical outcomes may include impairment of gross motor function and intellectual abilities, visual impairment and epilepsy. The aim of this study was to examine the relationships among gestational age, type of CP, functional ability and associated conditions. **Methods.** The sample size was 206 children with CP. The data were obtained from medical records and included gestational age at birth, clinical characteristics of CP and associated conditions. Clinical CP type was determined according to *Surveillance of Cerebral Palsy in Europe* (SCPE) and topographically. Gross motor function abilities were evaluated according to the Gross Motor Function Classification System (GMFCS). **Results.** More than half of the children with CP were born prematurely (54.4%). Statistically significant difference was noted with respect to the distribution of various clinical types of CP in relation to gestational age ($p < 0.001$). In the group with spastic bilateral CP type, there is a greater proportion of children born preterm. Statistically significant difference was noted in the functional classification based on GMFCS in terms of gestational age ($p = 0.049$), children born at earlier gestational age are classified at a higher GMFCS level of functional limitation. The greatest percentage of children (70.0%) affected by two or more associated conditions was found in the group that had extremely preterm birth, and that number declined with increasing maturity at birth. Epilepsy was more prevalent in children born at greater gestational age, and this difference in distribution was statistically significant ($p = 0.032$). **Conclusion.** The application of antenatal and postnatal protection of preterm children should be a significant component of the CP prevention strategy.

Key words:
Cerebral palsy; risk factors; infant, premature.

Apstrakt

Uvod/Cilj. Cerebralna paraliza (CP) jedan je od najčešćih uzroka nastanka neuroloških oštećenja u dečjem uzrastu. Prevremen porođaj je značajan faktor rizika od pojave CP. Kliničke posledice mogu biti oštećenje grube motoričke funkcije, intelektualnih sposobnosti, poremećaj vida i epilepsija. Cilj rada bio je da se istraži odnos između gestacijske starosti, oblika CP, funkcionalnih sposobnosti i pridruženih oboljenja. **Metode.** Uzorak se sastojao od ukupno 206 dece. Iz medicinske dokumentacije dobijeni su podaci o gestacijskoj starosti, kliničkim karakteristikama CP i pridruženim oboljenjima. Klinički tip CP određivan je prema preporuci *Surveillance of Cerebral Palsy in Europe* (SCPE) i topografski. Grube motoričke funkcije procenjivane su na osnovu istoimenog klasifikacionog sistema *Gross Motor Function Classification System* (GMFCS). **Rezultati.** Više od polovine dece sa CP prevremeno su rođena (54,4%). Registrovana je statistički značajna razlika u distribuciji kliničkih tipova CP u odnosu na gestacijsku starost ($p < 0,001$); kod dece sa spastičnim bilateralnim tipom CP više su zastupljena prevemeno rođena deca. Utvrđena je statistički značajna razlika u funkcionalnoj klasifikaciji na osnovu GMFCS u odnosu na gestacijsku starost ($p = 0,049$); deca koja su manje gestacijske starosti imaju viši stepen prema GMFCS-u. Najveći procenat dece (70,0%) koja imaju dva ili više pridruženih oboljenja utvrđen je kod izrazito prevremeno rođene dece i ovaj broj opada što je termin bliži normalnom terminu porođaja. Epilepsija je bila češća kod dece sa većom gestacijskom starosti i ova razlika u distribuciji bila je statistički značajna ($p = 0,032$). **Zaključak.** Primena antenatalne i postnatalne zaštite preterminski rođene dece trebalo bi da bude značajna komponenta strategije prevencije CP.

Ključne reči:
paraliza, cerebralna; faktori rizika; nedonošče.

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Introduction

Cerebral palsy (CP) is one of the leading causes of neurological impairment in childhood [1, 2]. Worldwide studies of the prevalence of this disorder indicate that it is much higher in developing countries and its incidence varies from 1.5 to 3 *per* 1,000 live births [2, 3]. In some regions, CP prevalence has historically remained stable [3]. However, in most studies, changes were noted in the CP prevalence rates in indifferent risk groups, such as low birthweight infants (with body mass below 1,000 or 1,500 g), or those born prematurely (before 32 weeks of gestation) [4]. Improved healthcare system can reduce CP prevalence by minimizing the effects of prenatal, perinatal and postnatal damage. On the other hand, by increasing survival rates of preterm infants, CP prevalence is likely to increase [5].

Preterm birth is a significant risk factor in the occurrence of CP [6, 7]. In preterm infants, secondary postnatal complications can develop, as a result of immaturity, or preexisting brain damage that occurred during prenatal or perinatal period [6]. Specific perinatal risk factors for CP in preterm infants are maternal-fetal infection, neonatal sepsis, and other serious conditions that can develop during the neonatal period. Brain damage resulting from periventricular hemorrhage, periventricular cystic leucomalacia and posthemorrhagic hydrocephalus in particular, are reliable predictors of future neurodevelopmental issues, and thus CP [8]. According to O'Callaghan et al. [7], preterm birth, intrauterine fetal growth restriction, perinatal infection, and multiple births are the leading risk factors for developing CP.

Prematurely born infants (preterm neonates) are babies born at gestational age below 37 weeks and can be classified into three groups, namely those born at 32−36, 28–31, and before 28 weeks of gestation [9]. Survival and neurological development of preterm children born after 32 weeks of gestation is similar to that of those carried to full term. However, the period from 20 to 32 weeks post-conception is characterized by rapid brain growth and development. Illness, undernourishment and infection during this period may compromise fetal neurodevelopmental progress, resulting in clinical outcomes that may include CP, visual and hearing impairment, learning difficulties, as well as behavioral, psychological and social deficits [8].

As preterm infants are at a greater risk of developing CP, the aim of this study was to examine the relationships among gestational age, type of CP, functional ability and associated conditions.

Methods

This qualitative study, clinical-epidemiological, classical type was conducted at the Clinic for Child Habilitation and Rehabilitation, the Institute for Child and Youth Healthcare of Vojvodina in Novi Sad. The study was approved by the Ethics Committee of the Institute and the Faculty of Medicine, University of Novi Sad. The analysis included all patients in whom the CP diagnosis had been established in 1990−2009 period, resulting in a sample of 206 children. A part of this comprehensive study was presented in this paper. Initially, medical history of all patients was reviewed in order to ascertain their gestational age at birth, as well as their CP clinical characteristics and associated conditions. Clinical CP type was determined according to *Surveillance of Cerebral Palsy in Europe* (SCPE) and topographically [10]. Based on the clinical presentation, gross motor function classification was performed, according to the five-level Gross Motor Function Classification System (GMFCS), whereby Level I indicates the highest functional ability, and Level V most severely limited motor function. For each of the five levels, the description of gross motor function is given for four age groups: before the 2nd birthday, between the 2nd and the 4th birthday, between the 4th and the 6th birthday, and between the 6th and the 12th birthday [11]. Data on associated conditions (mental deficit, epilepsy, visual impairment) was sourced from the reports provided by the child's neurologist, neuropediatrician, psychologist, speech therapist and ophthalmologist.

The collected data was input into a database specifically designed for the purpose of this study. The subsequent data analysis consisted of descriptive and inferential statistics. Attributive characteristics were presented through frequencies and percentages. Comparison of the observed and expected attributive characteristics frequencies was performed *via* χ^2 test, whereas analysis of the correlation between two characteristics was conducted by applying Spearman correlation coefficient. All statistical analyses were performed using SPSS Statistics 17.0 computer software.

Results

Of the 145 children diagnosed with CP for whom gestational age at birth was documented, more than half were born prematurely (54.4%). More specifically, 30.3% were born at 32−36 weeks of gestation, 17.2% between 28 and 31, and in 6.9% cases gestational period was shorter than 28 weeks. The distribution difference was found to be statistically significant ($\chi^2 = 48.572$, $p < 0.001$) (Table 1).

Table 1
Distribution of children with cerebral palsy (CP) by gestational age

Gestational age (weeks)	Total numbers of patients, n (%)	Patients with complete data (%)
< 28	10 (4.9)	6.9
28−31	25 (12.1)	17.3
32−36	44 (21.4)	30.3
> 36	66 (32.0)	45.5
Total	145 (70.4)	100.0
No data	61 (29.6)	
Total	206 (100.0)	

Of the 109 children classified as having spastic bilateral type of CP (of the 116 in the sample), more than half (n = 64; 58.7%) had diplegia, and the remaining 45 (41.3%) quadriplegia. While nearly 1/3 of the studied children had diplegia, spastic unilateral CP was somewhat less prevalent (affecting 29.5% of the children). In addition, while

quadriplegia affected nearly quarter of the sample (23.3%), 8.8% children were diagnosed with dyskinetic CP type, and 5.2% had ataxic CP (Table 2).

In children diagnosed with CP, a statistically significant difference was noted with respect to the distribution of various clinical types of CP in relation to gestational age (χ^2 = 33.448, $p < 0.001$). More than 2/3 of the children diagnosed with spastic unilateral, dyskinetic and ataxic type of CP were born at full term (71.8%, 69.2% and 66.7%, respectively). In contrast, in the group with spastic bilateral CP type, there was a greater proportion of children born preterm, which constituted the majority of the group diagnosed with diplegia (85.4%), whereas among those affected by quadriplegia, more than half (51.6%) were prematurely born children. The data is presented in Table 3.

A statistically significant difference was noted in the functional classification based on GMFCS in terms of gestational age (χ^2 = 15.541, p = 0.049). As can be seen in Table 4, in full-term children, Level I is most prevalent, accounting for more than a third of the sample (36.9%), while fewer prematurely born children are classified as having this level of functionality (14.3% of children born before 32 weeks of gestation, and 17.1% of those born at 32−36 weeks of gestation). The analysis of relationships between gestational age and GMFCS classification presented in Table 4 revealed negative correlation (nonparametric Spearman), which was not statistically significant (p = 0.751). This finding implies that children born at earlier gestational age are classified at a higher GMFCS level of functional limitation.

The majority of children delivered after 28 weeks of gestation have moderate or borderline mental deficit, which affects 41.2% of those born at 28–31 gestational age, 48.6% of those born at 32–36 weeks of gestation, and 40.0% full-term children. In the group born before 28 weeks of gestation, 75% of children have mental deficit, with equal proportion of those affected by the severe and moderate/borderline form (37.5%). In line with the mental deficit, the distribution of the visual impairment severity in relation to gestational age is also evident. Severe visual impairment is most prevalent among children born before 28 weeks of gestation (20.0%), while the mild/moderate form is more frequent among those born at 28–31 gestational age (28.0%), those born at 32–36 gestational age (22.7%), and full-term children (19.7%). Epilepsy is more prevalent in children born at greater gestational age, and this difference in distribution (Table 5) is statistically significant (χ^2 =8.795, p = 0.032).

Table 2

Distribution of cerebral palsy (CP) types

Clynical type of CP	Total number of patients n (%)	Patients with complete data (%)
Spastic unilateral	57 (27.7)	29.5
SB* diplegia	64 (31.1)	33.2
SB* quadriplegia	45 (21.8)	23.3
Dyskinetic	17 (8.3)	8.8
Ataxic	10 (4.9)	5.2
Total	193 (93.7)	100.0
No data	13 (6.3)	
Total	206 (100.0)	

***SB – spastic bilateral.**

Table 3

Distribution of cerebral palsy (CP) types by gestational age

Clinical type of CP	Preterm birth n (%)	Term birth n (%)	Total n (%)
Spastic unilateral	11 (28.2)	28 (71.8)	39 (100.0)
SB diplegia	41 (85.4)	7 (14.6)	48 (100.0)
SB quadriplegia	16 (51.6)	15 (48.4)	31 (100.0)
Dyskinetic	4 (30.8)	9 (69.2)	13 (100.0)
Ataxic	2 (33.3)	4 (66.7)	6 (100.0)
Total	74 (54.0)	63 (46.0)	137 (100.0)

SP − spastic bilateral; n – number of children.

Table 4

Distribution of Gross Motor Function Classification System (GMFCS) levels by gestational age

Gestational age (weeks)	GMFCS, n (%)					
	1	2	3	4	5	Total
< 32	5 (14.3)	11 (31.4)	9 (25.7)	6 (17.1)	4 (11.4)	35 (100.0)
32−36	7 (17.1)	12 (29.3)	8 (19.5)	8 (19.5)	6 (14.6)	41 (100.0)
> 36	24 (36.9)	8 (12.3)	7 (10.8)	14 (21.5)	12 (18.5)	65 (100.0)
Total	36 (25.5)	31 (22.0)	24 (17.0)	28 (19.9)	22)15.6)	141 (100.0)

n – number of children.

Table 5

Distribution of associated impairments/conditions by gestational age

Gestational age (weeks)	Intellectual impairment		Visual impairment		Epilepsy
	Severe	Moderate/mild	Severe	Moderate/mild	
< 28	37.5	37.5	20.0	10.0	10.0
28−31	11.8	41.2	0.0	28.0	12.0
32−36	25.7	48.6	9.1	22.7	27.3
> 36	18.0	40.0	0.0	19.7	39.4
Total	20.9	42.7	4.1	21.4	29.0

The results are given as percentage of children.

The greatest percentage of children (70.0%) affected by two or more concomitant conditions is found in the group that had extremely preterm birth (< 28 gestational weeks), and this number declines with increasing maturity at birth. Similarly, the number of children without any concomitant conditions increases with gestational age (from 10% for extremely preterm children to 25.8% for those born at full term). However, the differences in the total number of concomitant conditions in relation to gestational age were not statistically significant (p = 0.200). The data is shown in Table 6.

Table 6

Number of associated impairments by gestational age

Gestational age (weeks)	Associated impairments, n (%)			
	0	1	2 or more	Total
< 28	1 (10.0)	2 (20.0)	7 (70.0)	10 (100.0)
28−31	3 (12.0)	11 (44.0)	11 (44.0)	25 (100.0)
32−36	5 (11.4)	12 (27.3)	27 (61.4)	44 (100.0)
> 36	17 (25.8)	16 (24.2)	33 (50.0)	66 (100.0)
Total	26 (17.9)	41 (28.3)	78 (53.8)	145 (100.0)

n – number of children.

Discussion

Preterm birth is recognized as the key risk factor for developing CP [7, 12, 13], in particular if it occurs prior to 32 weeks of gestation [13]. A meta analysis of 26 studies revealed the CP prevalence of 14.6% for children born at 22−27 gestational age, 6.2% for those born at 28−31, 0.7% for those born at 32–36 weeks of gestation, and 0.1% for full-term children [14]. Our results indicate that about half of the children affected by CP were born preterm, of whom 17% were delivered at 28–32 weeks of gestation and 7% before 28 weeks of gestation. Preterm children with CP have periventricular white matter damage. The damage to posterior thalamic region is most prominent, which correlates with reduced sensory-motor ability at the contralateral side. In rare cases, descendent corticospinal tract is also affected [15]. The presence of prenatal infection contributes to the increased risk of CP in prematurely born children. One of the mechanisms responsible for its development is neurotoxicity of cytokines that initiate chain reaction, resulting in apoptosis of various brain cells, predominantly oligodendrocytes, which are responsible for myelination [16]. Advancements in neonatal intensive care have contributed to the reduction in prenatal and neonatal death rates over time; however, most children are affected by the harmful noxa prenatally, which partly explains the relatively high CP prevalence in the extremely premature neonates [17].

Diplegia is the dominant CP subtype, and in more than 50% of cases, it occurs in preterm infants [5, 17]. In the present study, 33.2% of children included in the sample had diplegia, of whom 55.4% were born preterm, and 80% had low birthweight. Variations in the percentage participation of the diplegic CP form reported in the extant literature (35−39.8%) can potentially be explained by the variable prevalence of preterm neonates in the total population of children with CP [5, 17].

Our study findings indicate that quadriplegia is present in 23.3% children, while other authors reported somewhat lower values, ranging from 15% to 20% [18]. This disparity may be due to the different treatment provided to neonates in the intensive care unit, therapies offered, as well as differences in prenatal care. According to our findings, quadriplegia is equally prevalent among full-term and preterm children in our sample. One group of authors reported that the dyskinetic form was present in 11−13% of children with CP [2, 19]. In our sample, this percentage is somewhat lower (8.3%), most likely due to the recently adopted practice of making the diagnosis based on the dominant symptom. In that case, children with the dyskinetic form of CP that also present with spasticity are classified as having spastic bilateral CP type [2]. In the study conducted by Serdaroglu et al. [5], dyskinetic form of CP was found in 6.4% children. Our results indicate that the dyskinetic form primarily occurred in full-term children born with normal body mass. Ataxic CP form is least prevalent and occurs in 4.7−5.9% cases, which is in accordance with our findings (4.9%) [5, 17, 18].

Our findings revealed a statistically significant difference in the functional ability distribution, as determined according to GMFCS, in relation to gestational age. Level I was most prevalent among the full-term children, while

prematurely born children were assigned higher GMFCS levels. According to numerous studies, children with hemiplegia, most of whom were born at full term, predominantly exhibit Level I functional ability [20–22]. In our study, slightly more than 50% of children were born preterm. According to several authors, the most severe form of CP is linked to the higher GMFCS levels, predominantly Level V [23–25].

Our findings indicate that children born before 28 weeks of gestation presented with the greatest number of concomitant conditions, which is in line with the results reported in the literature [19, 26, 27]. Marlow et al. [27] reported that cognitive and neurological impairments were most prevalent among children born before 26 weeks of gestation. In the study conducted by Laptook et al. [26], nearly 30% of children born on the average at 26 weeks of gestation, with normal brain ultrasound findings, were subsequently diagnosed with CP or had learning difficulties. Mongan et al. [28] reported that, in their study, 56% of the children with CP had intellectual impairment, which was severe in 35% of those cases. The authors also concluded that the probability of children of normal birthweight developing intellectual impairment was twice as high as that found for those with low birthweight. Our findings indicate that severe mental deficit was most prevalent in children born before 28 weeks of gestation. When discussing the developmental prognosis with the parents, it is necessary to consider the associated cognitive impairment, as severe intellectual disability can significantly reduce the likelihood of the child learning to walk [29]. Among children with CP, nonverbal learning deficits, accompanied by limited visual-spatial ability, are common [30]. According to the literature reports, 22–41% children with CP have epilepsy, the prevalence of which varies across subtypes [28, 30, 31]. Our findings indicate that epilepsy mostly occurred in children born at or close to full term. On the other hand, greater number of prematurely born children suffered from retinopathy, cortical visual impairment and strabismus, compared to the children with CP born at full term [32]. In our study, severe visual impairment was most prevalent among children born before 28 weeks of gestation (20%). In prematurely born children, while compromised sight is mostly due to the retinopathy of prematurity (ROP), it can also result from cortical damage. ROP typically occurs in children born before 32 weeks of gestation and its incidence and severity are inversely proportional to gestational age [8].

Conclusion

Among the prematurely born children included in this study, bilateral spastic CP type and higher GMFCS level of functional limitation were most prevalent. Children born before 28 weeks of gestation had the greatest number of associated conditions, as well as greatest prevalence of more severe cognitive and visual impairment. Providing antenatal and postnatal care to preterm infants should certainly be an important component of the CP prevention strategy.

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REFERENCES

1. *Stelmach T, Pisarev H, Talvik T.* Ante- and perinatal factors for cerebral palsy: case-control study in Estonia. J Child Neurol 2005; 20(8): 654–60.
2. *Himmelmann K, Hagberg G, Wiklund LM, Eek MN, Uvebrant P.* Dyskinetic cerebral palsy: a population-based study of children born between 1991 and 1998. Dev Med Child Neurol 2007; 49(4): 246–51.
3. *Winter S, Autry A, Boyle C, Yeargin-Allsopp M.* Trends in the prevalence of cerebral palsy in a population-based study. Pediatrics 2002; 110(6): 1220–5.
4. *Pakula AT, Van Braun NK, Yeargin-Allsopp M.* Cerebral palsy: classification and epidemiology. Phys Med Rehabil Clin N Am 2009; 20(3): 425–52.
5. *Serdaroğlu A, Cansu A, Ozkan S, Tezcan S.* Prevalence of cerebral palsy in Turkish children between the ages of 2 and 16 years. Dev Med Child Neurol 2006; 48(6): 413–6.
6. *Dolk H, Parkes J, Hill N.* Trends in the prevalence of cerebral palsy in Northern Ireland, 1981-1997. Dev Med Child Neurol 2006; 48(6): 406–12.
7. *O'Callaghan ME, MacLennan AH, Gibson CS, McMichael GL, Haan EA, Broadbent JL,* et al. Epidemiologic associations with cerebral palsy. Obstet Gynecol 2011; 118(3): 576–82.
8. *Colvin M, McGuire W, Fowlie PW.* Neurodevelopmental outcomes after preterm birth. BMJ 2004; 329(7479): 1390–3.
9. *Venkateswaran S, Shevell MI.* Comorbidities and clinical determinants of outcome in children with spastic quadriplegic cerebral palsy. Dev Med Child Neurol 2008; 50(3): 216–22.
10. *Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B,* et al. Proposed definition and classification of cerebral palsy, April 2005. Dev Med Child Neurol 2005; 47(8): 571–6.
11. *Palisano RJ, Cameron D, Rosenbaum PL, Walter SD, Russell D.* Stability of the Gross Motor Function Classification System. Dev Med Child Neurol 2006; 48(6): 424–8.
12. *Bonellie SR, Currie D, Chalmers J.* Comparison of risk factors for cerebral palsy in twins and singletons. Dev Med Child Neurol 2005; 47(9): 587–91.
13. *Thorngren-Jerneck K, Herbst A.* Perinatal factors associated with cerebral palsy in children born in Sweden. Obstet Gynecol 2006; 108(6): 1499–505.
14. *Himpens E, van den Broeck C, Oostra A, Calders P, Vanhaesebrouck P.* Prevalence, type, distribution, and severity of cerebral palsy in relation to gestational age: a meta-analytic review. Dev Med Child Neurol 2008; 50(5): 334–40.
15. *Hoon AH, Stashinko EE, Nagae LM, Lin DD, Keller J, Bastian A,* et al. Sensory and motor deficits in children with cerebral palsy born preterm correlate with diffusion tensor imaging abnormalities in thalamocortical pathways. Dev Med Child Neurol 2009; 51(9): 697–704.
16. *Marret S, Zupan V, Gressens P, Lagercrantz H, Evrard P.* Periventricular leukomalacia. I. Histological and pathophysiological aspects. Arch Pediatr 1998; 5(5): 525–37.
17. *Sigurdardóttir S, Thórkelsson T, Halldórsdóttir M, Thorarensen O, Vik T.* Trends in prevalence and characteristics of cerebral

palsy among Icelandic children born 1990 to 2003. Dev Med Child Neurol 2009; 51(5): 356−63.
18. *Andersen GL, Irgens LM, Haagaas I, Skranes JS, Meberg AE, Vik T.* Cerebral palsy in Norway: prevalence, subtypes and severity. Eur J Paediatr Neurol 2008; 12(1): 4−13.
19. *Himmelman K.* Cerebral Palsy in Western Sweden-Epidemiology and Function. Sweden: The Sahlgrenska Academy at Goteborg University; 2006.
20. *Beckung E, Carlsson G, Carlsdotter S, Uvebrant P.* The natural history of gross motor development in children with cerebral palsy aged 1 to 15 years. Dev Med Child Neurol 2007; 49(10): 751−6.
21. *Voorman JM, Dallmeijer AJ, Knol DL, Lankhorst GJ, Becher JG.* Prospective longitudinal study of gross motor function in children with cerebral palsy. Arch Phys Med Rehabil 2007; 88(7): 871−6.
22. Caram LH, Funayama CA, Spina CI, Giuliani Ld, de Pina NJM. Investigation of neurodevelopment delay etiology: resources and challenges. Arq Neuropsiquiatr 2006; 64(2B): 466−72.
23. *Pfeifer LI, Silva DB, Funayama CA, Santos JL.* Classification of cerebral palsy: association between gender, age, motor type, topography and Gross Motor Function. Arq Neuropsiquiatr 2009; 67(4): 1057−61.
24. *Himmelmann K, Beckung E, Hagberg G, Uvebrant P.* Gross and fine motor function and accompanying impairments in cerebral palsy. Dev Med Child Neurol 2006; 48(6): 417−23.
25. *Howard J, Soo B, Graham KH, Boyd RN, Reid S, Lanigan A*, et al. Cerebral palsy in Victoria: Motor types, topography and gross motor function. J Paediatr Child Health 2005; 41(9−10): 479−83.
26. *Laptook AR, O'Shea MT, Shankaran S, Bhaskar B.* Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents. Pediatrics 2005; 115(3): 673−80.
27. *Marlow N, Wolke D, Bracewell MA, Samara M.* Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med 2005; 352(1): 9−19.
28. *Mongan D, Dunne K, O'Nuallain S, Gaffney G.* Prevalence of cerebral palsy in the West of Ireland 1990-1999. Dev Med Child Neurol 2006; 48(11): 892−5.
29. *Beckung E, Hagberg G, Uldall P, Cans C.* Surveillance of Cerebral Palsy in Europe. Probability of walking in children with cerebral palsy in Europe. Pediatrics 2008; 121(1): e187−92.
30. *Sigurdardottir S, Eiriksdottir A, Gunnarsdottir E, Meintema M, Arnadottir U, Vik T.* Cognitive profile in young Icelandic children with cerebral palsy. Dev Med Child Neurol 2008; 50(5): 357−62.
31. *Odding E, Roebroeck ME, Stam HJ.* The epidemiology of cerebral palsy: incidence, impairments and risk factors. Disabil Rehabil 2006; 28(4): 183−91.
32. *Pennefather PM, Tin W.* Ocular abnormalities associated with cerebral palsy after preterm birth. Eye (Lond) 2000; 14(Pt 1): 78−81.

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The Fisher Grade in predicting a degree of cerebral vasospasm in patients after intracranial aneurysm rupture

Fišerova klasifikacija u predviđanju nastanka cerebralnog vazospazma kod bolesnika nakon rupture intrakranijalne aneurizme

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Abstract

Background/Aim. Intracranial aneurysms are pathological enlargement of the wall of cerebral arteries. Intracranial aneurysms rupture is a dramatic event with a significant morbidity and mortality. The Fisher Grade is widely accepted in assessment of the extensiveness of aneurysmal subarachnoid hemorrhage (aSAH) and the presence of other intracranial hemorrhage on the computed tomography (CT) scan. Significant early complication of a aSAH may be a cerebral vasospasm. The aim of this study was to determine the relationship between the extensiveness of aSAH, assessed by the Fisher Grade on admission, with the intensity of cerebral vasospasm in patients with ruptured intracranial aneurysm. **Methods.** This prospective clinical study included 50 patients with aSAH hospitalized at the Clinic of Neurosurgery, Clinical Center of Vojvodina, Novi Sad, Serbia. All the patients underwent 256-layer cranial CT and CT angiography on admission and on the day 9. Based on native CT scans, they were classified according to the Fisher Grade. On CT angiography images, intensity of cerebral vasospasm was determined. **Results.** On the basis of admission CT images, 24% of patients were classified into the Fisher Grade group 2, while 34% and 42% were in the groups 3 and 4, respectively. A positive correlation of the Fischer Grade on admission with the intensity of cerebral vasospasm was established, but with no statistical significance (ϱ = 0.273, p = 0.160). **Conclusion.** This study showed that the Fisher Grade is not significant in predicting the intensity of cerebral vasospasm in patients hospitalized with intracranial aneurysm rupture.

Key words:
intracranial aneurysm; subarachnoid hemorrhage; cerebral arteries; vasospasm, intracranial; disease progression; severity of illness index; tomography, x-ray computed; angiography; predictive value of tests.

Apstrakt

Uvod/Cilj. Intrakranijalne aneurizme su patološka proširenja zida moždanih arterijskih krvnih sudova. Ruptura intrakranijalnih aneurizmi je dramatičan događaj sa značajnim morbiditetom i mortalitetom. Za procenu opsežnosti subarahnoidalne hemoragije aneurizmatske etiologije (aneurizmatska subarahnoidalna hemoragija aSAH) i prisustva drugih intrakranijalnih hemoragija na nalazu kompjuterizovane tomografije (CT) široko je prihvaćena Fišerova klasifikacija. Značajna rana komplikacija aSAH je cerebralni vazospazam. Cilj rada bio je da se utvrdi korelacija obima aSAH vrednovanog Fišerovim stepenom na prijemu sa intenzitetom cerebralnog vazospazma kod bolesnika sa rupturiranom intrakranijalnom aneurizmom. **Metode.** Ovo prospektivno kliničko istraživanje, obuhvatilo je 50 bolesnika sa aSAH lečenih na Klinici za neurohirurgiju Kliničkog centra Vojvodine. Kod svih bolesnika je na prijemu i najčešće 9. dana od prijema, urađen CT i CT angiografski pregled glave na 256-slojnom CT aparatu. Na osnovu nativnih CT pregleda izvršena je klasifikacija po Fišerovoj skali. Na CT angiografskim pregledima određivan je intenzitet cerebralnog vazospazma. **Rezultati.** Na osnovu CT nalaza na prijemu, 24% bolesnika klasifikovano je u grupu 2 po Fišerovoj skali, 34% bolesnika u grupu 3, i 42% bolesnika u grupu 4. Utvrđena je pozitivna korelacija Fišerove skale na prijemu sa intenzitetom vazospazma, ali ta korelacija nije bila statistički značajna (ϱ = 0,273, p = 0,160). **Zaključak.** Ova studija je pokazala da Fišerova klasifikacija na prijemu bolesnika nakon rupture intrakranijalne aneurizme nije statistički značajna za predviđanje intenziteta cerebralnog vazospazma.

Ključne reči:
aneurizma, intrakranijalna; krvarenje, subarahnoidno; aa. cerebri; vazospazam, intrakranijalni; bolest, progresija; bolest, indeks težine; tomografija, kompjuterizovana, rendgenska; angiografija; testovi, prognostička vrednost.

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Introduction

Intracranial aneurysms (IA) are pathological enlargements of the wall of intracranial arteries. IA rupture and consequent bleeding in the subarachnoid, intraventricular or subdural space is a dramatic event with significant morbidity and mortality. Most studies show that the incidence of aneurysmal subarachnoid hemorrhage (aSAH) is 10–11 cases *per* 100,000 population *per* year, except in China where it is 2 and in Japan and Finland 16.8 to 18.33 [1]. Sudden intense headache that does not respond to painkillers occurs in about 80% of patients with aSAH. Symptoms that also indicate aSAH are nausea, vomiting, disturbance of consciousness, meningism, and focal neurological deficit [2].

The diagnosis of ruptured IA is made by anamnesis, physical, neurological and radiographic examination. Cranial computed tomography (CT) is the best diagnostic tool for the detection of intracranial hemorrhage, hydrocephalus and cerebral infarction. The Fisher Grade is widely accepted in the classification of the extensiveness of the aSAH and the presence of other intracranial hemorrhage on CT images [3]: Ficher Grade 1 – no SAH visualized; Ficher Grade 2 – thin layer of SAH, less than 1 mm thick; Ficher Grade 3 – focal or diffuse layer of SAH, greater than 3 mm in thickness; Ficher Grade 4 – intracerebral or intraventricular clots with or without SAH.

Complications after aneurysm rupture may be early and late. Early complications include aneurysm re-ruptures, hydrocephalus and cerebral vasospasm (CV), while the late ones include general medical complications, late hydrocephalus and epilepsy.

CV is a pathologic, reversible narrowing of the cerebral arteries, which develops a few days after SAH. Symptomatic vasospasm presents narrowing of the arteries of the brain, which results in cerebral ischemia with associated clinical symptoms and signs.

Based on the intensity, CV is classified into mild (0–33%), moderate (34–66%) and severe (67–100%) [4].

CV involves intracerebral part of cerebral arteries. The distribution and degree of vasospasm correlates with the location and size of aSAH. Delayed onset and relative ability to predict CV offers a chance to act in a therapeutic window that is still narrow. Determining the precise mechanism of vasospasm remains an objective to enable a complete pharmacological preventive treatment [5].

The incidence of vasospasm after SAH is about 60%, with data from the literature ranging from 20% to 97% as a result of unequal criteria for determination of vasospasm [6].

There is a proven correlation between the quantity and location of subarachnoid blood with the risk of occurrence and distribution of angiographic vasospasm. If the initial CT scans show a significant amount of SAH (Fisher Grade III), a significant vasospasm develops in 60% of patients. Latest multivariate studies have shown that only the presence of blood in the subarachnoid space is a significant factor for the occurrence of vasospasm [7].

The aim of this study was to determine the relationship between the extensiveness of aSAH, determined by the Fisher Grade on admission, with the intensity of CV in patients with ruptured IA.

Methods

This prospective clinical study included a total of 50 patients hospitalized at the, Clinic of Neurosurgery, Clinical Center of Vojvodina Novi Sad, Serbia, due to aSAH. On admission, all patients underwent cranial CT and CT angiography (CTA) with 256-layer "dual energy" CT (Siemens) by the special protocol. All images were processed and reviewed on a workstation "Syngo.via" Siemens.

Initially, a native cranial CT scan was made. Scans were performed from the level of the foramen magnum to the top of the vertex with axial sections at 5 mm and subsequent reconstruction on 1mm. Acquisition parameters were 120 kVA, EffmAs to 320 mA, collimation 0.6 and a matrix of at least 512 × 512 pixels. Native CT examinations were analyzed with triplanar reconstruction in a standard window for brain parenchyma 80 WW and 40 WL. Based on CT images the radiologist determined the extensiveness of SAH and the presence of other intracranial hemorrhages and classified patients according to the Fisher Grade.

CTA of cerebral vessels were performed on admission and on the day 9 or 10 of hospitalization. Two patients underwent CTA on the day 5 and 8, respectively, due to worsening of neurological condition. CTA was performed by scanning in the same given range as native CT, using non-ionic, isoosmolar, iodine contrast (Iohexol, Omnipaque 350, GE Healthcare). The contrast was injected mechanically by "two-headed" Optivanatage DH injector, 1–1.5 mL *per* kg of body weight. Axial cross-section at the level of the Circle of Willis was recorded after 10 s delay from the beginning of the application and then successively, every two seconds, until the appearance of contrast in the internal carotid artery and Circle of Willis, when the scanning was initiated.

CTA images were analyzed with triplanar reconstruction in "angio window", 700 WW and 80 WL, maximum intensity projection (MIP) reconstruction of axial, coronal and sagittal sections, "Volume rendering" reconstructions, as well as with software application "syngo CT vascular analysis" to determine the diameter of a blood vessel. Before measuring a blood vessel, every single analyzed artery was labeled automatically and set aside in a separate window on the screen, where possible, with additional tools to improve image and determine accurately the appropriate position for the measurement. Then, the automatic determination of the boundaries of the vessel was checked and, if necessary, corrections were made (minimum and maximum Housfield units – HU values).

On CTA scans on admission and control CTA scans, seven segments of the proximal blood vessels of the brain in their distal parts were measured: suprasellar parts of internal carotid artery; M1 segments of the middle cerebral artery, A1 segments of the anterior cerebral artery; A2 segments of the anterior cerebral artery; P1 segment of the posterior cerebral artery; vertebral arteries and basilar arteries. If there were evident signs of vasospasm in the distal circulation (M2 and M3), these data were noted and taken into statistical analysis.

The presence of CV was determined on the measured values of arterial narrowing of blood vessels. The presence

of CV was classified into: present (degree of narrowing of blood vessels 5–100%, taking into account the possibility of error in the measurement), and absent (0–5%). Narrowing of the arteries on CTA images were classified into: mild (5–33%), moderate (34–66%) and severe (67–100%).

Results

On the basis of CT findings at admission, 24% of the patients were classified as the Fisher Grade 2, 34% of the patients as the Fisher Grade 3 and 42% of the patients as the Fisher Grade 4. On control CTA scans, mild degree of CV was detected in 36% of the patients, moderate in 46 % and severe in 9% of the patients.

In the Fisher Grade 2 group, a significant (severe) vasospasm was observed in 7.5% of the patients. In the Fisher Grade 3 group, 29.4% of the patients had a significant vasospasm. This shows a clear correlation, indicating that there are notably more patients with significant vasospasm in the Fisher Grade 3 group, than in the Fisher Grade 2 group (Table 1).

Table 1
Comparison of the Fishers Grade at admission with the intensity of cerebral vasospasm (CV)

Fisher Grade	Intensity of CV (% of patients)		
	Mild	Moderate	Severe
2	30	62.5	7.5
3	17.6	52.9	29.4
4	52.4	33.3	14.3

A positive correlation with no statistical significance of the Fisher Grade at admission with the intensity of vasospasm in the whole study group of 50 patients is shown in Figure 1 ($\rho = 0.273$, $p = 0.160$).

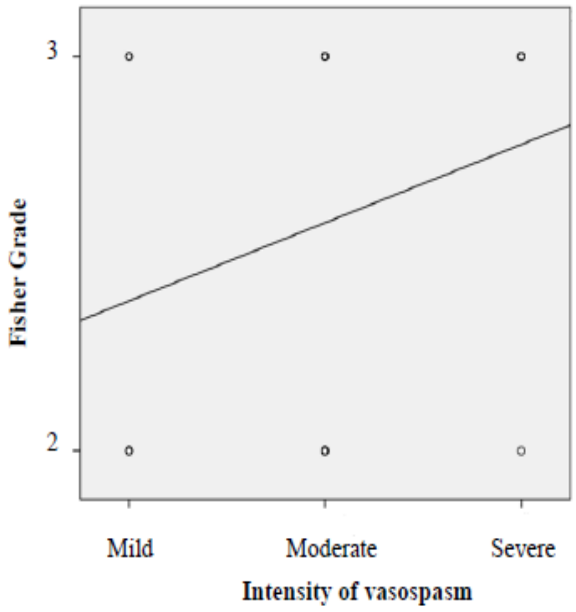

Fig. 1 – Correlation between the Fisher Grade at admission with the intensity of cerebral vasospasm.

Discussion

The Fisher Grade was established in 1980 with the assumption that it is valid enough to predict the level of CV after aSAH. This assumption was confirmed by the small sample size (41 patients) in 1983 [8]. However, recently in the larger, 2005 series, it has been found that this correlation exists, but it is not significant [9].

In this study, we determined a positive, but not significant correlation between the Fisher Grade and the intensity of vasospasm.

The Fisher Grade was created in the age when the resolution of imaging technology was about ten times lower than today. Descriptions of the amount of blood in the subarachnoid space in scans from those years could not be considered valid. The biggest difference is in the description of the thickness of SAH less than 1 mm, because in today's CT images that thick collection of SAH is almost always visible. One of the shortcomings of the Fischer Grade is that not all forms of intracranial hemorrhage after IA rupture are defined and classified. The classification of patients with small focal SAH and intracerebral hemorrhage (ICH)/intraventricular hemorrhage (IVH) is unclear, because the Fisher Grade 4 includes only patients with diffuse layer of SAH or without SAH and ICH/IVH. Also, it is not clear into which group to classify patients with SAH and subdural hematoma. Analyzing the admission CT findings in this study, 24% of the patients were classified in the Fisher Grade 2, 34% of the patients in the Fisher Grade 3 and 42% of patients in the Fisher Grade 4. The patients with ICH and the IVC, regardless the amount of SAH, were classified into the Fisher Grade 4.

Angiographic vasospasm was defined as narrowing of the contrast in the main cerebral arteries, which is usually focal, but may be diffuse, as well [10]. A large number of studies have classified the CV as mild (0–30%); moderate (30–50%) and severe or significant (above 50% narrowing of cerebral arteries) [11, 12]. The degree of CV in this study was classified into mild (0–33%), moderate (34–66%) and severe (67–100%), according to the 2008 study of Macdonald et al. [4]. After that study, most studies accepted the new classification. Frequently, it is stated that angiographic vasospasm develops in approximately 67% of patients with aSAH, which is characterized by a higher degree than the mild one [13]. Based on CTA findings, this study determined a mild degree of CV in 36% of the patients, moderate in 46% and severe of CV in 9% of the patients. In our study, 55% of the patients had moderate or severe (significant) CV.

Due to the lack of statistical correlation between the Fisher Grade and the intensity of CV, the modified Fisher Grade was proposed in 2006, which correlates significantly with the intensity of vasospasm and classifies logically the cerebral hemorrhage on CT findings (Table 2) [14].

The disadvantage of this classification, and probably the reason why the modified Fisher Grade is not widely accepted in neurosurgical clinical practice, is because it is complex and difficult to remember.

Table 2

Classification of aneurysmal subarachnoid hemorrhage (SAH) on cranial computed tomography (CT) scans by the modified Fisher Grade [14]

CT finding	Intraventricular hemorrhage	Modified Fisher Grade
Thick diffuse SAH	present	4
	absent	3
Thick focal SAH	present	4
	absent	3
Thin diffuse SAH	present	2
	absent	1
Thin focal SAH	present	2
	absent	1
No signs of SAH	present	2
	absent	0

Conclusion

This study points out the advantages and disadvantages of the original classification by Fisher. The Fisher Grade is in a positive, statistically nonsignificant correlation with the degree of cerebral vasospasm, but it is widely accepted and used in neurosurgical practice. The modified Fischer Grade is in a significant correlation with the intensity of vasospasm, but it is more complex and therefore much less applicable. In the future, it would be desirable to modify both Fisher Grades and combine them into one simple, but accurate classification.

REFERENCES

1. *Sekhar LN, Heros RC.* Origin, growth, and rupture of saccular aneurysms: a review. Neurosurgery 1981; 8(2): 248−60.
2. *Weir B.* Aneurysms affecting the nervous system. Baltimore, MD: Williams & Wilkins; 1987.
3. *Fisher CM, Kistler JP, Davis JM.* Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980; 6(1): 1−9.
4. *Macdonald RL, Kassell NF, Mayer S, Ruefenacht D, Schmiedek P, Weidauer S*, et al. Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. Stroke 2008; 39(11): 3015−21.
5. *Findlay MJ.* Cerebral vasospasm. In: *Win RH*, editor. Youmans neurological surgery. Philadelphia: Saunders; 2004. p. 1840.
6. *Dorsch NW, King MT.* A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. J Clin Neurosci 1994; 1(1): 19−26.
7. *Hatiboglu MA, Bikmaz K, Iplikcioglu AC, Turgut N.* Evaluating the factors affecting cerebral vasospasm in patients after aneurysmal subarachonoid haemorrhage. Acta Neurochir Suppl 2008; 104: 363−655.
8. *Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zervas T*, et al. The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: a prospective study. Neurology 1983; 33(4): 424−36.
9. *Smith ML, Abrahams JM, Chandela S, Smith MJ, Hurst RW, Le Roux PD.* Subarachnoid hemorrhage on computed tomography scanning and the development of cerebral vasospasm: the Fisher grade revisited. Surg Neurol 2005; 63(3): 229−34; discussion 234−5.
10. *Kassell NF, Sasaki T, Colohan AR, Nazar G.* Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 1985; 16(4): 562−72.
11. *Ferguson SD, Rosen DS, Bardo D, Macdonald RL.* Arterial diameters on catheter and computed tomographic angiography. World Neurosurg 2010; 73(3): 165−73; discussion e25.
12. *Ionita CC, Graffagnino C, Alexander MJ, Zaidat OO.* The value of CT angiography and transcranial doppler sonography in triaging suspected cerebral vasospasm in SAH prior to endovascular therapy. Neurocrit Care 2008; 9(1): 8−12.
13. *Loch Macdonald R.* Management of cerebral vasospasm. Neurosurg Rev 2006; 29(3): 179−93.
14. *Frontera JA, Claassen J, Schmidt JM, Wartenberg KE, Temes R, Connolly ES Jr,* et al. Prediction of symptomatic vasospasm after subarachnoid hemorrhage: the modified fisher scale. Neurosurgery 2006; 59(1): 21−7.

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Differences in temperament and character dimensions in adolescents with various conduct disorders

Razlike u temperamentu i karakteru kod adolescenata sa raznim poremećajima ponašanja

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Abstract

Background/Aim. Adolescence is characterized both by a large developmental potential and by an increased risk for emergence of different forms of psychopathology. International classifications of mental disorders recognize the psychopathology of adolescence at the age of 15−18 through the categories of conduct disorders and some forms of addiction: chemical and non-chemical. The aim of this research was to analyse the personality structure among four groups of adolescents manifesting different types of conduct disorder based on Cloninger's Psychobiological theory of personality. **Methods.** The research sample consisted of 140 respondents at the age of 16−18, divided into five groups: 30 respondents manifesting socialized conduct disorder, 20 adolescents in conflict with the law, 30 respondents manifesting abuse of psychoactive substances, 30 respondents with the problem of the Internet addiction and 30 from general population. The Belgrade Adolescent Personality Inventory (BAPI) questionnaire was used for the purpose of assessment of personality. Multivariate analysis of variance (MANOVA), followed by univariate analysis of variance (ANOVA) was used to examine differences between the given groups of adolescents. **Results.** The results of MANOVA show differences in the personality structure among the groups, both in the dimensions of temperament, F (20,418.84) = 2.71, $p < 0.001$, Wilks's lambda 0.67, and in the dimensions of character, F (12,344.24) = 3.27, $p < 0.001$, Wilks's lambda is 0.75. Socialized conduct disorder is characterized by low self-directedness and average cooperativeness. Adolescents in conflict with the law have the lowest persistence, together with low self-directedness and cooperativeness. Adolescents abusing psychoactive substances have low harm avoidance and self-transcendence. Adolescents with Internet addiction are characterized by high novelty seeking (impulsivity and curiosity), low self-directedness and the lowest cooperativeness. **Conclusion.** The results show that the dimensions of personality can play an important role in etiopathogenesis of various disorders in adolescents.

Key words:
mental disorders; adolescent; substance-related disorders; character; personality.

Apstrakt

Uvod/Cilj. Adolescenciju karakteriše veliki razvojni potencijal, ali i pojačani rizik od formiranja različitih vidova psihopatologije. Psihopatologiju adolescencije uzrasta od 15 do 18 godina, međunarodne klasifikacije mentalnih poremećaja prepoznaju kroz kategorije poremećaja ponašanja i neke vidove bolesti zavisnosti: hemijskih i nehemijskih. Cilj istraživanja bio je da se na osnovu Klonindžerove psihobiološke teorije ličnosti analizira struktura ličnosti kod četiri grupe adolescenata koji manifestuju različite oblike poremećaja ponašanja. **Metode.** Uzorak istraživanja sastojao se od 140 ispitanika uzrasta od 16 do 18 godina, podeljenih u pet grupa: 30 ispitanika koji su manifestovali socijalizovani poremećaj ponašanja, 20 adolescenata sa poremećajem ponašanja u sukobu sa zakonom, 30 ispitanika sa problemom zloupotrebe psihoaktivnih supstanci, 30 ispitanika sa problemom zavisnosti od interneta i 30 ispitanika kontrolne grupe. Za procenu strukture ličnosti korišćen je upitnik *Belgrade Adolescent Personality Inventory* (BAPI). Razlike između grupa adolescenata ispitane su multivarijatnom (MANOVA) i univarijatnom (ANOVA) analizom varijanse. **Rezultati.** Rezultati multivarijatne analize varijanse pokazuju da postoje razlike u strukturi ličnosti između svih grupa, kako na dimenzijama temperamenta, F

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(20,418.84) = 2.71, $p < 0.001$, Vilksova lambda 0,67, tako i na dimenzijama karaktera, F (12,344.24) = 3,27, $p < 0,001$, Vilksova lambda 0,75. Socijalizovani poremećaj ponašanja karakteriše niska samousmerenost, ali prosečna kooperativnost. Adolescenti u sukobu sa zakonom imaju najnižu perzistenciju, kao i nisku samousmerenost i kooperativnost. Adolescenti koji zloupotrebljavaju psihoaktivne supstance imaju nisko izbegavanje štete i autotranscedenciju. Za adolescente sa internet zavisnošću karakteristična je visoka potraga za novinama (impulsivnost i radoznalost), niska samousmerenost i najniža kooperativnost. **Zaključak.** Rezultati ukazuju da dimenzije ličnosti mogu igrati značajnu ulogu u etiopatogenezi različitih poremećaja adolescenata.

Ključne reči:
ponašanje, poremećaji; adolescenti; poremećaji izazvani supstancama; karakter; ličnost.

Introduction

Adolescence is characterized both by a large developmental potential and by the risk for the emergence and structuring of various forms of psychopathology [1]. International classifications of mental disorders (ICD 10 and DSM IV) mostly recognize the psychopathology of adolescence of the age between 15 and 18 through the categories of conduct disorders and some forms of addiction [2, 3].

Conduct disorders are a heterogeneous group characterized by a broad spectrum of repetitive and persistent antisocial patterns, creating a continuum [4]. At one pole of the continuum are mild disorders which can be pre-delinquent, situation-caused or a part of developmental crisis and need not to be based on an antisocial construction. The ICD 10 Classification recognizes them as socialized conduct disorder. On the other pole of the continuum are the modes of conduct having the characteristic of juvenile delinquency, revealed already in early childhood, with a tendency to being transformed into the antisocial personality disorder [5]. Conduct disorders also involving a conflict with the law make a non-socialized form of disorders. They are characterized by the lack of actual integration into the peer group, with the presence of more aggressive forms of delinquency and the disturbance of relations with adults in the form of hostility and frustration. Accordingly, the American DSM IV Classification distinguishes two models of understanding the antisocial conduct and antisocial personality disorder after the age of 18: children and adolescent types of conduct disorder [6, 7]. Both forms of disorders are significantly more present in males. The children type of conduct disorder inclines towards the antisocial personality disorder. The adolescent type of conduct disorder can persist as a disorder only in the period of adolescence.

Another large group of adolescence problems is the abuse of and the addiction from psychoactive substances, together with relatively new forms of non-chemical addiction, which unofficial name "Internet addiction", became common in the professional and popular literature. Causes of occurrence and course of psychoactive substances abuse (PAS) are often connected to the problem of intensive short or permanent anxiety. The emergence of problems related to abuse of PAS goes through early, middle and late phases [8]. The early phase is characterized by the abuse of PAS manifested through "experimenting" ("trying") or "social use". The middle phase is characterized by a slow transition from abuse to addiction, whereas the late phase is characterized by addiction.

Under the influence of the development of electronic communications and the accompanying forms of entertainment offered through new communication patterns, new disorders are emerging – particularly emphasized in the adolescence period [9, 10]. They have not been officially included into the diagnostic classifications, although the coming American DSM V Classification brings clearer guidelines for diagnosing the disorder. For the time being, the clinical practice and professional literature recognize the problem under the name of "Internet addiction" [11]. One group of experts perceives the problem in the light of behavioral, non-chemical or even technological addiction, whereas the other group comprehends the problem in the light of impulse control disorder [12]. There is an agreement among both groups of experts about the fact that the Internet by itself does not create "addiction" but it is the individual personality which finds specific forms of satisfaction in the "cyberspace" [13, 14].

The personality assessment has always been determined by the use of certain questionnaires and the theory in the background thereof. In this research, the adolescence subject matter was analyzed through the prism of the psychobiological theory of personality of Robert Cloninger. This theory defines the personality through interactive shaping of biological and social factors, describing seven dimensions of personality: four dimensions of temperament and three dimensions of character [15–17].

The paper deals with determining the relation between the basic dimensions of personality defined by the Cloninger's personality model and various forms of disorders in the adolescence period having both similarities and specific differences: socialized conduct disorder, conduct disorder that includes the conflict with the law, abuse of PAS and Internet addiction. In accordance with the chosen subject matter, the research was aimed at determining specificities and differences in the personality structure among the four groups of adolescents expressing the aforementioned forms of conduct disorders and the differences in the personality structure between the groups of adolescents with conduct disorders in comparison with the adolescents from the general population.

Methods

Sample

The research was conducted in the period between January and November 2012 in public health institutions, private hospitals, and correctional institution for adolescents in conflict with law in Belgrade, Niš and Knjaževac, as well as in Belgrade secondary schools. Inclusion of a large number of institutions into the research was necessary due to the formation of different research groups. All respondents and

their parents (parents' consent was necessary due to the juvenile age of the respondents) signed the Form of informed consent. The sample consisted of 140 respondents in total, between 16 and 18 years of age (M_{age} = 16.86), out of which 72 (51.4%) male and 68 (48.6%) female. The sample encompassed five groups of respondents: 30 respondents manifesting socialized conduct disorder (diagnosed socialized conduct disorder according to the ICD 10 Classification from the scope of the diagnosis F91.2); 20 respondents with conduct disorder in conflict with the law (pronounced judicial measure of committal to an educational institution); 30 respondents with the problem of abuse of PAS (fulfillment of criteria of substance abuse according to the ICD 10 Classification); 30 respondents with the problem of Internet addiction (fulfillment of criteria on the basis of the Young's Internet Addiction Test (IAT); and 30 respondents from the control group. The fifth group consisted of adolescents from the general population who did not manifest conduct problems or some form of addiction, nor other forms of psychopathological manifestation. Additional criteria for all respondents were the absence of psychotic manifestations, central nervous system (CNS) injuries or other somatic illnesses, the absence of problems in intellectual development and voluntary acceptance of participation in the research for all respondents.

Procedure

The adolescents with socialized conduct disorder were tested in Belgrade secondary schools and additionally in private psychiatric surgeries, upon the received approval from the school principal, Parents council and with the agreement of the private surgeries owners. The research was conducted in the two central Belgrade high schools: XIV Belgrade High School and Zemun High School; in one suburban secondary school – Grocka Secondary School, and in one vocational secondary school in Belgrade: Architecture and Technical Secondary School. The participants were initially selected by their head teachers, then they had an interview with school psychologists and at the end with the members of the research team within the school premises. The members of the research team conducted a typical psychiatric interview on the basis of which the diagnosis of social conduct disorder was established. The tests were distributed by the school psychologists and by the members of the research team in the school psychologist's premises.

The adolescents with conduct disorder in conflict with the law (pronounced judicial measure of committal to an educational institution), were tested within the PIKS project, (PIKS − *Program intervencija u kriznim situacijama* – Intervention programme in critical situations), conducted by the Republic Institute for Social Protection. The programme has obtained an approved by the Ministry of Labour and Social Issues, as well as special contracts with each of the three above-mentioned institutions – correctional institutions (Belgrade, Niš, Knjaževac). Tests have been distributed by the psychologists in these institutions.

The adolescents with the problem of PAS abuse were tested in the Special Hospital for Substance Abuse, as well as in two specialized private hospitals for substance abuse (private hospitals "Lorijan" and "Netrex zone", both from Belgrade), all upon the obtained official consent and permission for research conducting, as well as the consent of the Ethic board in the public health institution / the private hospitals owners.

The adolescents with non-chemical addiction were tested in the Day Hospital for Non-Chemical Addiction of the Special Hospital for Substance Abuse, upon the obtained official consent and approval for the research, as well as the consent of the Ethic board in the above-mentioned Belgrade secondary schools, following the above-stated procedure. The adolescents with non-chemical addiction filled in tests in the psychiatric surgery, in the public health institutions, and in the school psychologist's premises.

The control group adolescents were tested in the above-stated Belgrade secondary schools following the already stated procedure. The tests for the adolescents were distributed by the members of the research team during specially organized classes agreed with the head teachers and the school psychologists.

The time necessary for the tests filling in was form 45 to 90 minutes, approximately up to 60 minutes. The research in the above-stated schools lasted one school class or one school class and a break.

Instruments

Since the respondents were classified into groups on the basis of the ICD-10 Classification criteria, the test criterion was used only for the group of adolescents with Internet addiction problem, considering that this disorder had not yet been included into the official classification. To that end, the Young's Internet Addiction Test (IAT) consisting of 20 items was used for self-assessment of the problems related to the use of Internet. The following classification of scores was used in this paper: the score of 20−39 average use; 40−69 frequent problems due to the use of Internet; 70−100 significant problems due to the use of Internet [18]. The adolescents classified into the category of "Internet addicts" had IAT score above 70.

For the purpose of personality assessment we used the BAPI, a questionnaire constructed on the basis of Cloninger's theory of personality, which measures four dimensions of temperament and three dimensions of character. The BAPI consists of 46 items grouped into 4 temperament scales: Novelty Seeking (NS) divided into two subscales − Impulsivity (NS Im) and Explorative Curiosity (NS Ec); Harm Avoidance (HA); Reward Dependence (RD) and Persistence (P) and 3 character scales: Self-Directedness (SD); Cooperativeness (C) and Self-Transcendence (ST). The respondents answered on a 5-level Likert-type scale. BAPI is created for adolescents from 14 to 18 years of age [19, 20]. The questionnaire's scale showed a satisfactory reliability (0.66–0.80), particularly having in mind a small number of items (5–6) *per* scale. Confirmatory factor analysis in principle confirmed the two main dimensions of temperament and character as defined by Cloninger. The only exception was the case of NS, for which two-factor solution is more conveni-

ent. NS is composed of Explorative Curiosity and Impulsivity which differs "good" (curious, interested, creative) and "bad" (impulsive, impatient, chaotic) features of this dimension.

Statistics

Data analysis was performed by SPSS. Multivariate analysis of variance (MANOVA), followed by univariate analysis of variance (ANOVA) was used to examine differences between the given groups of adolescents. Before the analysis, the basic psychometric characteristics of BAPI were examined.

Results

The obtained results show the satisfactory psychometric characteristic of the BAPI scale on this sample, having in mind only 6 items *per* scale. Cronbach's alpha ranges from 0.70 to 0.82, apart from the Reward Dependence (RD) scale with a marginal reliability (0.60). Even the NS subscales, Impulsivity and Curiosity with 5 items each have an acceptable reliability (0.66 and 0.67) (Table 1).

The results of multivariate analysis of variance (MANOVA) show significant differences in personality structure among all the four groups of adolecesents, both in dimensions of temperament, F (20,418.84) = 2.71, $p < 0.001$, Wilk's lambda is 0.67, and in dimensions of character, F (12,344.24) = 3.27; $p < 0.001$, Wilk's lambda is 0.75.

When observing differences in particular scales, significant differences notably exist at all scales except for the RD scale and the NS Curiosity subscale (Table 2).

The dimensions of personality that differ the groups are mainly the Impulsivity (NSI) subscale, Persistence (P) and Harm Avoidance (HA) scales, representing the dimensions of temperament which in fact essentially optimize the development of a stable, mature character. In relation thereto, significant differences among the groups are shown in the Self-Directedness (SD) and Cooperativeness (C) dimensions of character which, together with Persistence (P), are the highest in the group of adolescents from the general population (Figure 1).

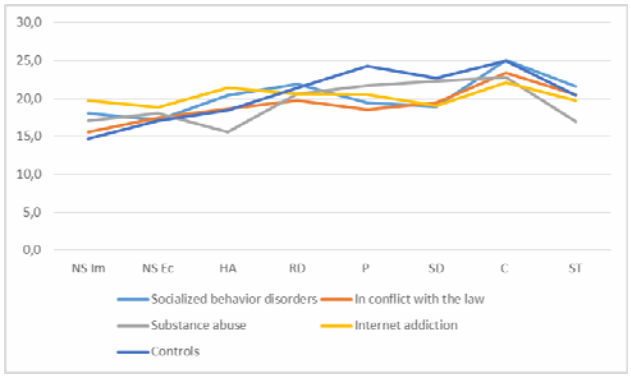

Fig. 1 – Groups profiles in temperament and character dimensions.
NSIm – Novelity Seeking Impulsivity; RD – Reward Dependence; P – Persistence; SD – Self-Directedness; C – Cooperativeness; ST – Self-Transcendence.

Discussion

The lack of adequate instruments for assessment of adolescents' personality and the open issues about the development of adolescents' personality structure point to theoretical and practical problems in the research. The BAPI is a new

Table 1

Descriptive statistics and adolescents temperament and character scales validity

Temperament and character dimension	x̄	SD	Min	Max	Sk	Ku	Number of items	α
Impulsivity (NS Im)	17.19	4.21	5	25	-0.39	-0.22	5	0.66
Explorative Curiosity (NS Ec)	17.69	3.55	8	23	-0.70	0.00	5	0.67
Harm avoidance (HA)	18.96	6.14	6	30	-0.10	-0.89	6	0.82
Reward dependence (RD)	21.11	3.93	11	29	-0.32	-0.67	6	0.60
Persistence (P)	21.48	5.31	7	30	-0.38	-0.42	6	0.86
Self-directedness (SD)	20.72	5.14	6	30	-0.68	0.37	6	0.75
Cooperativeness (C)	23.73	3.70	16	30	-0.10	-1.06	6	0.67
Self-transcendence (ST)	19.68	6.16	6	30	-0.44	-0.48	6	0.83

NS – Novelity Seeking.

Table 2

Differences between the groups in temperament and character dimensions

Temperament and character dimension	Socialized behavior disorders	In conflict with the law	Substance abuse	Internet addiction	Controls	Group comparison				
	x̄ ± SD	x̄ ± SD	x̄ ± SD	x̄ ± SD	x̄ ± SD	F	df_w	dfb	p	η^2
Impulsivity (NS Im)	18.13 ± 3.60	15.62 ± 4.31	17.07 ± 3.47	19.70 ± 3.70	14.67 ± 4.40	7.38	4	128	0.00	0.19
Explorative Curiosity (NS Ec)	17.17 ± 3.74	17.54 ± 4.43	18.10 ± 4.04	18.83 ± 3.17	17.07 ± 2.89	1.24	4	128	0.299	0.04
Harm avoidance (HA)	20.43 ± 5.32	18.62 ± 5.98	15.60 ± 6.34	21.37 ± 5.46	18.43 ± 6.00	4.34	4	128	0.003	0.12
Reward dependence (RD)	21.87 ± 4.38	19.69 ± 3.59	20.63 ± 4.16	20.57 ± 3.93	21.37 ± 3.17	0.97	4	128	0.434	0.03
Persistence (P)	19.43 ± 5.96	18.46 ± 4.77	21.67 ± 4.47	20.53 ± 5.49	24.30 ± 4.08	4.94	4	128	0.001	0.13
Self-directedness (SD)	18.90 ± 5.40	19.41 ± 4.05	22.27 ± 4.84	19.03 ± 5.08	22.67 ± 4.16	4.29	4	132	0.003	0.12
Cooperativeness (C)	25.07 ± 3.97	23.35 ± 4.08	22.77 ± 3.30	22.07 ± 3.12	25.00 ± 3.54	4.21	4	132	0.003	0.11
Self-transcendence (ST)	21.63 ± 5.86	20.53 ± 3.39	17.03 ± 7.03	19.67 ± 5.79	20.37 ± 5.17	2.65	4	132	0.036	0.07

NS – Novelity Seeking.

form of questionnaire for temperament and character assessment based on the Robert Cloninger's Psychobiological model of temperament and character. It has been constructed in our region and is fully adjusted to our population in the cultural aspect. Its satisfactory psychometric characteristics have in several occasions been confirmed in the process of construction [20], as well as in this report.

The adolescents with socialized conduct disorder showed an emphasized novelty seeking in the sense of impulsivity, reduced persistence, i.e. weak endurance and low self-directedness, however without a weak cooperativeness, which points to their good inclusion into the society, i.e. their peer group. This group of adolescents represents a milder form of conduct disorder, characterized by running away from school and/or home, in fact running away from the source of problem as a "coping" strategy, while remaining good social contact with the peer group of similar tendencies. The problem appears when all possibilities for running away from problems are exhausted and when pressure emerges both from the parents and from the school for the accumulated problems to be resolved. Low Self-Directedness confirms the difficulties in adjusting the behavior to the situation, weakly defined goals and feeling of responsibility for own acts. Particularly high scores in the reward dependence indicate social and emotional sensitivity. This finding, together with the accompanying cooperativeness, point to the socialization potential which can act as a protective factor for development of subsequent behavioral disorders, therefore transitional adolescent crises which are to be overcome in future are more typical for this group.

The adolescents with conduct disorder including the conflict with the law have similar characteristics. They score the lowest persistence, which indicates the difficulties in endurance and easily giving up. Also, they have reduced harm avoidance as a reflection of absence of fear from punishment which does not inhibit their behavior, and lower cooperativeness in relation to the group with socialized form of behavioral disorder, having in mind their antisocial behavioral tendencies. A specific problem of this group is the lowest reward dependence (although not significantly), as a reflection of weaker social and emotional responsiveness. Within a reduced response to reward and punishment (reduced scores on RD and HA), a successful implementation of educational methods is difficult and requires more intensive therapeutic interventions.

For the adolescents with the abuse of PAS it is characteristic that in comparison with other groups of respondents they have the lowest scores in Harm Avoidance (HA), reflecting their inclination towards a risky and auto-destructive behavior which is not inhibited by fear from harmful consequences. This contradicts to the earlier researches of abuse of and dependence from PAS, which emphasized a high level of harm avoidance [21]. It is possible that exactly the abuse of PAS has the function to reduce the anxiety related to high scores in this dimension, or that – which was not the case in our sample – anxiety and depression (high HA scores) appear later, in developed addiction phase, as consequences of secondary addiction-related problems. Another specificity is lower scores in self-transcendence. This can be related to the experience of the absence of sense or spiritual values, leading to the need for escape from reality through the abuse of PAS. In the selected sample of respondents, the abuse of substances did not achieve the level of dependence, increasing the chances for therapeutic success [8]. Many researches emphasize the importance of personality in emergence of problems with use and abuse of PAS and not only the availability of the substances, whereas additional family and other environmental factors can play a decisive role in further deepening of the disorder to the level of addiction.

The adolescents who developed Internet addiction have the lowest Self-Directedness (SD) and Cooperativeness (C) character dimensions, however rather of asocial than of antisocial type. Considering that this group also has the highest scores in Harm Avoidance, the combination of these two dimensions can point to the actual social anxiety and avoidance of society through protective isolation and withdrawal into the virtual world. In addition, this group is also characterized with the highest scores in both components of Novelty Seeking (NS), Impulsivity (NS Im), and Explorative curiosity (NS Ec). This is in accordance with the typical Internet-related conduct problem, emphasizing the impulse control disorder [11] regardless of the reason this control has been disturbed. It is possible that the technological progress brings a new opportunity for expressing impulsivity in the virtual reality, or, which is more probable, long sitting in front of the computer and seizing into often violent contents lead to irritability, with a tendency for its impulsive relieve. The results of researches carried out so far indicate that Internet by itself does not create the "addiction" but it is the individual personality which finds specific forms of satisfaction in the "cyberspace" [12]. Observed as a whole, this group of adolescents has the most risky profile, with the lowest character and the most unstable temperament characteristics, particularly in relation to internal conflict of contradictory aspirations such are the aspirations towards novelties (high NS scores) and inhibiting anxiety in front of them (high HA scores).

The group of adolescents from the general population is very heterogeneous, except when it is about the absence of manifestation of psychopathological forms of behavior; therefore, any common description of personality would be senseless. However, their scores in personality dimensions point to the most mature personality structure, in the sense of the most developed character dimensions and the highest Persistence (P), the lowest Impulsivity (NS Im), moderate Harm Avoidance (HA) and increased Reward Dependence (RD), being an optimum combination for the development of a stable and mature personality.

On the basis of these results all the examined groups of adolescents differ *per* low scores in character dimensions in relation to young people from the general population, while temperament dimension specifically distinguishes particular groups of youth with behavioral problems. On the basis of the obtained results we can say that personality dimensions can play a significant role in etiopathogenesis of different adolescents' disorders. The results can be a base for psychodiagnostic assessment, as well as for creating programs and interventions for prevention adolescence prob-

lems. Socialized conduct disorder can be not an ephemeral developmental phase in adolescence, however, the preventive work in this phase can prevent complications of the problem and deepening of disorders within the framework of other unfavorable circumstances. Among the adolescents in conflict with the law, the conduct disorder have already achieved the level of problems requesting educational court measures; however, the personality profile has no characteristics of a serious antisocial disorder and intensive, integrative therapeutic interventions might yield promising results. The abuse of substances is still only the first phase in addictive disorders and there are possibilities for termination of further development of the problem, which requires timely implementation of the existing preventive programs and creation of new ones. The adolescents with Internet addiction problem in our research have the most extreme scores in personality dimensions, which might represent a significant factor of psychopathological risk.

The research results confirmed the hypotheses of this paper that the clinical groups of adolescents differ from the group from general population, which is in line with earlier findings of Cloninger et al. [17] on the basis of the Self-Directedness and Cooperativeness character dimensions which differentiate the respondents along the normal-pathological dimension [22–24]. The character dimensions' scores are the highest on the scales of the group general population. The results also confirmed the other hypotheses of the study, showing that different personality profiles can be connected with specific disorder manifestations.

This study has some limitations that should be taken into account when interpreting its findings and conclusions. First, it is a descriptive, cross-sectional study, which limits the ability to determine causal relationships between the personality and various conduct disorders. Second, research sample, primarly because of the size, may not be representative of all the adolescents in Serbia.

Conclusion

It can be concluded that the dimensions of personality can play an important role in etiopathogenesis of different adolescence disorders. Assessment of these dimensions of personality could be useful in psychodiagnostic evaluation, risk assessment of development problems in adolescence, as well as in considering therapeutic goals. These findings suggest the need for further longitudinal-type studies on larger samples as well as the need for creation more efficient preventive and therapeutic programs, and their more systematic and coordinated implementation.

REFERENCES

1. *Erikson HE*. Identity and life cycle. Belgrade: Zavod za udžbenike; 2008. (Serbian)
2. *Dukanac V*. Correlation of socio-pathological and adolescent psychopathology with the structure of the personality of parents and family functioning. Belgrade: Faculty of Special Education and Rehabilitation; 2013. (Serbian)
3. *World Health Organization*. ICD-10 International Classification of Diseases. Belgrade: Zavod za udžbenike i nastavna sredstva; 1992. (Serbian)
4. *Pejović-Milovančević M*. Conduct disorder. In: *Bojanin S, Popović-Deušić S*, editors. Developmental psychiatry. Belgrade: Institute of Mental Health; 2012. p. 361–72. (Serbian)
5. *Radulović D*. Conceptual framework of conduct disorder - psychological approach. In: *Radovanović D*, editor. Conduct disorder and juvenile delinquency: Special-pedagogical discourse. Belgrade: University of Belgrade, Faculty of Special Education and Rehabilitation; 2007. p. 11–29. (Serbian)
6. *Moffitt TE*. Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy. Psychol Rev 1993; 100(4): 674–701.
7. *Loeber R*. Development and risk factors of juvenile antisocial behavior and delinquency. Clin Psychol Rev 1990; 10(1): 1–41.
8. *Ramah A*. Substance abuse in children and adolescents. In: *Bojanin S, Popović-Deušić S*, editors. Developmental psychiatry. Belgrade: Institute of Mental Health; 2012. p. 435–54. (Serbian)
9. *Grüsser SM, Thalemann R, Griffiths MD*. Excessive computer game playing: evidence for addiction and aggression. Cyberpsychol Behav 2007; 10(2): 290–2.
10. *Batthyany D, Muller KW, Benker F, Wolfling K*. Computer game playing: clinical characteristics of dependence and abuse among adolescents. Wien Klin Wochenschr 2009; 121(15–16): 483–5.
11. *Young KS*. Internet addiction: the emergence of a new clinical disorder. Cyberpsychol Behav 2008; 1(3): 237–44.
12. *Shapira NA, Lessig MC, Goldsmith TD, Szabo ST, Lazoritz M, Gold MS,* et al. Problematic - internet use: proposed classification and diagnostic criteria. Depress Anxiety 2003; 17(4): 207–16.
13. *Popović-Ćitić B, Marković M, Popović V*. Internet addiction – current knowledge and dillemas. In: *Kordić B, Kovačević A, Banović B*, editors. Responding to security risks in educational institutions. Belgrade: Faculty of Security Studies; 2012. p. 261–71. (Serbian)
14. *Elder GH, Nguyen TV, Caspi A*. Linking family hardship to children's lives. Child Dev 1985; 56(2): 361–75.
15. *Cloninger R, Przybeck T, Svrakic D, Wetzel R*. The Temperament and Character Inventory (TCI): A guide to its development and use. St Louis: Washington University; 1994.
16. *Dzamonja-Ignjatovic T, Svrakic DM, Svrakic N, Jovanovic MD, Cloninger RC*. Cross-cultural validation of the revised Temperament and Character Inventory: Serbian data. Compr Psychiatry 2010; 51(6): 649–55.
17. *Cloninger CR, Svrakic NM, Svrakic DM*. Role of personality self-organization in development of mental order and disorder. Dev Psychopathol 1997; 9(4): 881–906.
18. *Chang MK, Law SP*. Factor structure for Young's Internet Addiction Test: A confirmatory study. Comput Human Behav 2008; 24(6): 2597–619.
19. *Dukanac V, Džamonja-Ignjatović T, Milanović M*. Construction and psychometric checking of Adolescent Temperament and Character Inventory ATCI-80. Engrami 2011; 33(3): 5–17. (Serbian)
20. *Džamonja-Ignjatović T, Milanović M, Dukanac V*. Belgrade Adolescent Personality Inventory: bridging the gap between personality assessment in children and adults. Psihološka istraživanja 2014; 17(1): 35–53.
21. *Svrakic D, Cloninger CR, Svrakic N, Lazic B, Milivojevic D, Nastasic P*. Drug addiction and choice of drugs: Temperament and

personality as risk factors. Ser J Exp Clin Res 2010; 11(3): 93−8.

22. *Svrakic DM, Whitehead C, Przybeck TR, Cloninger CR*. Differential diagnosis of personality disorders by the seven-factor model of temperament and character. Arch Gen Psychiatry 1993; 50(12): 991−9.
23. *Svrakic D, Cloninger R*. Personality disorder. In: *Kaplan H, Sadock B*, editors. Comprehensive Textbook of Psychiatry. Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo: Williams and Wilkins; 2005. p. 2063−104.
24. *Ignjatovic-Dzamonja T, Svrakic D*. Western personality models applied in Eastern Europe: Yugoslav data. Compr Psychiatry 2003; 44(1): 51−9.

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Organized colorectal cancer screening in Serbia – The first round within 2013–2014

Organizovano ispitivanje kolorektalnog karcinoma – prvi ciklus tokom 2013–2014.

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Abstract

Background/Aim. The National Organized Colorectal Cancer Screening Program was conducted in the Republic of Serbia during 2013–2014 covering the population of both genders, aged 50 to 74 years, in 28 municipalities out of 180, with the target population of 651,445 people. This organized colorectal cancer screening aim is to reduce mortality from colorectal cancer in the target population. The aim of this study was to show the results of organized screening for colorectal cancer during the first biannual round in Serbia. **Methods.** General practitioners from the primary health centers, invited target population by mail and by phone to perform immunochemical fecal occult blood test. Persons with a positive test results were referred to the colonoscopy. The database of health insurance and other citizens of the target population was used for invitation for screening in primary health centers. Descriptive statistical analysis of the results in organized colorectal cancer screening in the first round was performed for the key screening indicators. **Results.** In the first round a total of 99,592 persons were invited. The participation rate was 62.5%. Colonoscopy was performed in 1,554 persons. Adenomas were found in 586 persons (0.9% of all the tested), e.g. 37.7 % of all colonoscopied. In 129 persons colorectal cancer was diagnosed (0.2% of all the tested), e.g. 8.3% of all the colonoscopied. In the left half of the colon (rectum, sigmoid and descending colon) there were 70.4% diagnosed polyps and 77.3% carcinomas, while 29.6% of polyps and 22.7% carcinomas were found in the proximal parts of the colon. **Conclusion.** In the first round of the organized colorectal cancer screening in Serbia the participation rate of the targeted population was high and gave encouraging result. It was expected that in the forthcoming rounds even higher coverage of the target population would be accomplished. A positive predictive value of the completed colonoscopies showed that further observing the stages of diagnosed adenomas and carcinomas would reach the goals of the expected improvement in early detection of colorectal cancer in Serbia.

Key words:
colorectal neoplasms; serbia; mass screening; diagnosis; occult blood; predictive value of tests; colonoscopy; adenoma.

Apstrakt

Uvod/Cilj. Nacionalni program organizovanog ispitivanja kolorektalnog karcinoma sproveden je u Republici Srbiji tokom 2013. i 2014. godine, obuhvatajući stanovništvo oba pola, starosti 50–74 godine, u 28 od 180 opština, sa ciljnom populacijom od 651 445 ljudi. Program ima za cilj sniženje mortaliteta od kolorektalnog karcinoma u ciljnoj populaciji. Cilj rada bio je da se prikažu rezultati organizovanog ispitivanja kolorektalnog karcinoma tokom dvogodišnjeg perioda u Srbiji. **Metode.** Izabrani lekari iz domova zdravlja pozivali su pismom i telefonom ciljnu populaciju da urade imunohemijski FOB test za otkrivanje okultnog krvarenja u stolici. Osobe sa pozitivnim nalazom testa upućivane su na kolonoskopiju. Korišćena je baza podataka osiguranika i ostalih građana ciljne populacije u pozivanju na snimanje u domovima zdravlja. Rađena je deskriptivna statistička analiza rezultata u organizovanom ispitivanju kolorektalnog karcinoma u prvom dvogodišnjem ciklusu, za ključne indikatore ispitivanja. **Rezultati.** U prvom ciklusu bile su pozvane ukupno 99 592 osobe. Odziv na testiranje bio je 62,5%. Kolonoskopija je urađena kod 1 554 osobe. Adenomi su otkriveni kod 586 osoba (0,9% svih testiranih), tj. 37,7% svih kolonoskopiranih. Kod 129 osoba otkriven je kolorektalni karcinom (0,2% svih testiranih), tj. kod 8,3% svih kolonoskopiranih. U levoj polovini kolona (rektumu, sigmoidnom i descedentnom kolonu) bilo je 70,4% dijagnostikovanih polipa i 77,3% karcinoma, dok je 29,6% polipa i 22,7% karcinoma bilo u proksimalnim delovima ko-

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lona. **Zaključak.** U prvom ciklusu organizovanog ispitivanja kolorektalnog karcinoma u Srbiji odziv ciljne populacije na testiranje bio je visok i predstavljao je ohrabrujući rezultat. Očekuje se da će u narednim ciklusima biti postignuta čak i veća pokrivenost ciljne populacije. Pozitivna vrednost predviđanja urađenih kolonoskopija pokazuje da će se daljim radom na praćenju stadijuma otkrivenih adenoma i karcinoma dostići ciljevi očekivanog napretka u ranoj dijagnozi kolorektalnog karcinoma u Srbiji.

Ključne reči:
kolorektalne neoplazme; srbija; masovno isitivanje; dijagnoza; okultna krv; testovi, prognostička vrednost; kolonoskopija; adenom.

Introduction

Epidemiology

In Serbia, as in developed countries, colorectal cancer represents a very significant public health problem because of the high frequency and high mortality. Organized screening showed to be a powerful weapon against this disease [1].

According to the data of Globocan from 2012, colorectal cancer in Serbia is the second most frequent malignant tumor in men, after lung cancer, since the number of newly discovered cases is around 3,400, and the standardized incidence rate is 43.4 *per* 100,000 males. In females, colorectal cancer is the fourth most frequent malignant tumor, after the breast, lung and cervical cancer. The number of the newly discovered cases is around 21,00 and the standardized incidence rate is 22.3 *per* 100 000 females. According to the same source, regarding mortality from malignant diseases in Serbia, colorectal cancer stands in the second place after lung cancer, with the standardized mortality rate of 22.8 *per* 100 000 males, with around 1,900 deaths *per* year, while in females it is the third most frequent cause of death from malignant diseases, after the breast and lung cancer, with a standardized mortality rate of 11.5 *per* 100 000 and 1,200 death cases *per* year [2].

The data of the Register for Cancer of the Institute for Public Health of Serbia "Dr Milan Jovanović Batut" for the year 2012 are almost similar, showing that colorectal cancer in Serbia is the second most frequent cancer in males for both incidence and mortality. In females it is also the second leading cause of mortality due to malignancy. According to this data, in 2012 there were 4,080 newly diagnosed colorectal cancers in Serbia (2,495 males and 1,585 females) while 2,652 patients died (1,579 males and 1,073 females) [3].

According to the data of the International Agency for Research on Cancer (IARC) from the year of 2012, Serbia is in the 13th place among the European countries regarding the incidence of colorectal cancer, while it is in the 6th place in Europe, after Hungary, Croatia, Slovakia, Slovenia and the Czech Republic regarding mortality from colorectal cancer. This information shows that colorectal cancer in Serbia is frequently detected in advanced stage when chances for cure are significantly reduced.

Organized Colorectal Cancer Screening Program in Serbia

In order to reduce the incidence and the mortality from colorectal cancer, experts of the Ministry of Health of the Republic of Serbia, in cooperation with the most eminent experts in this area, led by Academician Prof. Dr. Zoran Krivokapić, in a decade long work, created and improved Organized Colorectal Cancer Screening Program in Serbia, and coordinated it with the European Guidelines for Quality Assurance of Colorectal Cancer of Screening (year 2010.) [4], which utilize programs of screening of most of the European countries. This program was realized in coordination with National program for early detection of colorectal cancer (Official Gazette of the Republic of Serbia 73/2013) [5].

The goal of the organized colorectal cancer screening program is to reduce the incidence and the mortality of colorectal cancer by early detection, in precancerous stadium, the stadium of benign polyps (whose removal prevents transformation into colorectal cancer), or detection of colorectal cancer in an early phase when it is localized and a complete cure is possible in almost 90% of cases.

Due to its slow progression (adenoma-carcinoma sequence), colorectal cancer is an ideal target for early discovery and prevention thorough organized screening [6].

In developed countries, in which screening programs have been successfully utilized for several decades, a significant decrease in the mortality from colorectal cancer has been recorded [2, 4].

The aim of this study was to show the results of organized screening for colorectal cancer during the first biannual round in Serbia.

Methods

The database of those with health insurance and other citizens of the targeted population was used for invitations to screening, planning to include 75% of the target population.

The selected process indicators of the results of screening were analyzed from the screening reports from 20 municipalities in Serbia in the year 2013, as well as additional 8 municipalities included in 2014, with a total of 28 municipalities in the course of the first biannual round of the organized colorectal cancer screening.

A model of the Organized Colorectal Screening Program in the Republic of Serbia

A National Program of the Organized Colorectal Cancer Screening is conducted by the Ministry of Health in association with the Institute for Public Health of Serbia and the Republican Fund for Health Insurance.

Screening for colorectal cancer is conducted on the territory of the Republic of Serbia as an organized decentralized program. The target population were men and women from 50 to 74 years of age in a round of screening for two calendar years. Screening test was immunochemical fecal occult blood (iFOB) test. In all cases of positive iFOB test colonoscopy was performed.

The end of the process of screening was defined as: cases with negative iFOB test; in case of positive iFOB test colonoscopy was performed (with endoscopic polypectomy or biopsy depending on findings and histopathological report) [5].

The participants in organized colorectal cancer screening were: Ministry of Health, Republican Expert Commission for Implementation of the Program for Early Detection of Malignant Diseases in the Republic of Serbia, Republican Fund for Health Insurance, Cancer Screening Office and local health institutions such as primary health centers as basic carriers of screening, as well as general hospitals, health centers, clinical hospital centers, clinical centers and the Institute for Public Health in Serbia "Dr Milan Jovanović Batut" with a network of 24 institutes of public health in each of the districts.

Invitations to the target population by letters and by phone were conducted by the primary health care centers in cooperation with district institutions of public health. The tests were delivered by the general practitioners (GPs) in the primary health care centers, while analysis of the results of iFOB tests was done in the laboratories of the primary health care centers. Colonoscopies for all the participants in the screening with positive iFOB test were performed in the closest district hospitals and colonoscopy units [5].

In 2013 and 2014, 27 primary health care centers, in cooperation with secondary and tertiary health care institutions, conducted the organized colorectal cancer screening with their available capacities and scope which did not affect their primary occupation.

As above mentioned the year 2013, the target population from 20 municipalities from the territory of the Republic of Serbia was invited and tested under the organized screening for colorectal cancer in 19 primary health care centers. During 2014, the scope of invitations and testing of the target population was expanded with 8 additional municipalities, which included a total of 28 municipalities in the first biannual round out of 180 municipalities in Serbia.

Statistics

Descriptive statistical analysis of the basic epidemiological indicators was performed. The process indicators, coverage by invitations, participation rate, coverage by iFOB test, percentage of positive iFOB tests, colonoscopy compliance rate, adenoma and cancer detection rate, as well as positive predictive value for detection of adenoma and colorectal cancer were calculated.

Results

Participation rate

In the first round during 2013 and 2014 there were 99,592 invited persons of the target population, i.e. 19% of the total target population in the municipalities in which organized colorectal cancer screening was conducted (Table 1). A total of 62,252 persons performed and returned the test, so the participation rate was 62.5% of all the invited persons. The number of positive iFOB test was 3,690 representing 5.9% of all the tested persons. Colonoscopy was performed in 42.1% of those with positive iFOB test.

Adenomas were found in 586 (37.7%) of all the colonoscopied, while in 129 (8.3%) colorectal cancer was found.

The coverage by invitations and the participation rate of the target population was higher in the year 2014, as shown in the Figures 1 and 2.

Results of colorectal cancer (CRC) screeing programme performed during two-years period according to the different municipalities are shown in Table 2.

Table 1

Basic indicators of organized colorectal cancer (CRC) screening

Indicator	Persons, n (%)			Relative to issue
	2013	2014	Total	
Invited	38,290 (15.3)	61,302 (20.5)	99,592 (19)	Eligable
Tested iFOBT	23,761 (62.1)	38,491 (62.8)	62,252 (62.5)	Invited
Positive iFOBT	887 (3.7)	2,803 (7.3)	3,690 (5.9)	Tested
Colonoscopied	463 (52.2)	1,091 (38.9)	1,554 (42.1)	Positive iFOBT
Adenom detected	145 (31.3)	441 (40.4)	586 (37.7)	Colonoscopy
CRC diagnosed	41 (8.9)	88 (8.1)	129 (8.3)	Colonoscopy

iFOBT – immunochemical fecal occult blood test.

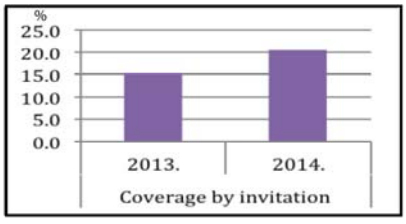

Fig. 1 – Proportion of persons of the target population invited to participate in the program according to years.

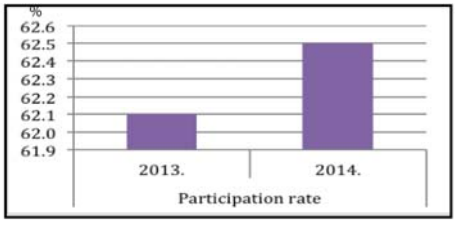

Fig. 2 – Participation rate by year.

Table 2

Distribution of invited persons, participation rate, positive immunochemical fecal occult blood (iFOB) test, screening colonoscopies and the persons with detected adenomas and colorectal cancer (CRC) in municipalities in Serbia, 2013–2014

Municipality	Invited persons	Participation rate	Positive iFOB test		Colonosocopy	Adenoma		CRC	
	n	%	n	%	n	n	% of colonoscopy	n	% of colonoscopy
Zemun	2,580	41.6	76	7.1	45	17	37.8	2	4.4
Voždovac	6,774	92.1	298	4.8	96	42	43.8	15	15.6
Čukarica	9,939	70.3	615	8.8	465	212	45.6	37	8
Barajevo	751	73.8	114	20.6	17	2	11.8	0	0
Sopot	2,808	67.7	122	6.4	34	10	29.4	5	14.7
Novi Bečej	1,460	59.2	110	12.7	41	20	48.8	1	2.4
Sečanj	2,110	75	72	4.6	49	28	57.1	5	10.2
Pančevo	12,461	61.4	337	4.4	176	47	26.7	7	4
Kragujevac	4,637	51.4	200	8.4	52	21	40.4	3	5.8
Topola	887	71.5	39	6.2	15	2	13.3	4	26.7
Knić	15,96	60.2	24	2.5	7	2	28.6	0	0
Niš	22,162	43.7	597	6.2	127	44	34.6	5	3.9
Doljevac	1,478	89	45	3.4	15	5	33.3	5	33.3
Kosjerić	1,803	64.4	14	1.2	12	3	25	1	8.3
Požega	3,954	54.5	25	1.2	17	1	5.9	1	5.9
Leskovac	3,793	83.4	140	4.4	43	9	20.9	9	20.9
Vlasotince	1,589	65.8	57	5.5	31	11	35.5	8	25.8
Lebane	1,795	53.4	14	1.5	7	2	28.6	2	28.6
Medveđa	1,236	67.9	15	1.8	8	5	62.5	1	12.5
Mali Iđoš	1,083	57.7	30	4.8	15	7	46.7	0	0
Sr. Mitrovica	2,730	47.7	89	6.8	28	2	7.1	3	10.7
Lučani	1,238	70.4	16	1.8	2	0	0	0	0
Ivanjica	854	46.7	9	2.3	3	1	33.3	0	0
Ćuprija	1,017	79.1	178	22.1	50	21	42	1	2
Valjevo	3,045	93.1	230	8.1	77	19	24.7	10	13
Sombor	4,052	66.8	76	2.8	30	19	63.3	2	6.7
Kraljevo	1,760	88.5	148	9.5	92	34	37	2	2.2
Total	99,592	62.5	3,690	5.9	1554	586	37.7	129	8.3

Participation rate

In the first biannual round of the organized colorectal cancer screening, participation rate was high (62.5%) and there was no statistically significant difference in participation rate of the target population between municipalities in Serbia (Figure 3).

Positive iFOB test rate

A total number of persons with positive iFOB test was 3,690 which makes 5.9% of the tested. The highest percentage of positive iFOB tests was recorded in the town Cuprija (22.1%) and Barajevo (20.6%), and the lowest in the town of Kosjerić and Požega (1.2%). A large difference in the per-

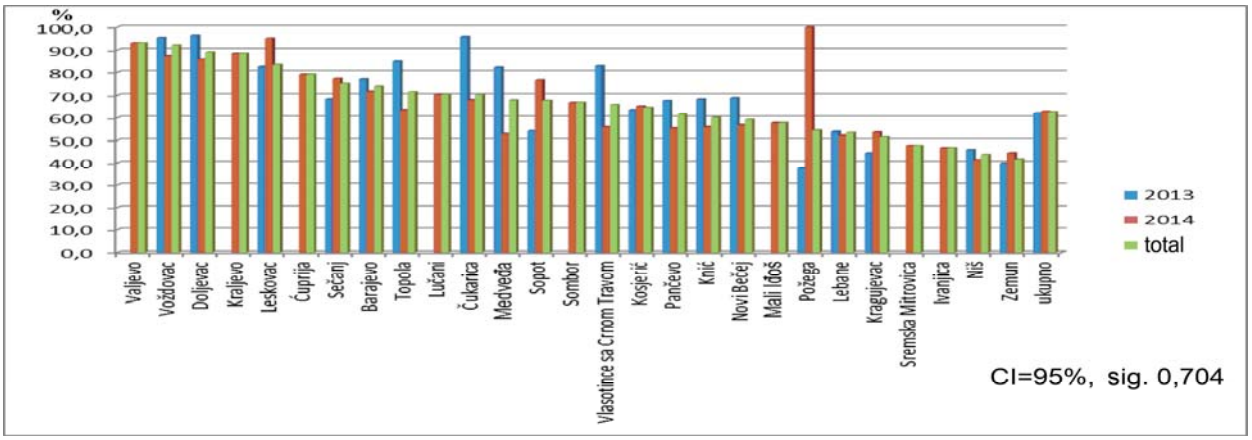

Fig. 3 – Participation rate of the target population in different municipaties in Serbia.

centage of positive iFOB test findings between municipalities urged further investigation, considering that primary health care centers until then did not use the same iFOB test. There was no statistically significant difference in positive iFOB test rate in different municipalities in Serbia (Figure 4).

Colonoscopy compliance rate

Colonoscopy was performed in 1,554 out of 3,690 persons with positive iFOB test, so colonoscopy compliance rate was 42.1%.

In the group of 1,554 persons in whom during the first round of organized colorectal cancer screening colonoscopy was performed, 586 (37.7%) persons of all colonoscopied, i.e. 0.9% of all the tested, adenomas were diagnosed and treated with endoscopic polypectomy, while in 129 (8.3%) of all colonoscopied, i.e. 0.2 of those tested, colorectal cancer was found.

Adenoma detection rate

Adenoma detection rate was 9‰. It represents proportion of persons with at least one detected adenoma in relation to complete number of tested persons in the observation period, calculated *per* 1,000 participants. There was no statistically significant difference in adenoma detection rate in different municipalities in Serbia (Figure 5).

Positive predictive value (PPV) for detection of adenoma (percentage of persons with at least one detected adenoma in relationship with persons with positive FOB test who undertook colonoscopy within the period of observation) measured 37.7%. There was no statistically significant difference in PPV for detection of adenoma in different municipalities in Serbia (Figure 6).

Cancer detection rate

The cancer detection rate was 2‰. In different municipalities in Serbia there was no statistically significant difference in the cancer detection rate (Figure 7).

PPV for detection of cancer

PPV for detection of cancer was 8.3%. There was no statistically significant difference in PPV for detection of cancer in different municipalities in Serbia (Figure 8).

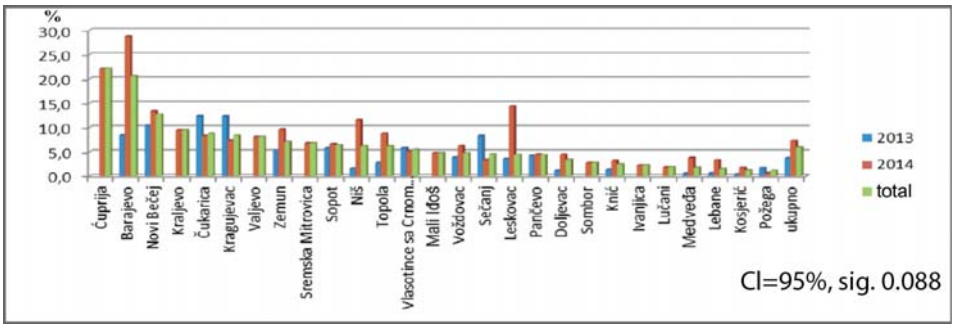

Fig. 4 – Positive immunochemical fecal occult blood test administered to persons in different municipalities in Serbia.

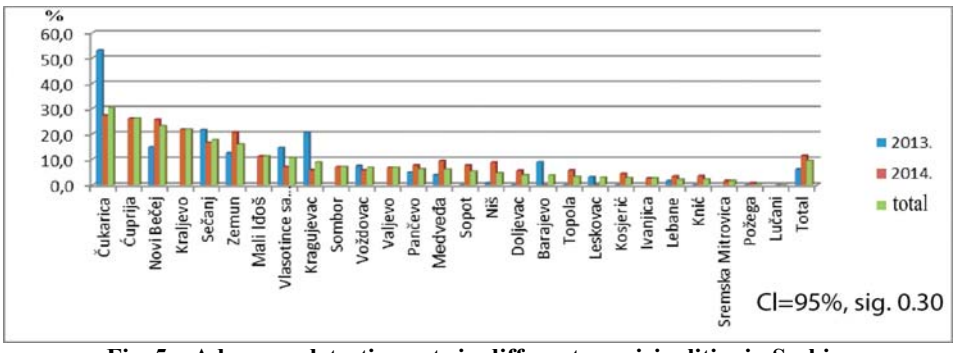

Fig. 5 – Adenoma detection rate in different municipalities in Serbia.

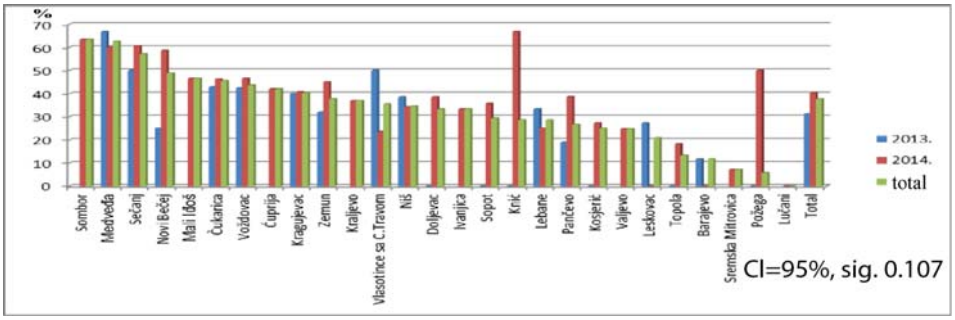

Fig. 6 – Positive predictive value for detection of adenoma in different municipalities in Serbia.

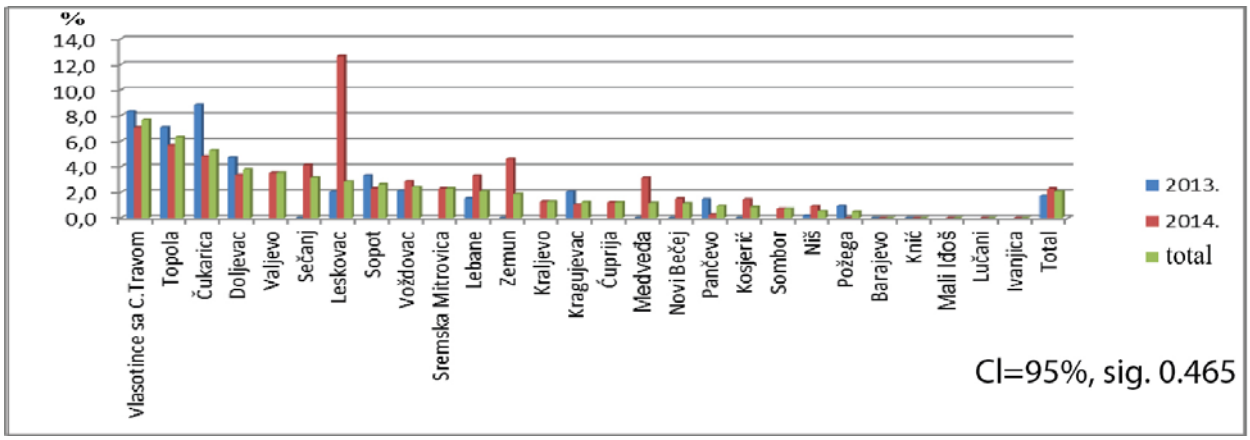

Fig. 7 – Positive predictive value for detection of cancer in different municipalities in Serbia.

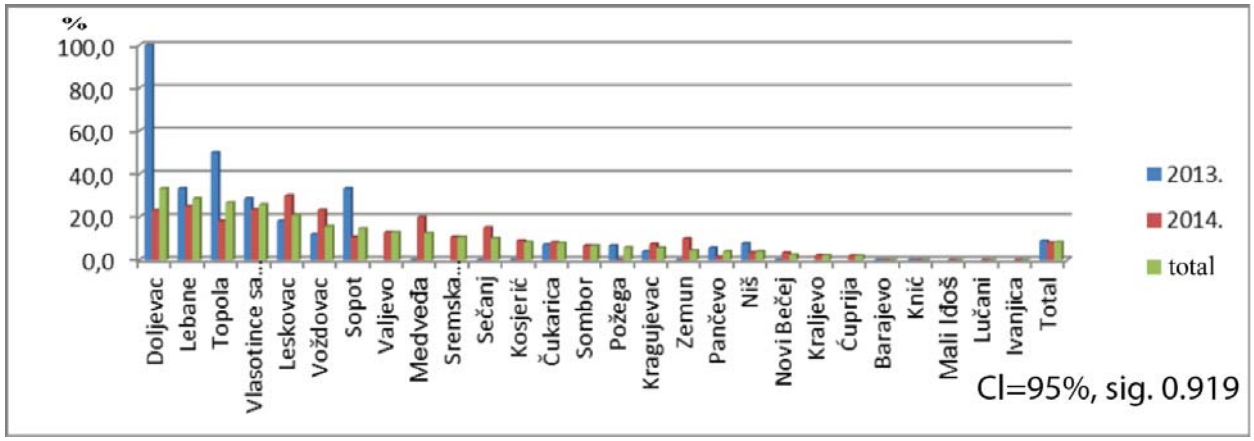

Fig. 8 – Positive predictive value for detection of cancer in different municipalities in Serbia.

Localization of adenomas and colorectal carcinoma

Totally 70.4% of diagnosed polyps and 77.3% of carcinomas were in the left half of the colon (in rectum, sigmoidal and descending colon), while 29.6% of polyps and 22.7% of the carcinomas were in the proximal parts of the colon (in cecum, ascending and transversal colon). This was observed on a sample of 40% of persons with the positive diagnosis on colonoscopy (Table 3).

Discussion

Participation rate

This study, conducted after the first round indicated high participation rate of the target population, which showed an increase in the second year of screening round, like in studies conducted in Canada, New York and in Sweden [7–9]. Implementation of the National program in which GPs administered

Table 3

Localization of detected polyps and colorectal carcinoma (CRC)

Part of colon	Polyps (%)			CRC (%)
	localization	≤ 10 mm	≥ 10 mm	localization
Left				
rectum	11.2	12.9	10.9	27.3
sigmo-rectum	5.9	5.7	6.7	12.1
sigmoid	43.1	38.1	53.8	30.3
descending	10.1	11.1	6.7	7.6
total	70.4	67.8	78.1	77.3
Right				
transverse	15.9	16.8	14.3	4.5
ascending	7.2	9.2	4.2	16.7
cecum	6.6	6.2	3.4	1.5
total	29.6	32.2	21.9	22.7

iFOB tests after issuing letters of invitation was 62%, confirming such recommendations as sucsesful [10]. In the first round of colorectal cancer screening with FOB tests in Finland, it was 62–68% in males and 77–80% in females [6].

In Ontario, Canada, where guaiac FOB test was used in 2005 and 2006 when the program of organized screening of colorectal carcinoma was introduced, participation rate was 26%, and in the period of 2010–2012 increased to 63% [9, 11, 12]. Such uptake was accomplished in the first round in Serbia, as well.

The participation rate in such a program in Ireland was 51% [13]. In this program of the organized colorectal cancer screening in the second year there was an increase in uptake. These results are in concordance with the results of the organized screening presented in this study.

Although with a far wider scope, organized colorectal cancer screening in France in the first biannual round with a primary guaiac FOB test achieved uptake of 34%. Compared to the uptake in Serbia, which was almost two times higher (although on a smaller sample of target population), it is shown that the delivery of the test from a general physician after a written or telephone invitation is far more effective than the mailing of the test with an invitation and instructions [14].

Faivre and Hamza [15] has pointed out the paramount importance of the response higher than 50% for the success of the screening and effectiveness in lowering of mortality of the colorectal cancer. That is the reason for the results of the first cycle of screening in Serbia with the response rate of 62% of the target population to be very encouraging.

In the course of the four-year cycle of testing in Korea, the uptake of the target population rose from 10% at the beginning to 20% at the end of the organized colorectal cancer screening round with the use of iFOB test [16].

The coverage of the population with invitations and testing continues to be low despite all the European and American Cancer Society recommendations. Coverage of target population in various European countries varies from 7.2 to 90% when the initial FOB test was used and between 7% and 55% when colonoscopy was used [2, 4].

Positive iFOB test

Results from different municipalities in Serbia showed that in the first cycle, there were on the average 5.9% of persons with positive findings. In 2013, the percentage of persons with positive findings of FOB test was 4.3%, while in the year 2014, it was 7.3%. In the first round of the organized colorectal cancer screening, a high percentage of persons with positive findings of iFOBT, particularly in some municipalities, according to other authors could have been connected with invitations to persons who did not belong to the group of average risk [17]. In the research of Benson International Colorectal Cancer Screening Network from the year 2012, which included 26 programs of organized screening for colorectal cancer and 9 pilot programs in 24 countries, it is shown that the results from Serbia are in concord with programs in Europe, America and the countries of the West Pacific, which had used iFOB test. The percentage of positive iFOBT was 6.1% [18]. Compared to the iFOB test, guaiac test has less percentage of positive findings, which on the average is 4.6% [17, 18].

In the first round of the organized screening in Serbia, the percentage of positive iFOB test was 4.4–11.1%. The results achieved in Serbia are within the established and recommended values of the European recommendation [4].

Adenoma detection rate and PPV for the detection of adenoma

We recorded a somewhat lower rate of adenoma detection (9‰) which was slightly less compared to previous Euro-plan programs (13.3–22.3‰) [4]. On the other hand, the PPV for the detection of adenoma in all the colonoscopied was 37.7% in the first round of organized colorectal cancer screening. It was found that PPV for detection of adenoma in our study is in concordance with the European recommendations (19.6–40.3%) [4].

It was shown that colonoscopy had high potential for detecting adenomas. These data are comparable with the results of organized screening program in Hungary, which also has a high incidence of and mortality from colorectal cancer, and used iFOB test, as well as in Italy [19]. Although the PPV for detection of adenomas was in line with previous results of the European population based screening program, adenoma detection rate was lower, caused by low colonoscopy compliance rate. The results from the first round of colorectal cancer screening for five provincial programs in Canada have shown that a high colonoscopy compliance rate (80.5%) produced the increased adenoma detection rate which was 16.9‰ [20].

Cancer detection rate and PPV for the detection of cancer

The PPV for the detection of cancer in Serbia was 8.3%, slightly higher compared to the results after the first two-year round in France (7.5%) and Canada (4.4%). Despite low a colonoscopy compliance rate, the cancer detection rate in Serbia was 2%, similar to that in France (1.9%) and Canada (1.8%), where colonoscopy compliance rate was as almost two times higher, 80.5% in Canada, and 88.5% in France [14, 20]. Cancer detection rate in our study is in concordance with European programs conducted (1.8–9.5‰) [4], as well as PPV for detection of cancer in our study is in the concordance with the European recommenoations (4.5–8.6%) [4].

These results indicate that Serbia needs to continue with education of the target population regarding the importance of colonoscopy for the detection of the cause of fecal bleeding in patients with a positive iFOB test, in order to increase colonoscopy compliance rate and the number of persons with detected adenomas and colorectal cancer.

Localization of polyps and carcinoma

A total of 70.4% of detected polyps were located in the left colon, which is slightly more than proportion of detected polyps in the left colon in Croatia (64%) [17].

A total of 77.3% of detected carcinomas at colonoscopy were left-sided, which is identical to the results of organized screening for colorectal cancer in England [21].

Conclusion

According to the data from the first biannual round of organized colorectal cancer screening in Serbia, the participation rate of the target population to testing was high, that is encouraging. We believe that in the forthcoming rounds, we could increase the coverage of the target population through organized screening for colorectal cancer. The established positive predictive value of completed colonoscopies shows that in further observing the stages of the diagnosed adenomas and carcinomas, we could reach the expected improvement regarding earlier detection of colorectal cancer in Serbia.

R E F E R E N C E S

1. *Bastos J, Peleteiro B, Gouveia J, Coleman MP, Lunet N.* The state of the art of cancer control in 30 European countries in 2008. Int J Cancer 2010; 126(11): 2700−15.
2. *Altobelli E, Lattanzi A, Paduano R, Varassi G, di Orio F.* Colorectal cancer prevention in Europe: Burden of disease and status of screening programs. Prev Med 2014; 62: 132−41.
3. Institute of Public Health of Serbia "Dr Milan Jovanovic Batut". Cancer incidence and mortality in central Serbia 2012. In: Cancer registry of central Serbia. Report. Belgrade: Institute of Public Health of Serbia "Dr Milan Jovanovic Batut"; 2012.
4. *von Karsa L, Patnick J, Segnan N.* European guidelines for quality assurance in colorectal cancer screening and diagnosis. 1st ed.Executive summary. Endoscopy 2012; 44 Suppl 3: SE1−8.
5. Regulation on the National Programe on early detection of colorectal cancer. "Official Gazette of RS" 2013/73. (Serbian)
6. *Levin TR, Jamieson L, Burley DA, Reyes J, Oehrli M, Caldwell C.* Organized Colorectal Cancer Screening in Integrated Health Care Systems. Epidemiol Rev 2011; 33(1): 101−10.
7. *Blom J, Kilpelainen S, Hultcrantz R, Tornberg S.* Five-year experience of organized colorectal cancer screening in a Swedish population - increased compliance with age, female gender, and subsequent screening round. J Med Screen 2014; 21(3): 144−50.
8. *Guy GP, Richardson LC, Pignone MP, Plescia M.* Costs and benefits of an organized fecal immunochemical test-based colorectal cancer screening program in the United States. Cancer 2014; 120(15): 2308−15.
9. *Rabeneck L, Tinmouth JM, Paszat LF, Baxter NN, Marrett LD, Ruco A,* et al. Ontario's ColonCancerCheck: results from canada's first province-wide colorectal cancer screening program. Cancer Epidemiol Biomarkers Prev 2014; 23(3): 508−15.
10. *Stock C, Holleczek B, Hoffmeister M, Stolz T, Stegmaier C, Brenner H.* Adherence to Physician Recommendations for Surveillance in Opportunistic Colorectal Cancer Screening: The Necessity of Organized Surveillance. PLoS One 2013; 8(12): e82676.
11. *Charters TJ, Strumpf EC, Sewitch MJ.* Effectiveness of an organized colorectal cancer screening program on increasing adherence in asymptomatic average-risk Canadians. BMC Health Serv Res 2013; 13(1): 449.
12. *Spayne M, Rabeneck L, Guerriero L.* Successes and Challenges in Population-Based Cancer Screening. Healthc Q 2014; 17(SP): 16−22.
13. *McNamara D, Qasim A, Lee N, Condon C, O'Morain C.* Round one of the Adelaide and Meath Hospital/Trinity College Colorectal Cancer Screening Programme: programme report and analysis based on established international key performance indices. Ir J Med Sci 2011; 180(2): 549−52.
14. *Leuraud K, Jezewski-Serra D, Viguier J, Salines E.* Colorectal cancer screening by guaiac faecal occult blood test in France: Evaluation of the programme two years after launching. Cancer Epidemiol 2013; 37(6): 959−67.
15. *Faivre J, Hamza S.* Organised screening for colorectal cancer: current position and futureprospects. Oncologie 2010; 12(10): 579−83.
16. *Choi KS, Lee H, Jun JK, Shin A, Park E.* Adherence to follow-up after a positive fecal occult blood test in an organized colorectal cancer screening program in Korea, 2004-2008. J Gastroenterol Hepatol 2012; 27(6): 1070−7.
17. *Katičić M, Antoljak N, Kujundžić M, Stamenić V, Skoko Poljak D, Kramarić D,* et al. Results of National Colorectal Cancer Screening Program in Croatia (2007-2011). World J Gastroenterol 2012; 18(32): 4300−7.
18. *Benson VS, Atkin WS, Green J, Nadel MR, Patnick J, Smith RA,* et al. Toward standardizing and reporting colorectal cancer screening indicators on an international level: The international colorectal cancer screening network. Int J Cancer 2011; 130(12): 2961−73.
19. *Segnan N, Senore C, Andreoni B, Azzoni A, Bisanti L, Cardelli A,* et al. Comparing Attendance and Detection Rate of Colonoscopy With Sigmoidoscopy and FIT for Colorectal Cancer Screening. Gastroenterology 2007; 132(7): 2304−12.
20. *Major D, Bryant H, Delaney M, Fekete S, Gentile L, Harrison M,* et al. Colorectal cancer screening in Canada: results from the first round of screening for five provincial programs. Curr Oncol 2013; 20(5): 252−7.
21. *Logan RF, Patnick J, Nickerson C, Coleman L, Rutter MD, von Wagner C.* Outcomes of the Bowel Cancer Screening Programme (BCSP) in England after the first 1 million tests. Gut 2012; 61(10): 1439−46.

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GENERAL REVIEW

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Biomarkers in aortic dissection, including specific causes of troponin elevation

Biomarkeri u disekciji aorte, uključujući specifične uzroke povišenja troponina

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Key words:
aortic aneurysm; aneurysm, dissecting; myocardial infarction; acute coronary syndrome; stroke; biological markers; troponin t; c-reactive protein; fibrin fibrinogen degradation products.

Ključne reči:
aorta, aneurizma; aneurizma, disekantna; infarkt miokarda; akutni koronarni sindrom; cerebrovaskularni insult; biološki pokazatelji; troponin t; c-reaktivni protein; fibrin fibrinogen, produkti degradacije.

Introduction

Serum/plasma cardiac troponin (cTn) has been recognized as highly sensitive and specific marker of the damage to cardiomyocytes, with no information on the etiopathogenesis of this damage [1]. The expression "false positive cTn" should be reserved for preanalytical and analytic false positives [i.e. when cTn elevation is not really present, but it is due to methodological problem(s)] [2]. cTn is also useful prognosticator in acute myocardial infarction (AMI) [3]. cTn predicts outcome in stable coronary artery disease (CAD), too [4], as well as in many non-ischemic diseases, which increase cTn concentration [5, 6].

Aortic dissection (AoD) is a catastrophic disease of the aorta with the highest mortality rate, which reaches 1% *per* hour in the first 48 h, if left untreated [7]. Initial misdiagnosis of AoD was ≈30% (24% of AoD patients were misdiagnosed as AMI, and 6% as stroke) [8]. Elevated cTn at admission (together with electrocardiography – ECG) sugestive of [acute coronary syndrome (ACS), dyspnea and pleural effusion] may increase time to AoD diagnosis [9]. Indeed, the highest lethality occurs when AoD causes ST segment elevation myocardial infarction (STEMI) and chemical reperfusion (thrombolysis) is used for STEMI, with AoD as the cause being overlooked. Proximal AoD usually arises above right sinus of Valsalva and more likely affects the right coronary artery [10]. If AoD causes AMI, ostium of the right coronary artery is most commonly affected. This is by definition known as right ventricular (RV) AMI. Consequently, in this type of AMI, RV AMI, we should be probably more vigilant to search for AoD as a cause of AMI [11].

Patients who receive thrombolysis (by mistake) for AoD-induced STEMI, have been described even in the cardiologic institutions with very high reputation. It is sometimes "unavoidable" [12], because it is extremely difficult to make in the emergency department differentiation between AMI due to atherothrombosis and due to AoD [13]. In AoD antithrombotic and thrombolytic treatments double hemorrhagic complications and mortality, which approaches 69–100% [14]. Although thrombolytic and anticoagulant agents might temporary improve symptoms and signs of AMI, the mortality rate climbs > 70%, mostly due to hemorrhage into paricardium, which progresses to tamponade [10]. Contemporary expansion of prehospital thrombolysis is rational and life-saving for many STEMI patients. Regrettably, it might enhance the number of thrombolysis in rare cases with AoD-induced STEMI [15].

The motivation for this paper comes from the following: cTn has been increasingly used in the emergency room to im-

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prove detection of the diseases with myocardial necrosis, AMI at the first place. Elevated cTn in a patient with chest pain has been frequently (but erroneously) interpreted as a proof against AoD. It is important for practice that elevated cTn level does not exclude AoD at all. Namely, as much as > 20% of acute ascending AoD patients may have increased cTn values [16]. It could be helpful for practitioners who interpret cTn results to be aware of the following possibilities of elevated cTn in patients with chest pain. Namely, the patient may have AoD and some of the conditions with increased cTn, which could erroneously point toward AMI as the single disease.

CTn can be raised in AoD patients due to various reasons:

When AoD causes AMI

The prevalence of AMI among almost 1,000 AoD patients was 7% [17]. In 1/7 of AoD patients coronary arteries are affected [18]. Up to 1/4 AoD patients have increased cTn concentration, and they have 4-fold higher mortality risk [16]. Additionally, 3% of AoD patients have STEMI, as seen in the large International Registry of Aortic Dissection (IRAD) [19]. Such patients often present with strong retrosternal chest pain, with ST-segment abnormalities, and with increased serum cTn level, all common signs of AMI. It really is AMI, but not usual one, type 1 AMI (due to coronary artery thrombosis), i.e. „classical" STEMI, but caused by AoD [10]. Some quickly performed simple procedures can be life-saving if they help detect AoD as the cause of AMI: comparing pulses and pressures between the arms, discovery of aortic regurgitation murmur, transthoracic echocardiogram in the emergency room or in the coronary care unit. Therefore, high-risk predisposing condition (such as bicuspid aortic valve, aortic aneurysm or manipulation, Marfan syndrome, or positive familly history), high-risk pain characteristics (abrupt, severe, tearing pain), above mentioned high-risk examination features (plus hypotension / focal neurologic deficit) were recognized in the latest European Guidelines as suggestive of AoD.

AoD may cause AMI by compromising coronary artery blood flow directly (mechanically) in 5 ways [15]. Neri recognized 3 main types of lesion: Type A – AoD with disruption of the inner layer, limited to the area of coronary ostium; Type B – AoD with coronary false channel, is a retrograde extension of the AoD into coronary artery wall; Type C – circumferential detachment, with inner cylinder intussuception [20, 21].

Other authors reported somewhat different potential mechanisms of coronary artery obstruction: bulging of dissected false lumen, producing occlusion at the coronary orifice, with possible subsequent distal thrombosis; intimal detachment of coronary orifice and dominant blood flow *via* false lumen; dissection extending into the coronary artery, producing obstruction beyond the coronary orifice; extension of the proximal AoD flap into the right coronary artery; blocking the coronary ostia during diastole, either completely (if the flap reaches the middle portion of the left ventricle) or only partially (if flap is shorter) [10, 21]. Many other mechanisms in AoD can be operative resulting in AMI: stress-induced tachycardia, high blood pressure, etc. It is type 2 AMI [14]. Namely, tachycardia is common in AoD patients, due to excruciating pain and subsequent sympathetic activation. High blood pressure can be expected, too, unless there is, e.g. bleeding or blood leakage from the aorta. All such disturbances cause mismatch between myocardial oxygen consumption and supply, which can lead to ischemic myocardial necrosis (and cTn raise). This scenario occurs typically in AoD patients who also have CAD, but CAD is not *conditio sine qua non*.

When AoD causes the disease, other than AMI (eg, stroke), which can increase cTn concentration

AoD can cease arterial circulation to the brain by obstructing carotid arteries, with resulting ischemic stroke. Of 1,873 patients with type A AoD, enrolled in the large International Registry for Acute Dissection, 4.7% presented with cerebrovascular accident and 2.9% with coma [22]. It is an old clinical rule that we should think of AoD when a patient presents with chest pain and neurologic problems, e.g. syncope. We have to avoid thrombolysis, which is contraindicated if ischemic stroke is caused by AoD (because thrombolysis may increase hemorrhage into the aortic media and promote aortic rupture) [23]. In several studies, cTn concentration was found to correlate significantly positively with the increased volume of the cerebral lesion in ischemic stroke, demonstrated by CT scan. In 15 studies 18.1% [(95% confidence interval (CI) 13.6–22.6)] of 2,901 stroke patients had an increased cTn level [24]. Thus, AoD causes ischemic stroke, which can increase cTn concentration. In addition, approximately half of AoD patients have (acute) aortic insufficiency [13]. This may result in acute heart failure, capable of raising cTn [1, 25].

When the disease increases (cTn) level and contributes to AoD (chronically or acutely)

Renal failure has been known to enhance cTn values, especially of cardiac troponin T (cTnT) [2, 16]. Asymptomatic patients with end-stage renal disease had increased cTnT in 12%–66% and cardiac troponin I (cTnI) in 0.4–38%. Elevated cTnT significantly correlated with all-cause mortality [relative risk (RR) 2.64; 95% CI: 2.17–3.20] and with cardiac death (RR 2.55; 95% CI: 1.93 to 3.37), as demonstrated in a meta-analysis [2, 16]. The exact mechanism is not definitely elucidated, but current explanations for cTn elevations are: asymptomatic necrosis of cardiomyocytes, left vetricle (LV) hypertrophy, LV systolic dysfunction, enhanced cardiac preload accompanied by myocardial stretch, endothelial dysfunction, microvascular derangement, episodes of hypotension during dialysis or myocardial lesion due to calcium and oxalate deposition [26]. Diminished renal clearance of cTn and its particles may also contribute. Increased cTnT could come from skeletal muscles (skeletal isoform) [26].

In 3,243 patients of Chronic Renal Insufficiency Cohort (CRIC), cTnT concentrations were mainly an indicator of

LV hypertrophy [27]. The great majority of renal failure patients have arterial hypertension (AHT), which is commonly difficult-to-treat and uncontrolled, representing chronic risk factor for AoD. Hypertensive crisis was listed as condition which can acutely raise cTn [2, 16]. It is due to LV pressure overload and tachycardia (with consequent increase in myocardial oxygen consumption), as well as to sympathetic and catecholaminergic enhancement of the tone of coronary arteries (with resultant decrease in coronary flow), etc. At the same time, hypertensive crisis can cause/contribute to AoD [17].

When other factor induce cTn elevation independently from AoD

AoD can occur in patients who already have raised cTn level. A list of conditions, characterized by cTn elevation is very long [2, 16, 17] and obviously not finished. Its simplification was published to make it easier for learning [1]. Moreover, it is important to recognize that diseases from each system of organs can raise cTn value. cTn and/or hs-cTn is solid prognosticator in many non-infarction causes of elevated cTn concentrations: congestive heart failure, pulmonary embolism, chronic obstructive pulmonary disease, sepsis, renal failure, critical illness and the perioperative period [6].

When cTn elevation is the result of analytical (laboratory) error (false positive cTn elevation)

False positive increases of cTn concentration are less frequently found now - with new, improved essays [2, 6, 16]. In order to diminish any potential of false positive cTn elevation (which is the result of the imprecision in the low concentration range), the aim is to have all cTn assays attain a 10% coefficient of variation (CV) at the 99th percentile reference limit [28]. Taken together, raised cTn concentration in chest pain patients should not automatically lead to the conclusion that it is AMI and to the administration of antithrombotic therapy. It may actually be AoD patient with any of the aforementioned 5 conditions.

Some obvious implications for practice and research

In patients with diagnosed AoD, high cTn should be interpreted having in mind the possibilities numbered in this paper. For example, angiotensin-converting enzyme (ACE) inhibitor may be administered in AoD if elevated cTn is a result of AoD-induced AMI, but not if cTn increase comes from acute kidney injury.

AoD may cause stroke. In patients with ischemic stroke, elevated cTn concentration is important for prognosis and imposes the need for cardiologic examination, including probably echocardiogram with focus also on aorta.

It should be investigated which of the etiopathogenic mechanisms of cTn raise in AoD are common and clinically relevant.

In patients with the diagnosed AoD, high cTn is believed to have prognostic value. Moreover, it is highly probable that various causes of elevated cTn in AoD are not equally good prognosticators; some (e.g., AMI–induced) may be valid, but others (e.g., false-positive) are certainly not.

In which of the etiopathogenic type of cTn increase in AoD (e.g. within AMI-induced cTn elevation), is cTn quantitatively related to bad prognosis (i.e. is there a threshold for cTn as a prognosticator)?

Important biomarkers in AoD: D dimer (DD)

Since early laboratory investigations in AoD, e.g. serum transaminase and lactic acid dehydrogenase, by Mold [29] in 1964 , many of biomarkers have been studied, including DD, C reactive protein (CRP), smooth muscle myosin heavy chain, calponin, soluble elastin fragments (sELAF), matrix metalloproteinases (MMPs), tenascin, transforming growth factor beta (TGF-b), N-terminal pro-brain natriuretic peptide (NT-proBNP), big endothelin-1 (Big ET-1), creatine kinase-BB isozyme, Notch signaling pathway, and genetic markers [30–33].

DD is now the most widely available and reliable biomarker for AoD, the only biomarker for AoD tending to become a gold standard [33, 34]. DD is the consequence of the fibrinolysis, it circulates in the blood several days after intravascular thrombus formation (the half-life is ≈ 8 hours) [35]. AoD activates inflammatory, coagulation and fibrinolytic cascades [36]. In AoD low admission platelet count is a sign of their massive consumption, possibly with similar consumption of fibrinogen and other coagulation factors [37].

Diagnostic potential of DD in AoD

Probably, the first paper about usefulness of DD in AoD was the one published by Weber et al. [38] in 2003. They analyzed 24 AoD patients: 12 with type A and 12 patients with type B. DD concentration was abnormal (> 500 ng/mL in all of them, with the average result of 9,400 ng/mL. In a meta-analysis, plasma DD for AoD has the high sensitivity (0.97; 95% CI, 0.94–0.99), but low specificity (0.56; 95% CI, 0.51–0.60) [39]. In a large cohort of patients with suspected AoD, ADD risk score 0 or 1 plus a negative DD accurately and efficiently ruled out AoD [40]. DD is better in the detection of AoD compared to the smooth myosin heavy chain and soluble elastin fragments, because of its longer half-life and higher sensitivity [30].

AoD patients with stroke had a significantly higher DD as compared with any other stroke subtypes and their B-type natriuretic peptide (BNP) concentrations were significantly lower in comparison to patients with cardioembolic stroke. Moreover, DD:BNP ratio was significantly higher in AoD patients with stroke than in patients with any other stroke subtypes (within 6 h of onset, sensitivity was 81.8% and specificity 96.4%) [41]. In a study using DD as a screening tool in patients not highly suspected of having AoD, the clinical picture and physician's judgment remained principal, despite a well-known high sensitivity of DD for AoD [42]. Although DD by itself is not able to discriminate AoD from PTE, if DD is very high, it may demand an urgent CT, aiming to detect AoD or PTE [34]. In general, raised DD concentrations, caused by tissue injury, show a trend for gradual decline within the first few days following the event [38].

Cut-off values of DD for AoD

Different DD cut-offs were used as upper normal limit in the medical literature (100–900 ng/mL) [39]. The lower the cut-off, the higher the sensitivity and the lower the specificity, but even with the cut-off concentration of 400 ng/mL, the sensitivity of DD for aortic intramural hematoma (IMH) was only 90% and insufficient to rule out the diagnosis of acute aortic syndrome (AAS), particularly if there is high clinical suspicion [43]. IRAD-Bio published 96.6% sensitivity and 46.6% specificity using a 500 ng/ml DD cut-off during the first day of symptom onset [34]. The specificity increased to 73% when the cutoff value was 626 ng/mL [30]. The largest study on the use of DD in AoD showed its marked elevation within 6 hours of the clinical event: 3,213 ± 1,465 ng/mL in type A and 3,574 ± 1,430 ng/mL in type B versus 2,452 ± 1,891 ng/mL in pulmonary thromboendarterectomy (PTE) patients, 1,459 ± 1,650 ng/mL in patients with myocardial infarction, and 760 ± 974 ng/mL in patients with angina pectoris. With the cut-off level of 500 ng/mL, within the first 6 h, sensitivity was 95.7% and specificity 61.3%. Importantly, AoD can be ruled in if DD concentration is > 1,600 ng/mL during the initial 6 hours [32].

The same 500 ng/mL DD cut-off value is also used for PTE, which is beneficial for simplicity and cost-effectiveness: with a single blood test, one can rule out AoD and PTE in patients at low risk [33]. DD is useful in AoD up to 10 days after the onset (with a cut-off of 1,600 ng/ml sensitivity was 95.3%). It is important that DD concentrations may be measured in 2 different units: DD unit (DDU) or fibrinogen equivalent unit (FEU). One DDU is equivalent to 2 FEU [44, 45]. Therefore, DD units should be reported in publications and different units (DDU or FEU) can not be used interchangeably.

False negatives DD in AoD

False negative results (when DD is normal in an AoD patient) were most likely in patients: 1) under 70 years old, with intromural hemorrhage (IMH), with thrombosed false lumen, and with shorter dissections (in length) [40, 44, 46, 47]. DD concentration may be within the normal limits in young patients with short dissection and a thrombosed false lumen, without penetrating atherosclerotic ulcer (PAU) [30]. The DD concentration < 100 ng/mL can exclude AoD, but not IMH or PAU [48, 49].

Prognostic potential and correlations of DD in AoD

In the study of Weber et al. [38] DD was not higher in AoD patients who died in hospital. To the contrary, in the study of Ohlmann et al. [43], the independent markers of in-hospital mortality were: pericardial effusion [odds ratio (OR), 6.80; CI, 1.87–27.60), DD > 5,200 ng/mL (OR, 5.38; CI, 1.27–30.87), and female gender (OR, 4.96; CI, 1.39–19.95) [43]. Serum DD ≥ 10,000 ng/mL resulted in the sensitivity of 61.5% and specificity of 84.1% for the prediction of in-hospital death [50]. DD was higher in patients with in-hospital death [51]. This is a consequence at least in a part of a positive correlation between the extent of AoD and DD concentration [30, 51, 52]. The average DD increased from DeBakey class II AoD, over class III, to class I AoD ($r = 0.63$; $p < 0.01$) [51]. DD decreased with longer duration from the onset of symptoms, allowing the differentiation of acute from chronic AoD [30, 52, 53].

DD values are lower in AoD patients with thrombosed false lumen in comparison to patients with partially thrombosed one (which predicts poor outcome) [30, 33, 34, 54]. Ohlmann et al. [43] suggested that DD increased proportionally to the surface of contact between the bloodstream and thrombogenic components of the patent false lumen, which triggers the coagulation cascade and thereby raises DD level. DD concentration can be normal in patients with IMH [30, 33, 43]. Re-elevation of DD is unfavorable. During in-hospital treatment of AoD, the coagulation cascade could be activated by aortic injury, prolonged bed rest, and hemoconcentration, due to increased vascular permeability, as the result of inflammatory response. Serial measurements of DD can be used for early detection of re-AoD or venous thromboembolism (VTE) during in-hospital management of Stanford type B AoD [53].

Final remarks on DD in AoD

DD may be used in the same fashion in AoD as in PTE: negative result renders the presence of the disease very unlikely [38]. DD to rule out AoD is most likely to be useful in individuals with low to moderate risk of AoD. Excluding patients from definitive imaging studies due to normal plasma DD may be unwise, especially in high-risk patients (those with Marfan syndrome or uncontrolled AHT) [39]. Current evidence supports routine measurement of DD for the suspected acute AoD: if DD is < 100 ng/mL, this excludes acute AoD in all cases with a low likelihood of disease [55].

DD < 500 ng/mL (this cut-off has been used to rule out PTE in patients with low likelihood), can almost exclude AoD during the first day of presentation (negative likelihood ratio of 0.07). At higher levels in patients presenting within 6 h of symptoms onset, DD may be used to rule in AoD [34]. Neema [56] suggested that the absence of both increased DD and ECG changes is considered specific to rule out AoD.

CRP in AoD

CRP is mostly synthesized in the liver by stimulation of many cytokines, particularly by interleukin (IL)-6 [30]. In acute inflammation (due to infection, trauma, etc.), CRP levels increase promptly within 4–6 h, and reaches its maximum concentration at 36–50 h, and then diminish as the inflammation response decreases [33, 57]. CRP levels are significantly higher in both acute AoD and chronic aortic aneurysm.

Mechanisms of CRP elevation in AoD

Increased levels of inflammatory markers [IL-6, CRP, tumor necrosis factor alpha (TNF-α) in plasma] and changes in MMP-9 are significantly associated with AoD, suggesting

the role for inflammatory process in the AoD pathogenesis [57]. CRP rise is believed to originate from systemic stress with systemic acute-phase response, and local aortic inflammatory processes [52, 58, 59]. CRP levels depend greatly on the time elapsed from the onset of symptoms. Plasma CRP concentrations are higher in patients with aortic aneurysms in comparison with controls, but the average CRP levels were similar in patients with AoD and chronic aortic aneurysms. On the other hand, the white blood cell (WBC) count was only elevated in AoD in at least two studies [52]. CRP concentrations are increased in patients with asymptomatic abdominal aortic aneurysm (AAA), and they correlate with the size of AAA.

Diagnostic potential of CRP in AoD

CRP level is seldom prominent within the first hours after AoD onset. Therefore, CRP is not useful to diagnose acute AoD. Moreover, CRP is not appropriate for the discrimination between acute AoD and chronic aneurysm, because in some studies CRP levels were found to similarly increase in both diseases [30]. Newer data demonstrate increased CRP values in acute AoD compared with chronic AoD and AHT, as well as with healthy subjects (13.48 ± 3.74 mg/L *vs* 4.12 ± 2.99 mg/L, *vs* 1.62 ± 0.65 mg/L, and 1.12 ± 0.35 mg/L, respectively; $p < 0.001$) [57].

Prognostic potential of CRP in AoD

In a study of Schillinger et al. [58] higher CRP concentrations predict higher mortality. Mortality hazard ratios in AoD patients with CRP levels from 2nd to 4th quartiles *versus* the first quartile were 0.7, 1.8, and 2.6, respectively. CRP concentration > 63.0 mg/L predicted high short-term mortality. The admission CRP > 68.0 mg/L in AoD and/or aortic aneurysm predicted a higher mortality rate both in operated and conservatively treated patients [60]. In type III AoD, a peak CRP value over 150 mg/L was a marker of a high risk for oxygenation impairment and adverse outcome [61]. Higher maximal CRP concentrations were found in AoD patients with adverse outcome (251 ± 123 mg/L), as compared to patients without them (161 ± 74 mg/L, $p = 0.010$) [61]. The highest quartile of the peak CRP level is associated with higher incidence of adverse long-term events in type B AoD. Since CRP is a nonspecific marker of inflammation, it reveals not only AoD itself, but complications (e.g., pneumonia), too [32, 62, 63].

Okina et al. [63] studied 240 AoD patients and found that CRP values at admission most often were negative (< 5.0 mg/L) in the uneventful subgroup. In the event-free subgroup, CRP demonstrated the peak on the 4th day after AoD onset (4.2 ± 3.9 days, the average CRP value 137 mg/L), then gradually decreased to the average of 46 mg/L 4 weeks later. On the other hand, in the subgroup with clinical events, there was prolonged CRP elevation and/or re-elevation, peak CRP was significantly delayed (time to the maximum CRP: 8.1 ± 5.1 days, $p < 0.05$ in comparison with the event-free subgroup). Namely, CRP continued to raise after the 4th day, and subsequently adverse cardiac events occurred in these patients. Thus, persistently elevated CRP may be a sign of inflammation and AoD progression, imposing the need for (repeated) application of magnetic resonance imaging (MRI) or computed tomography (CT), improved blood pressure (BP) control and/or an early cardiosurgery [63].

In analysis of 180 patients with aortic IMH, the maximal CRP was found 4 days after the onset of symptoms (124 ± 63 mg/L). Those with raised CRP concentration (≥ 72 mg/L) at 2 weeks had significantly more adverse aortic events ($p < 0.001$) [64]. Suzuki et al. [32] suggest that maximal CRP concentration reflects the degree of inflammatory reaction and the damage of the aortic wall [32]. One study in acute AoD showed higher CRP in patients with longer hospitalization, due to complications [57]. In chronic type B AoD increased CRP concentration pointed to adverse outcome, particularly in patients on conservative treatment in comparison with the operated ones [58]. CRP was suggested for possible monitoring of the course of false lumen thrombosis [32, 65] (Table 1).

Correlations of CRP in AoD

Plasma CRP levels decrease significantly when the time from the onset of acute AoD to hospitalization increase ($p = 0.013$) [57]. CRP value is usually a low level within 24 h from the first symptoms. The peak of CRP level in the study of

Table 1

Some of contemporary biomarkers for aortic dissection (AoD) patients

Biomarker	Origin of biomarker increase	Main purpose in AoD currently	Relevant references
DD	High DD is a consequence of fibrinolysis of hematoma in the aortic media	To help excluding AoD in low risk patients	34, 38–41, 66
CRP	CRP rise results from systemic stress and local aortic inflammatory processes	Prognostication	32, 57, 58, 61, 63–65
cTn	Elevated cTn concentration is due to acute myocardial infarction type II of type one, or due to renal failure, stroke, etc.	Indicator of possible coronary artery affection by AoD	9, 16, 21, 67

DD – D dimer; CRP-C – reactive protein; cTn – cardiac troponin.

Sugano et al. [61] was observed at 3 ± 1 days. In AoD, the injury of the vessel wall induces inflammatory response, including numerous humoral factors, capable of activating not only endothelium in lungs, but extensive resident neutrophil pool, as well. In AoD patients, maximal CRP concentration ≥ 150 mg/L was an independent predictor of forthcoming lung injury (relative risk = 12.6, $p = 0.001$) [61].

Final remarks on CRP in AoD

CRP does not increase within the first day from the AoD symptoms onset, and therefore CRP is not a valuable biomarker for diagnostic purposes. Markedly elevated CRP during in-hospital stay results probably either from extensive aortic damage or from significant comorbidity, making CRP suitable candidate for valid prognostic marker in AoD.

Conclusion

Elevated troponin concentration does not exclude AoD. Five categories of diseases/clinical situations are probable causes of troponin raise in patients with AoD. Laboratories made a tremendous progress in troponin estimation, many scientists proved its usefulness for diagnostic purposes and prognostication, and it is time for all of us to optimize troponin interpretation in everyday work.

DD is now the most widely available and reliable biomarker for AoD, the only biomarker for AoD tending to become the gold standard. DD may be used in the same fashion in AoD as in PTE: negative result renders the presence of the disease very unlikely.

CRP is not useful for the AoD diagnosis, but has a potential as a prognostic marker. More investigation is needed to confirm this and to find optimal cut-off concentration and adequate time for the measurement, since CRP concentration depends a lot on time (in days) from the AoD onset.

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R E F E R E N C E S

1. *Goran KP*. Simple and practical classification of elevated troponin values. Am J Emerg Med 2008; 26(8): 951−2.
2. *Koraćević G, Ćosić V, Stojanović I*. False positive troponin - true problem. J Med Biochem 2013; 32(3): 197−206.
3. *Koracevic GP*. Troponin units should be unified. J Emerg Med 2011; 40(4): 445−6.
4. *Tsounis D, Deftereos S, Bouras G, Giannopoulos G, Anatoliotakis N, Raisakis K,* et al. High Sensitivity Tro ponin in Cardiovascular Disease. Is There More Than a Marker of Myocardial Death. Curr Top Med Chem 2013; 13(2): 201−15.
5. *Iversen K, Køber L, Gøtze JP, Dalsgaard M, Nielsen H, Boesgaard S,* et al. Troponin T is a strong marker of mortality in hospitalized patients. Int J Cardiol 2013; 168(2): 818−24.
6. *Goran KP*. Diseases from every organ system can raise cardiac troponin concentration. Resuscitation 2010; 81(1): 128.
7. *Jánosi RA, Erbel R, Konorza TF*. Recent advances in the diagnosis of acute aortic syndromes. Expert Opin Med Diagn 2012; 6(6): 529−40.
8. *Asouhidou I, Asteri T*. Acute aortic dissection: be aware of misdiagnosis. BMC Res Notes 2009; 2(1): 25.
9. *Rapezzi C, Longhi S, Graziosi M, Biagini E, Terzi F, Cooke RM*, et al. Risk factors for diagnostic delay in acute aortic dissection. Am J Cardiol 2008; 102(10): 1399−406.
10. *Cai J, Cao Y, Yuan H, Yang K, Zhu Y*. Inferior myocardial infarction secondary to aortic dissection associated with bicuspid aortic valve. J Cardiovasc Dis Res 2012; 3(2): 138−42.
11. *Koracevic GP*. Right ventricular myocardial infarction: an additional reason to search for aortic dissection as a possible cause. J Emerg Med 2013; 44(1): 191.
12. *Erbel R, Alfonso F, Boileau C, Dirsch O, Eber B, Haverich A,* et al. Diagnosis and management of aortic dissection. Eur Heart J 2001; 22(18): 1642−81.
13. *Güvenç TS, Erer HB, Çetin R, Hasdemir H, İlhan E, Türkkan C,* et al.. Acute Aortic Regurgitation with Myocardial Infarction: An Important Clue for Aortic Dissection. J Emerg Med 2013; 44(1): 5−8.
14. *Goran KP*. Suggestion to list acute aortic dissection as a possible cause of type 2 myocardial infarction (according to the universal definition). Eur Heart J 2008; 29(22): 2819−20.
15. *Koracevic GP*. Prehospital thrombolysis expansion may raise the rate of its inappropriate administration in ST-elevation acute myocardial infarction induced by aortic dissection. Am J Emerg Med 2013; 31(3): 628−9.
16. *Newby KL, Jesse RL, Babb JD, Christenson RH, De Fer TM, Diamond GA,* et al. ACCF 2012 expert consensus document on practical clinical considerations in the interpretation of troponin elevations: a report of the American College of Cardiology Foundation task force on Clinical Expert Consensus Documents. J Am Coll Cardiol 2012; 60(23): 2427−63.
17. *Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE*, et al. 2010 ACCF/AHA/AATS/ACR/ASA /SCA/SCAI/SIR/STS/SVM Guidelines for the Diagnosis and Management of Patients with Thoracic Aortic Disease. J Am Coll Cardiol 2010; 55(14): 27−129.
18. *Evangelista A, Flachskampf FA, Erbel R, Antonini-Canterin F, Vlachopoulos C, Rocchi G,* et al. Echocardiography in aortic diseases: EAE recommendations for clinical practice. Eur J Echocardiogr 2010; 11(8): 645−58.
19. *Armstrong EJ, Kulkarni AR, Hoffmayer KS, Bhave PD, MacGregor JS, Hsue P,* et al. Delaying primary percutaneous coronary intervention for computed tomographic scans in the emergency department. Am J Cardiol 2012; 110(3): 345−9.
20. *Neri E, Toscano T, Papalia U, Frati G, Massetti M, Capannini G,* et al. Proximal aortic dissection with coronary malperfusion: Presentation, management, and outcome. J Thorac Cardiovasc Surg 2001; 121(3): 552−60.
21. *Lentini S, Perrotta S*. Aortic dissection with concomitant acute myocardial infarction: From diagnosis to management. J Emerg Trauma Shock 2011; 4(2): 273−8.
22. *di Eusanio M, Patel HJ, Nienaber CA, Montgomery DM, Korach A, Sundt TM,* et al. Patients with type A acute aortic dissection presenting with major brain injury: Should we operate on them. J Thorac Cardiovasc Surg 2013; 145(3): 213−21.
23. *Koracevic GP*. Excluding aortic dissection before thrombolysis in patients with ischemic stroke has been insufficiently advised. J Stroke Cerebrovasc Dis 2011; 20(4): 384.

24. *Kerr G, Ray G, Wu O, Stott DJ, Langhorne P.* Elevated troponin after stroke: a systematic review. Cerebrovasc Dis 2009; 28(3): 220−6.
25. *Metra M, Bettari L, Pagani F, Lazzarini V, Lombardi C, Carubelli V,* et al. Troponin T levels in patients with acute heart failure: clinical and prognostic significance of their detection and release during hospitalisation. Clin Res Cardiol 2012; 101(8): 663−72.
26. *Kalaji FR, Albitar S.* Predictive value of cardiac troponin T and I in hemodialysis patients. Saudi J Kidney Dis Transpl 2012; 23(5): 939−45.
27. *Mishra RK, Li Y, DeFilippi C, Fischer MJ, Yang W, Keane M,* et al. Association of cardiac troponin T with left ventricular structure and function in CKD. Am J Kidney Dis 2013; 61(5): 701−9.
28. *Apple FS, Jesse RL, Newby L, Wu AH, Christenson RH, Cannon CP,* et al. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: analytical issues for biochemical markers of acute coronary syndromes. Clin Chem 2007; 53(4): 547−51.
29. *Mold JW.* High serum transaminase and lactic acid dehydrogenase levels in dissecting aneurysm of aorta. N Y State J Med 1964; 64: 1747−9.
30. *Wen D, Zhou X, Li J, Hui R.* Biomarkers in aortic dissection. Clin Chim Acta 2011; 412(9−10): 688−95.
31. *Nozato T, Sato A, Hirose S, Hikita H, Takahashi A, Endo H,* et al. Preliminary study of serum tenascin-C levels as a diagnostic or prognostic biomarker of type B acute aortic dissection. Int J Cardiol 2013; 168(4): 4267−9.
32. *Suzuki T, Bossone E, Sawaki D, Jánosi RA, Erbel R, Eagle K,* et al.. Biomarkers of aortic diseases. Am Heart J 2013; 165(1): 15−25.
33. *Segreto A, Chiusaroli A, de Salvatore S, Bizzarri F.* Biomarkers for the diagnosis of aortic dissection. J Card Surg 2014; 29(4): 507−11.
34. *Listijono DR, Pepper JR.* Current imaging techniques and potential biomarkers in the diagnosis of acute aortic dissection. JRSM Short Rep 2012; 3(11): 76.
35. *Antovic JP, Höög HK, Forslund G, Eintrei J, Sten-Linder M.* Comparison of five point-of-care D-dimer assays with the standard laboratory method. Int J Lab Hematol 2012; 34(5): 495−501.
36. *Krüger T, Conzelmann LO, Bonser RS, Borger MA, Czerny M, Wildhirt S*, et al. Acute aortic dissection type A. Br J Surg 2012; 99(10): 1331−44.
37. *Huang B, Tian L, Fan X, Zhu J, Liang Y, Yang Y.* Low admission platelet counts predicts increased risk of in-hospital mortality in patients with type A acute aortic dissection. Int J Cardiol 2014; 172(3): 484−6.
38. *Weber T, Högler S, Auer J, Berent R, Lassnig E, Kvas E,* et al. D-dimer in acute aortic dissection. Chest 2003; 123(5): 1375−8.
39. *Shimony A, Filion KB, Mottillo S, Dourian T, Eisenberg MJ.* Meta-analysis of usefulness of d-dimer to diagnose acute aortic dissection. Am J Cardiol 2011; 107(8): 1227−34.
40. *Nazerian P, Morello F, Vanni S, Bono A, Castelli M, Forno D,* et al. Combined use of aortic dissection detection risk score and D-dimer in the diagnostic workup of suspected acute aortic dissection. Int J Cardiol 2014; 175(1): 78−82.
41. *Okazaki T, Yamamoto Y, Yoda K, Nagahiro S.* The ratio of D-dimer to brain natriuretic peptide may help to differentiate between cerebral infarction with and without acute aortic dissection. J Neurol Sci 2014; 340(1−2): 133−8.
42. *Moysidis T, Lohmann M, Lutkewitz S, Kemmeries G, Kröger K.* Cost associated with D-Dimer screening for acute aortic dissection. Adv Ther 2011; 28(11): 1038−44.
43. *Ohlmann P, Faure A, Morel O, Petit H, Kabbaj H, Meyer N,* et al. Diagnostic and prognostic value of circulating D-Dimers in patients with acute aortic dissection. Crit Care Med 2006; 34(5): 1358−64.
44. *Albini P, Barshes NR, Russell L, Wu D, Coselli JS, Shen YH,* et al. D-dimer levels remain elevated in acute aortic dissection after 24 h. J Surg Res 2014; 191(1): 58−63.
45. *Halaby R, Popma CJ, Cohen A, Chi G, Zacarkim MR, Romero G,* et al. d-Dimer elevation and adverse outcomes. J Thromb Thrombolysis 2014; 39(1): 55−9.
46. *Coyle S, Moriarty T, Melody L, Ryan D.* Diagnostic Testing in Acute Aortic Dissection. Curr Emerg Hosp Med Rep 2014; 2(2): 97−103.
47. *Iyano K, Kawada T, Aiba M, Takaba T.* Correlation of hemostatic molecular markers and morphology of the residual false lumen in chronic aortic dissection. Ann Thorac Cardiovasc Surg 2004; 10(2): 106−12.
48. *Semsroth S, Dumfarth J, Schachner T, Grimm M, Domanovits H.* Das akute Aortensyndrom. Med Klin Intensivmed Notfmed 2014; 109(5): 371−84.
49. *Sodeck G, Domanovits H, Schillinger M, Ehrlich MP, Endler G, Herkner H,* et al. D-dimer in ruling out acute aortic dissection: a systematic review and prospective cohort study. Eur Heart J 2007; 28(24): 3067−75.
50. *Wen P, Zhu Q, Zhou X, Chai Z.* A Simple Emergency Prediction Tool for Acute Aortic Dissection. Iranian J Publ Health 2013; 42(10): 1085−91.
51. *Levčík M, Kettner J, Jabor A, Kautzner J.* Utility of plasma D-dimer levels in the diagnosis of acute aortic dissection. Cor et Vasa 2013; 55(6): e510−4.
52. *Sbarouni E, Georgiadou P, Marathias A, Geroulanos S, Kremastinos DT.* D-dimer and BNP levels in acute aortic dissection. Int J Cardiol 2007; 122(2): 170−2.
53. *Ferrera C, Vilacosta I, Suárez-Barrientos A, Vivas D, Fortuny E, Martin A,* et al. Aortic intramural hematoma. All-in-one complications. Int J Cardiol 2013; 167(4): 97−8.
54. *Jo Y, Anzai T, Ueno K, Kaneko H, Kohno T, Sugano Y,* et al. Re-elevation of D-dimer as a predictor of re-dissection and venous thromboembolism after Stanford type B acute aortic dissection. Heart Vessels 2010; 25(6): 509−14.
55. *Zhan S, Hong S, Shan-Shan L, Chen-Ling Y, Lai W, Dong-Wei S,* et al. Misdiagnosis of Aortic Dissection: Experience of 361 Patients. J Clin Hypertens (Greenwich) 2012; 14(4): 256−60.
56. *Neema PK.* Acute aortic dissection: Pitfalls in the diagnosis. Ann Card Anaesth 2013; 16(2): 83−5.
57. *Wen D, Zhou X, Li J, Luo F, Zhang L, Gao L,* et al. Plasma concentrations of interleukin-6, C-reactive protein, tumor necrosis factor-α and matrix metalloproteinase-9 in aortic dissection. Clin Chim Acta 2012; 413(1−2): 198−202.
58. *Schillinger M, Domanovits H, Bayegan K, Hölzenbein T, Grabenwöger M, Thoenissen J,* et al. C-reactive protein and mortality in patients with acute aortic disease. Intensive Care Med 2002; 28(6): 740−5.
59. *Lionakis N, Moyssakis I, Gialafos E, Dalianis N, Votteas V.* Aortic dissection and third-degree atrioventricular block in a patient with a hypertensive crisis. J Clin Hypertens (Greenwich) 2008; 10(1) :69−72.
60. *Perez A, Abbet P, Drescher MJ.* D-dimers in the emergency department evaluation of aortic dissection. Acad Emerg Med 2004; 11(4): 397−400.
61. *Sugano Y, Anzai T, Yoshikawa T, Satoh T, Iwanaga S, Hayashi T,* et al. Serum C-reactive protein elevation predicts poor clinical outcome in patients with distal type acute aortic dissection: association with the occurrence of oxygenation impairment. Int J Cardiol 2005; 102(1): 39−45.
62. *Sakakura K, Kubo N, Ako J, Wada H, Fujiwara N, Funayama H,* et al. Peak C-reactive protein level predicts long-term outcomes in type B acute aortic dissection. Hypertension 2010; 55(2): 422−9.

63. *Okina N, Ohuchida M, Takeuchi T, Fujiyama T, Satoh A, Sakamoto T,* et al. Utility of measuring C-reactive protein for prediction of in-hospital events in patients with acute aortic dissection. Heart Vessels 2013; 28(3): 330−5.
64. *Kitai T, Kaji S, Kim K, Ehara N, Tani T, Kinoshita M*, et al. Prognostic value of sustained elevated C-reactive protein levels in patients with acute aortic intramural hematoma. J Thorac Cardiovasc Surg 2014; 147(1): 326−31.
65. *Makita S, Ohira A, Tachieda R, Itoh S, Moriai Y, Yoshioka K,* et al. Behavior of C-reactive protein levels in medically treated aortic dissection and intramural hematoma. Am J Cardiol 2000; 86(2): 242−4.
66. *Paparella D, Malvindi PG, Scrascia G, de Ceglia D, Rotunno C, Tunzi F,* et al. D-dimers are not always elevated in patients with acute aortic dissection. J Cardiovasc Med (Hagerstown) 2009; 10(2): 212−4.
67. *Alkhateeb H, Said S, Cooper CJ, Rodriguez-Castro C, Dwivedi A, Onate E,* et al. Clinical profiles and outcomes of acute aortic dissection in a predominantly Hispanic population. Med Sci Monit 2014; 20: 747−51.

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Secondary hyperparathyroidism in chronic renal disease – etiopathogenesis, diagnosis and treatment

Sekundarni hiperparatireoidizam u hroničnoj bolesti bubrega – etiopatogeneza, dijagnostika i lečenje

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Introduction

Chronic renal failure (CRF) is usually slowly progressive and irreversible impairment of the functional unit of the kidney – the nephron. According to the agreed and established criteria (National Kidney Foundation / Kidney Disease Outcomes Quality Initiative – NKF / KDOQI) there is a five-stage classification, so that in I^0 stage glomerular filtration rate (GFR) > 90 mL/min/1.73m^2 and morphological and functional abnormalities may exist. Mild, moderate and pronounced reductions in GFR correspond with the stages II^0–IV^0, and GFR < 15 mL/min/1.73m^2 means end-stage renal failure [1].

Progressive decrease in GFR leads to a number of adaptive changes on tubular processes which maintain the external balance of substances and water. Biological 'price' of exhausted compensatory mechanisms is expressed as the disorder of internal balance – homeostasis along with the development of azotemia, hyperkalemia, hypocalcemia, hyperphosphatemia, and a number of consequential changes in all organs [2, 3].

Mineral and bone disorder (MBD) related to renal osteodystrophy (ROD), occurs relatively early in the course of chronic kidney disease (CKD-MBD) and includes: abnormal levels of serum calcium (Ca), phosphate (P), parathyroid hormone (PTH) and vitamin D; lower rates of bone turnover, mineralization, structure, strength and linear growth; vascular and soft tissue calcification [4].

Secondary hyperparathyroidism (SHPT) is a disorder of increased bone turnover, which is pathophysiological and can combine all the above mentioned disorders.

Etiopathogenesis of secondary hyperparathyroidism in chronic renal disease

According to the traditional concept of pathophysiology of SHPT, PTH hypersecretion is a compensatory measure for the correction of hypocalcemia resulted from transient hyperphosphatemia, which appears relatively early in the course of chronic renal disease (CRD) (GFR ≤ 60 mL/min/1.73m^2). PTH hypersecretion stimulates 1-α hydroxylase activity and enhances the synthesis of calcitriol, which promotes intestinal absorption of calcium and phosphorus. Synthesis of phosphaturic hormone is stimulated in osteocytes (FGF 23), and thus 'temporarily' disturbed homeostasis is restored.

With progression of chronic renal failure (CRF), hyperphosphataemia becomes more common, and after the reduction of GFR ≤ 25–30 mL/min/1.73m^2 it becomes permanent [5]. Serum calcium values therefore progressively decrease and it consequently lowers ionized calcium fraction (Ca^{2+}), thus stimulation of PTH synthesis and secretion becomes permanent, partly due to the absence of inhibitory effects of calcium and vitamin D for lower receptor density (CaSR/VDR) for these ligands in chief cells of the parathyroid gland [5].

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Reduced serum calcium and calcitriol values and increase in circulating PTH have been encountered in 40% of patients with GFR < 40 mL/min/1.73m^2 and in 80% of cases with GFR < 20 mL/min/1.73m [2, 3, 6].

Biological effects of parathyroid hormone

PTH is a polypeptide containing 84 amino acids, which is normally secreted by the chief cells of four parathyroid glands, 50–300 mg weight each. Proteolytic degradation of prepro-PTH in the endoplasmic reticulum and Golgi apparatus creates the final form ($^{1–84}$ iPTH) which is deposited on secretory granules. Release from the depot is encouraged by the same stimuli as its synthesis, primarily by low extracellular concentration of Ca^{2+} ions. It disintegrates rapidly in the liver and kidneys (T½ = 2–4 minutes) into biologically active N terminal amino acids (33-84, 36-84N), and various biologically inactive C carboxylate terminals ($^{7–84, 34–84, 37–84, 41–84, 43–84}$ C) [7, 8].

PTH exerts its effects on tissues through PTH_1/PTH_2 receptors. Correction of hypocalcemia involves rapid release of Ca^{2+} ions along with pumping-out a so-called 'bone fluid' located on the peripheral zone of bone matrix and slow-rate and long-term release of calcium deposited in the mineral matrix. Osteoclastic-induced enzymatic degradation of apatite is stimulated by cytokines (TNF-α and IL-1) and growth factors acting locally (TGFß, IGF, FGF). PTH stimulates reabsorption of Ca^{2+} and Mg^{2+} in kidney cells; it inhibits reabsorption of P and stimulates calcitriol synthesis by stimulating α 1-hydroxylase. Indirectly, stimulating the synthesis of vitamin D, PTH promotes the intestinal absorption of Ca^{2+} and P for the increased expression of genes responsible for NaPT2b transporter synthesis [9, 10].

Nontraditional target organs of PTH are other tissues and organs with PTH1/PTH2 receptors, which are not directly or indirectly involved in maintaining Ca^{2+} and P ion homeostasis. Maladaptive changes in blood vessels, myocyte hypertrophy, increased extracellular matrix, myocardial fibrosis, dyslipidemia, proatherogenic and proinflammatory effects, vascular and soft tissue calcifications, increased intracellular Ca^{2+} concentration, impaired compliance of arteries and arterioles are some of the harmful consequences of SHPT and related disorders leading to increased mortality in patients on dialysis [11, 12]. According to the relevant data from the DOPPS (Dialysis Outcomes and Practice Patterns Study), there was an increased mortality in patients on dialysis with Ca^{2+} > 10.0 mg/dL, P > 7 mg/dL, and PTH > 600 pg/mL [13].

Regulation of parathyroid hormone synthesis and secretion

Variation in the extracellular ionized Ca^{2+} concentrations is the most important mechanism of regulation of PTH synthesis and secretion. It is independent of the effects of vitamin D and is accomplished through a complex sensing receptor CaSR. Intracellular domain of the receptor complex (β-region) directs through adenylate cyclase the production of energy substrates ATP and cAMP which stimulate the synthesis of PTH under conditions of extracellular hypocalcemia. Otherwise, intracellular α- domain of CaSR and phospholipase C is activated, which finally inhibits mRNA transcription in PTH synthesis in a cascade process [14].

Experimental hyperphosphataemia stimulates PTH synthesis (and secretion), regardless of the concentration of calcium in the vicinity of parathyreocytes, by phospholipase A2-mediated post-transcriptional induction of PTH mRNA synthesis. Indirectly, it leads to hypocalcemia but also to stimulation of 1-α hydroxylase and the synthesis of phosphaturic hormone FGF23, thereby providing a feedback control of hyperphosphatemia [4].

Through its nuclear receptor (VDR) vitamin D directly inhibits PTH mRNA synthesis, and in different ways it indirectly controls biologically available PTH: it stimulates CaSR expression and the synthesis of FGF23 in osteocytes, encourages maturation of osteoblasts and synthesis of bone matrix (utilization of Ca^{2+} and P), stimulates osteoclastogenesis (liberation of Ca^{2+} and P), intestinal absorption of Ca^{2+} and P, renal reabsorption of Ca^{2+} and Mg, and excretion of phosphates [15].

Phosphaturic hormone FGF 23 is formed in the osteocytes and is stimulated by PTH, vitamin D and hyperphosphatemia. It inhibits PTH synthesis and secretion on post-transcriptional level through mitogen-activated protein kinase (MAPK). It exhibits phosphaturic effect through internalisation of phosphate-transport proteins (NaPT2a and NaPT2c) [5].

Permanent PTH hypersecretion in advanced CRF and in dialysis patients is encouraged by intrinsic change in parathyroid glands. Combining thropic stimuli enables diffuse (polyclonal) proliferation of parathyreocytes and consequently reduces the density of CaSR and VDR, so that individual clones proliferate uncontrollably, which eventually ends up in formation of micronodule or encapsulated macronodule. They are relatively insensitive to inhibitory effects of vitamin D and the concentration of ionized calcium in the environment, which shifts the 'sensitivity of Ca^{2+}' to the right, i.e. to inhibit PTH synthesis higher serum (and extracellular) calcium levels are needed [16].

Skeletal resistance to PTH indicates an inadequate response to its pro-calcemic effects and is associated with abnormalities in uremic environment: hyper-P, hypo-Ca, hypovitaminosis D, lower density of VDR and CaSR, accumulation of $^{7–84}$ PTH fragment exerting effects that are opposing to iPTH etc. In addition, the accumulation of osteoprotegerin (which inhibits osteoclast differentiation and maturation) and of BMP-7 bone morphogenetic protein (incorporation of phosphate into skeletal matrix) suppresses the function of osteoclasts and osteoblasts in different ways [8, 9].

The diagnosis of secondary hiperparathyroidism

Clinical manifestations of SHPT are: itching, nausea, vomiting, confusion, various clinical manifestations of soft tissue calcification, painful bones and joints, fractures, and other [17].

Laboratory analyses of relevant parameters are shown in ranges of normal serum/plasma values [7, 18]: 1) serum intact PTH (iPTH), 15–60 pg/mL (iPTH > 450 pg/mL indicates rapid bone turnover); 2) markers of bone formation are: total

alkaline phosphatase (ALP), 90–120 IU/L; bone-specific ALP (bALP), 5–15 μg/L; osteocalcin (OC-bGP), 38–202 ng/mL; matrix Gla protein (m-GP): 6.2 ± 3.5 nmol/L; 3) markers of bone resorption: tartrate-resistant acid phosphatase (TRAP), 2.5 to 45 IU/L; C - terminal of procollagen Type I (ICTP), 1.8–5.046 ng/mL; N - terminal type I collagen (NTX), 6.2 ± 19.0 nmol/L; 4) other parameters of bone metabolism are: total serum calcium: 2.10–2.60 mmol/L (8.5–10.5 mg/dL); ionized calcium ($Ca^{2+)}$: 1.15–1:35 mmol/L (4.6–5.4 mg/dL); serum phosphate 0.84–1.45 mmol/L (2.5–4.5 mg/dL); product (Ca × P): < 4.40 $mmol^2/L^2$ (< 55 mg^2/dL^2); fibroblast growth factor (FGF23), 29 ± 28 pg/mL; serum 25(OH)D, 20–70μg/L; serum $1.25(OH)_2D$ serum: 25–45 pg/mL.

Radiological diagnostics

Radiological skeletal survey (dominant hand bones, long bones, spine and synarthrosis) can identify signs of bone resorption (subperiosteal, intracortical, endosteal and subchondral) bone sclerosis (vertebrae) and calcification of the blood vessels (continuous in the intima - discontinuous in the media of the blood vessels) and parenchymal organs [19].

Ultrasound examination of the parathyroid glands reveals their position and size (volume), which correlates well with the degree of activity, and examination of the heart and blood vessels provides information on cardiovascular changes.

Scintigraphy of parathyroid glands with ^{99m}TC-methoxyisobutyl isonitrile (MIBI) allows additional visualizing the glands and morphofunctional analysis.

Computed tomography (electron beam – EBCT and multi-slice – MSCT) are useful for the assessment of severity of coronary and valve calcification, as well as for locating parathyroid glands and estimation of their size [19].

Biopsy is not a routine procedure and is applied in accordance with K/DIGO (Kidney Disease: Improving Global Outcomes) recommendations, along with two-dose tetracycline 500 mg administered 20 and 10 days before the biopsy [19, 20]. It is recommended in cases of inconsistent laboratory findings, unexplained bone pain or the occurrence of fractures, progressive vascular calcification, unexplained hypercalcemia, suspicion of aluminum or other metals intoxication, and it also should be considered before the application of bisphosphonates [20–22].

Prevention and treatment

Preventive measures include slowing the progression of kidney disease, timely commencement and periodic monitoring of indicators of mineral status thereafter, serum levels of regulatory hormones, biochemical markers of bone turnover, and timely diagnostic procedures, Table 1 [19].

Treatment involves the implementation of measures to normalize serum Ca^{2+} and P, correction of calcitriol deficiency and other procedures applied to suppress PTH synthesis and secretion.

Oral phosphate binder

Treatment of hyperphosphatemia begins by protein-adjusted restricted dietary phosphate intake of 800–1000 mg/daily, usually not before GFR is reduced to 50–60% of its normal values.

If serum P cannot be reduced to the recommended limit in this way or PTH is increased, the use of calcium, and optionally, non-calcium-based phosphate binders may be considered (Table 2) [19, 20, 23].

For this purpose, natural metabolites of vitamin D can be used [ergocalciferol (D_2), cholecalciferol (D_3), calcitriol]; synthetic analogues of vitamin D_2 (paricalcitol, falecalcitriol, doxercalciferol) synthetic analogues of vitamin D_3 (alfacalcidol, maxacalcitol).

The use of vitamin D in the treatment of secondary hyperparathyroidism

If hyperphosphatemia is corrected as described above and target serum Ca^{2+} levels achieved are not sufficient to suppress hypersecretion of PTH, application of vitamin D may be taken into consideration (Table 3) [24].

Ergocalciferol [Calcidol®, Calciferol®, Vitamin D2®, Drisdol® caps. 1.25 mg (50,000 IU); Ergocalciferol® amp. 200.000/300.000/600.000 in 1 mL or 2 mL is now rarely used, and priority is given to calcitriol and its synthetic derivatives.

General recommendations for use of vitamin D to suppress SHPT are: if, after the initial dose serum PTH increases remains unchanged, or is reduced to less than 50%, the dose should be increased; if PTH is reduced by more than 50%, the dose does

Table 1

Recommended reference intervals for monitoring biochemical parameters of mineral bone disorder in chronic kidney disease (CKD)

Parameter	Check-up interval (in months)		
	CKD–III[0] (GFR 59–30 mL/min)	CKD–IV[0] (GFR 29–15 mL/min)	CKD–V[0] (GFR < 15 mL/min)
Ca	6–12	3–6	1–3
P	6–12	3–6	1–3
PTH	basal	6–12	3–6
ALP	not recommended	6–12	3–6
bALP	basal- not recommended		
25 OH D_3	basal when PTH levels increase		when needed

GFR – glomerular filtration rate; Ca – serum calcium; P – serum phosphate; PTH – parathyroid hormone; ALP – serum alkaline phosphatase; bALP – bone alkaline phosphatase.

Table 2

Recommendations for the application of phosphate binder

Dosage	Phosphate binder
	Calcium carbonate
Initial dose	2–3 × 0.5–1.0 g (Calcium carbonate® tablets. 0.5/1.0 g)
Titration: dose/interval	0.5–1.0 g/1–2 weeks
D.Th. max.	5.0–6.0 g (2.0 g elemental Ca daily)
Notes	Constipation, hyper-Ca, soft-tissue calcification, proven ABD or PTH ≤ 150 pg/mL constantly
	Calcium acetate (Phoslo® capsules 667 mg; Phosex® tablets 1000 mg; Renacet® tablets 475 mg)
Initial dose	2 × 667 mg; 3 × 475 mg
Titration: dose/interval	475–667 mg/1–2 weeks
MDD	3–4.0 g (2.0 g elemental Ca daily).
Notes	Constipation, hyper-Ca, soft-tissue calcification, proven ABD or PTH ≤ 150 pg/mL constantly
	Combination 435 mg calcium acetate and 235 mg magnesium carbonate (Osvaren® tablets)
Initial dose	3 × 1 tabl.
Titration: dose/interval	1–2 tabl. /1–2 weeks
MDD	10–12 tab.
Notes	Mg > 2 mmol/L, AV-block III°, bradycardia/bradyarrhythmias, customize the dialysate magnesium content, myasthenia
	Sevelamer hydrochloride (Renagel® tablets 800 mg) **Sevelamer carbonate** (Renvela® tablets 800 mg)
Initial dose	3 × 1 (P: 1.76–2.42 mmol/L); 3 × 2 (P > 2.42 mmol/L).
Titration: dose/interval	1–2 tab/week
MDD	4–6 g/daily
Notes	Cholesterol binder, compensation of liposoluble vitamins, hypo-Ca (correction of $CaCO_3$, vitamin D)
	Lanatanum carbonate (Fosrenol® tablets 500/750/1000 mg)
Initial dose	1–2 tab/daily
Titration: dose/interval	1–2 tab/week
MDD	1.5–3.0 g/daily
Notes	Less hypocalcemic state, likely tissue accumulation

MDD – maximum daily dose.

Table 3

Recommendations for supplementation of vitamin D

Dosage	Vitamin D
	Calcitriol (Rocaltrol® tablets -0.25/0–5 μcg; Calcitriol® ampoules 1 μcg)
Initial dose	p.o.: 0.25 μcg/daily or 0.1 μg/кg/weekly in 3 doses i.v.: 1–2 μcg/HD.
Titration: dose/interval	p.o.: 0.25 μcg/2–4 weeks i.v.: 0.5–1 μcg/2–4 weeks
MDD	In accordance with parameters (Ca, P, Ca × P and PTH) – app. 10 μcg
Notes	General contraindications
	Alfacalcidol (One-Alpha® capsules 0.25/0.5/1.0 μcg; ampoules 1.0/2.0 μcg)
Initial dose	p.o.: 1,0 μcg/daily i.v.: 1.0 μcg/HD
Titration: dose/interval	p.o.: 0.25/0.5 μcg./2–4 weeks i.v.**:** 0.5–1.0 μcg./2–4 weeks
MDD	In accordance with parameters (Ca, P, Ca × P and PTH)
Notes	General contraindications
	22-oxacalcitriol-OCT (Maxacalcitol® Oxarol ®; ampoules 5.0/10.0 μcg)
Initial dose	i.v.**:** 5 μcg (PTH ≤ 500 pg/mL); 10 μcg (PTH > 500 pg/mL).
Titration: dose/interval	i.v.**:** 5.0 μcg/2–4 weeks
MDD	In accordance with parameters (Ca, P, Ca × P and PTH)
Notes	Lower levels of hyper-Ca and hyper-P in relation to calcitriol Better control of bone metabolism Useful in refractoriness to calcitriol General contraindications
	Paricalcitol (Zemplar® capsules 1/2/5 μcg; ampoules 5.0/10 μcg)
Initial dose	p.o.: 1 μcg/daily; 2 μcg 3 times weekly (PTH < 500 pg/mL), p.o.: 2 μcg/daily; 4 μcg 3 times weekly (PTH > 500 pg/mL), i.v.: 5 μcg/HD
Titration: dose/interval	p.o.: 1 μcg/2 μcg/2–4 weeks i.v.: 2.5 -5 μcg/2–4 weeks
MDD	p.o.: μcg dose = PTH (pg/mL) : 60; (≤ 32 μcg/weekly) i.v.: μcg dose = PTH (pg/mL) : 80, (≤ 40 μcg/weekly)
Notes	Less calcemic than calcitriol General contraindications
	Doxercalciferol (Hectorol® capsules 0.5/1.0/2.5 μcg; ampoules 2.0/4.0 μcg)
Initial dose	p.o.: 3 × 1–2.5-10 μcg. i.v.**:** 3 × 4 μcg
Titration: dose/interval	p.o.: 2.5–5.0 μcg/8 weeks i.v.**:** 3 × 1–2 μcg/8 weeks
MDD	p.o.: 60 μcg/weekly i.v.: 18 μcg/weekly
Notes	General contraindications

P.o. – *per os*; i.v. – intravenous; MDD – maximum daily dose; PTH – parathyroid hormone; Ca – serum calcium; P – serum phosphate.

not change if PTH > 150 pg/mL; if PTH < 150 pg/mL treatment will be suspended temporarily, and after 1–2 weeks it will continue at a lower dose. It will be necessary to periodically monitor: Ca, P, Ca × P (at every 7-14 days) and PTH (1-2 times a month.) [25, 26].

Contraindications to vitamin D are: hypercalcemia (≥ 2.60 mmol/L), hyperphosphatemia (≥ 1.70 mmol/L), iPTH < 150 pg/mL, adynamic bone biopsy-proven disease, vascular calcification and calciphylaxis.

Treatment of secondary hyperparathyroidism with calcimimetics

Calcimimetics are drugs that bind to the transmembrane domain of CaSR in parathyrocyte and increase its sensitivity to the existing extracellular Ca^{2+} concentration, and thereby suppress PTH synthesis independently of vitamin D. Long-term use of calcimimetics substantially reduces the need for parathyroidectomy (PTx), and the risk of fractures and cardiovascular complications [27].

The drugs are indicated for dialysis patients with refractory SHPT including those with calciphylaxis if serum PTH > 800–1000 pg/mL despite standard therapy and surgical treatment is impossible. The main contraindication is hypocalcemia.

Cinacalcet (Mimpara®; Sensipar® tabl. 30/60/90 mg) is used to treat SHPT with phosphate binders and vitamin D. The starting dose was 30 mg/day and progressively increased by 30 mg every 2–4 weeks to a maximum of 180 mg/day. During the titration phase PTH should be measured every month, and every 3 months when the maintenance dose is achieved. In case of hypo-Ca, calcium supplements and vitamin D should be administered and dialysate concentration of calcium should be corrected.

To change the dialysis regime means, besides the use of membrane with higher phosphate clearance and longer dialysis duration, more frequent or daily/nightly dialysis until hyperphosphatemia has been corrected. To provide a more liberal application of vitamin D or its analogues, and, consequently, a more efficient suppression of PTH, 1.5 mmol/L or 1.25 mmol/L calcium dialysate is commonly used [28].

Surgical treatment of secondary hyperparathyroidism

Surgical or sclerosing PTx is a procedure for treatment of advanced SHPT that is refractory to combined drug therapy [28].

Indications for PTx are: persistent hyper-Ca despite the complete suspension of Ca-phosphate binder; persistent hypersecretion PTH (≥ 1000 pg/mL) refractory to medical treatment, especially if the gland/nodule is greater than 0.5 cm; progressive and symptomatic soft-tissue calcifications including uremic arteriolopathy (calciphylaxis); unbearable itching with increased levels of PTH [29].

Surgical methods are subtotal or total parathyreidectomy. Total PTx means simultaneous autoimplantation of parathyroid tissue which is not of nodular hyperplastic gland. Subtotal PTx involves removing all located parathyroid glands except the least and anatomically the best one, which is partially resected and reconstructed [29].

Ultrasound-guided percutaneous ethanol injection therapy (PEIT) is used to selectively destroy nodular hyperplastic glands, while the rest of diffuse-type hyperplastic parathyroid tissue can be medically controlled. Complications include pain, bleeding and laryngeal nerve paresis. The same procedure may also be used with administration of injectable calcitriol or analogues and effectively suppress diffuse-type hyperplastic glands, through apoptosis induction or toxic necrosis [30].

Conclusion

Excessive secretion of parathyroid gland already develops in moderate renal failure, and severe clinical consequences are usually seen in patients treated with dialysis. Initially, transient hypocalcemia and hyperphosphatemia trigger the excessive synthesis and secretion of parathyroid hormone, and when they have turned into permanent condition, vitamin D deficiency is already present. Besides biochemical indicators that should be periodically monitored in accordance with the recommendations, a variety of visualization techniques can be used for localization and assessment of the gland activity. Correction of hyperphosphatemia and hypocalcemia using calcium-based phosphate binders and vitamin D leads to the suppression of PTH hypersecretion. In clinically well-defined patients, application of calcimimetics may be useful and, if associated with pulse doses of active metabolites of the vitamin D, can significantly reduce the need for parathyroidectomy.

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The authors stated that there are no conflicts of interest regarding the publication of this article.

R E F E R E N C E S

1. *Levey AS, de Jong PE, Coresh J, El-Nahas M, Astor BC, Matsushita K*, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011; 80(1): 17−28.
2. *Bricker NS*. On the pathogenesis of the uremic state. An exposition of the "trade-off hypothesis". N Engl J Med 1972; 286(20): 1093−9.
3. *Poskurica M*. Imbalance of phosphate, calcium and magnesium ions in development of chronic renal failure [dissertation]. Belgrade: School of Medicine, University of Belgrade; 1993. (Serbian)
4. *Lewin E, Rodriguez M*. Abnormal parathyroid gland function in CKD. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 77−108.
5. *Naveh-Many T, Silver J*. Regulation of parathyroid hormone biosynthesis and gene expression. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 61-75.
6. *Poskurica M*. Dynamics of fractional phosphate excretion in chronic renal failure [thesis]. Belgrade: School of Medicine, University of Belgrade; 1989.
7. *Ureña P, Olgaard K, Martin K*. Biochemical markers of bone metabolism in CKD. In: *Olgaard K, Salusky BI, Silver J,* editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 173−98.
8. *Ritter CS, Armbrecht HJ, Slatopolsky E, Brown AJ*. 25-Hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. Kidney Int 2006; 70(4): 654−9.
9. *Gonzaley AE, Martin JK*. Growth factors and cytokines in renal bone disease. In*: Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 137−44.
10. *Ott MS*. New aspects of normal bone biology. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 15−30.
11. *Petrovic D, Stojimirovic B*. Secondary hyperparathyroidism: risk factor for development of cardiovascular complications in patients on hemodialysis. Med Pregl 2010; 63(9−10): 674−80. (Serbian)
12. *Poskurica M, Lazarević M, Petrović D, Lazarević T, Milošević J*. Calcium and Phosphate Disturbances and its Regulating Hormones in Chronic Renal Failure. XXXVI Congress of EDTA-ERA, EDTA-ERA; Spain, Madrid; September 5–8: 1999; Abstract 38.
13. *Petrović D, Obrenović R, Trbojević-Stanković J, Majkić-Singh N, Stojimirović B*. Hyperphosphatemia - The Risk Factor for Adverse Outcome in Maintenance Hemodialysis Patients. J Med Biochem 2012; 31(3): 239−45. (Serbian)
14. *Cañadillas S, Canalejo A, Santamaría R, Rodríguez ME, Estepa JC, Martín-Malo A,* et al. Calcium-sensing receptor expression and parathyroid hormone secretion in hyperplastic parathyroid glands from humans. J Am Soc Nephrol 2005; 16(7): 2190−7.
15. *Koizumi M, Komaba H, Fukagawa M*. Parathyroid Function in Chronic Kidney Disease: Role of FGF23-Klotho Axis. In: *Razzaque MS*, editor. Phosphate and Vitamin D in Chronic Kidney Disease. Basel: Karger; 2013. p. 110−23.
16. *Lewin E, Garfia B, Almaden Y, Rodriguez M, Olgaard K*. Autoregulation in the parathyroid glands by PTH/PTHrP receptor ligands in normal and uremic rats. Kidney Int 2003; 64(1): 63−70.
17. *Levy J, Morgan J, Brown E*. Complications of ESRD: renal bone disease. In: *Levy J, Morgan J, Brown E*. Oxford handbook of dialysis. 2nd ed. New York: Oxford University Press; 2007. p. 653−82.
18. *Poskurica M*. Renal osteodystrophy in patients on peritoneal dialysis. In: *Nešić V*, editor. Peritoneal dialysis: A clinical problem. Belgrade: Faculty of Medicine; 2003. p. 167−95. (Serbian, English)
19. *Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group*. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009; (113): S1-130.
20. *National Kidney Foundation*. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 2003; 42(4 Suppl 3): S1−201.
21. *Poskurica M, Lazarević M, Grubor M, Laković S, Mitrović N, Milošević J*. Metastatic calcification of the heart in patients with terminal renal failure. Medicinska istraživanja 1999; 13(2): 109−13. (Serbian)
22. *Applbaum YH*. Imaging of the skeleton snd the joints in CKD. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 199−216.
23. *D'Haese PC, Spasovski GB, Sikole A, Hutchison A, Freemont TJ, Sulkova S,* et al. A multicenter study on the effects of lanthanum carbonate (Fosrenol) and calcium carbonate on renal bone disease in dialysis patients. Kidney Int Suppl 2003; (85): S73−8.
24. *Poskurica M*. The importance of vitamin D in preventing progression and complications of terminal renal failure. In: *Stolic R*, editor. School of dialysis Leskovac, "Preventive Nephrology. Nis: University of Nis; 2004. p. 229−36. (Serbian)
25. *Park J, Rhee CM, Lau WL, Kalantar-Zadeh K*. Clinical uses of 1-alpha-hydroxy-ergocalciferol. Curr Vasc Pharmacol 2014; 12(2): 306−12.
26. *Cunningham J, Lewin E, Silver J*. Medical therapy in chronic kidney disease. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 463−83.
27. *Nemeth EF*. Calcimimetics and calcilytics in the treatment of chronic kidney disease-mineral and bone disorder. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 443−61.
28. *Argilés A, Mourad G*. How do we have to use the calcium in the dialysate to optimize the management of secondary hyperparathyroidism? Nephrol Dial Transplant 1998; 13 Suppl 3: 62−4.
29. *Sarfati E, Drueke BT*. Surgical management of secondary hyperparathroidism. In: *Olgaard K, Salusky BI, Silver J*, editors. The spectrum of Mineral and Bone Disorders in Chronic Kidney Disease. 2nd ed. Oxford: University Press; 2010. p. 543−59.
30. *Ogata H, Koiwa F, Shishido K, Takahashi K, Ito H, Kinugasa E,* et al. Effects of 22-oxacalcitriol and calcitriol on PTH secretion and bone mineral metabolism in a crossover trial in hemodialysis patients with secondary hyperparathyroidism. Ther Apher Dial 2007; 11(3): 202−9.

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Cutaneous side effects caused by treatment for inflammatory bowel disease

Neželjeni efekti na koži bolesnika prouzrokovani lekovima za inflamatorne bolesti creva

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Key words:
inflammatory bowel diseases; therapeutics; drug hypersensitivity; skin; immunosuppressive agents; azathioprine; biological therapy; erythema nodosum; melanoma.

Ključne reči:
creva, zapaljenske bolesti; lečenje; lekovi, hipersenzitivnost; koža; imunosupresivi; azatioprin; biološka terapija; eritema nodozum; melanom.

Introduction

Treatment of inflammatory bowel disease (IBD) has significantly changed the shape and efficiency over the last 20 years. Cutaneous lesions could be part of extraintestinal manifestations of these disorders (like erythema nodosum or pyoderma gangrenosum), can occur as consequence of specific vitamin and micronutrient deficiencies (zinc and iron deficiency), or as complications of the drugs that are used with the intention to control inflammation. In fact, treatment with these drugs can cause paradoxical inflammatory dermatoses.

This article suggests how to recognize and treat cutaneous lesions that can occur during the treatment of IBD, especially how to diagnose them early (particularly skin cancer) and how to treat them most effectively with an emphasis on the importance of very close cooperation of gastroenterologists and dermatologists in the approach to these patients.

Prebiologic era

Before the advent of medications from the biological group, the following drugs were the mainstay of IBD treatment: 5-aminosalicylic acid (5-ASA) and mesalazine, corticosteroids and immunosuppressants − azathioprine, methotrexate (MTX) and cyclosporine.

5-ASA

Cutaneous side effects of 5-ASA are various forms of nonspecific exanthemas, which occur rarely and with mild intensity not requiring interruption of therapy. Serious adverse reactions are even more rare, but can occur as exfoliative dermatitis (erythroderma), Stevens-Johnson syndrome, and toxic epidermal necrolysis, mainly during sulfasalazine treatment [1]. These reactions occur within the first month of treatment. In the event of such a severe reaction it is necessary to immediately stop further implementation of the drug.

Corticosteroids

Methylprednisolone, hydrocortisone and prednisone have great potential in the treatment of IBD and are used in inducing remission in moderate to severe form of the disease. Corticosteroids suppress immune system by decreasing the number of activated T lymphocytes which have very important role in pathogenesis of IBD. But corticosteroids also carry a risk of side effects. The skin is prone to the following adverse effects from prolonged courses or high doses of systemic steroids: skin infections such are bacterial (*eg cellulitis*) and fungal infections (*eg* tinea, candida), skin atrophy resulting in easy bruising (purpura), skin tearing after minor

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injury and slow healing; these effects are most prominent on sun exposed areas particularly the backs of the hands and the forearms. Also, stretch marks (*striae*) can occur, particularly under the arms and in the groin. Steroid acnes are very common side effect with clusters of small spots on the face, chest and upper back, but without the usual open and closed comedones that are typical of juvenile acne (Figure 1). However, aggravation of preexisting acne in the young patients is also frequent. Hirsutism and androgenetic alopecia are also a common side effect of a prolonged steroid use [2]. These adverse effects are just one of the several reasons for contemporary treatment corticosteroids to be no longer recommended for a prolonged use. Its use should be limited to no longer than 3 months and repeated episodes of their application should be avoided, especially now with a large selection of effective drugs of a much better safety profile.

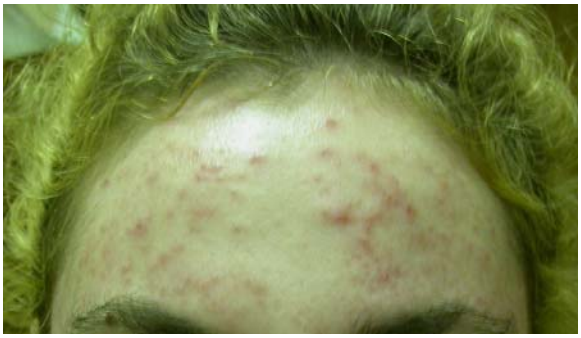

Fig. 1 − Steroid acne in a 19-year-old patient presented with severe form of ulcerative colitis. Treatment was started with 40 mg of methylprednisolone, and steroid acne developed thereafter, treated with topical antibiotic therapy. The lesions completely regressed after the discontinuation of systemic steroids.

Thiopurines

Thiopurine medications are used in the form of 6-mercaptopurine (not registrated in Serbia) and azathioprine. By blocking the synthesis of purines they represent antimetabolites which disrupt the synthesis of DNA and thereby block the differentiation and proliferation at the cell level, stimulate apoptosis of T lymphocytes, resulting in the reduction of the number of lymphocytes in peripheral blood and the number of natural killer cells [3].

After starting thiopurine medications, maculopapular rash can occur and lead to discontinuation of the treatment. Thiopurines also increases the risk of viral infections, such as herpes simplex (Figure 2) or herpes zoster. These infections are usually mild, although the presentation in immunocompromised patients may have an aggressive form [4, 5]. Regarding the patient presented to our clinic it is clear how a banal infection under the immunosuppressive therapy can take a more severe form which is harder and takes longer to treat.

This is the reason when under treatment with thiopurine with this type of skin changes, chemoprophylaxis is recommended for such patients with acyclovir 400 mg 2 times *per* day. In Serbia, the vaccination against varicella (chickenpox) is optional and according to the European Crohn's and Colitis

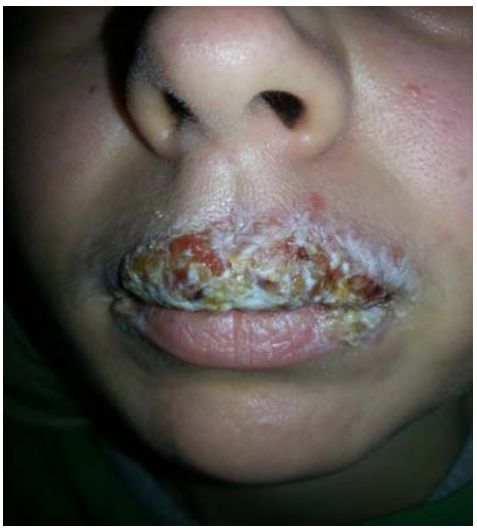

Fig. 2 − Labial bilateral herpes simplex in a 20-year-old patient with Crohn's disease treated with azathioprine and mesalazine. Oral and topical acyclovir were started and continued for 10 days, since the regression of the lesions was slow and prolonged. Azathioprine treatment was interrupted during the infection.

Organisation (ECCO) recommendations it is advised that nonvaccinated patients who are planned for application of immunosuppressive therapy are screened for susceptibility to primary varicella zoster virus (VZV) infection. Those without a clear history of chickenpox, shingles or receipt of two doses of varicella vaccine should be tested for VZV IgG. Where possible, seronegative patients should complete the two-dose course of varicella vaccine at least 3 weeks prior to commencement of immunomodulatory therapy [6].

Hypersensitivity skin reactions to thiopurine occur in about 12% of patients, most commonly manifested in the form of urticaria or maculopapular rash. Sweet syndrome (SS) (Figure 3) represents an eruption of painful erythematous plaques or nodules, accompanied by fever.

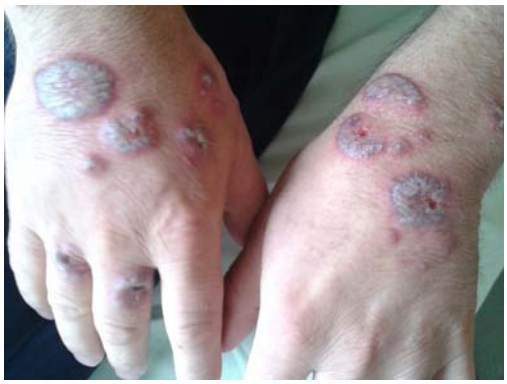

Fig. 3 − Neutrophilic dermatosis of the dorsal hands (localized form of Sweet's syndrome) in a 48-year-old patient with fistulized Crohn's disease treated with azathioprine and antibiotics. Skin changes presented as erythematous and edematous livid plaques, with ulcerations on the dorsal hands. Corticosteroid therapy (topical and oral) was started and complete resolution of the skin was achieved. Azathioprine was excluded and the treatment continued with anti-tumor necrosis factor (TNF) therapy.

Changes of this type occur in the first two weeks after starting thiopurine medication. It is important to emphasize that SS is also described as cutaneous extra-intestinal manifestation of IBD, but nevertheless there is sufficient evidence for the existence of azathioprine-induced SS that exists independently of confounding factors [7, 8].

Erythema nodosum (Figure 4) also occurs, although these changes may be a manifestation of an underlying IBD [9]. Common to all of these dermatoses is that after the cessation of drug administration, regression of skin lesions is evident. If cutaneous reaction to azathioprine is suspected, reintroduction of the drug can be dangerous because it can lead to more severe manifestations, so it should be avoided.

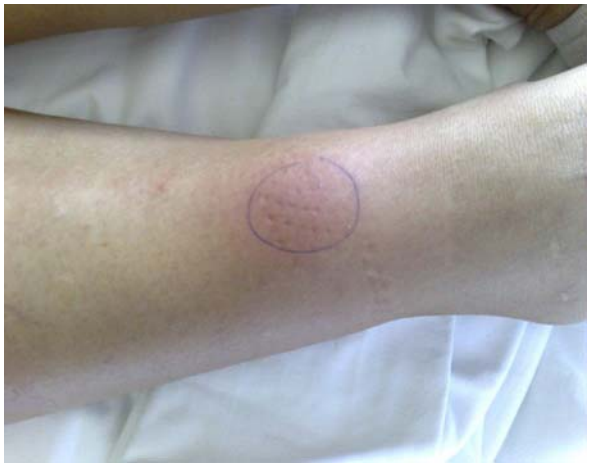

Fig. 4 − *Erythema nodosum* in a 27-year-old patient with first presentation of ulcerative colitis. At the onset of disease the patient was presented with barely raised, painful, tender, reddish nodules, below the knees, and treated with local and systemic steroids. While reaching clinical remission, skin changes completely disappeared.

Although thiopurine has been known for more than 30 years in the treatment of IBD it has been only recently noted that a prolonged use of this drug is associated with the appearance of skin cancer. It was originally observed in transplant patients. It is believed that patients who receive the drug for a prolonged period of time have a much higher risk of getting non-melanoma skin cancer (NMSC) than people in the general population [10]. NMSC is the most common cancer in industrialized world, and it presented as basal cell carcinoma (BCC) (Figure 5) and squamous cell carcinoma (SCC), usually in the ratio 1 : 4 and most commonly in the sun-exposed areas (face, lips, and backs of hands and forearms). Skin cancer develops as a consequence of accumulated 6-thioguanine (6-TG) that absorbs UV rays causing damage and instability formation of DNA and generates reactive oxygen species that can cause mutagenic and irreversible DNA damage [11].

A retrospective study on 53,377 patients found a positive correlation between exposure to azathioprine and NMSC after the minimum drug exposure of 90 days. The study points to the increased risk of incidence of NMSC (IRR, 1.64; 95% CI, 1.51−1.78), based primarily on the occurrence of immunological function disturbances which represent the basis of disorder in patients with IBD, as well as the use of immunosuppressive drugs. The risk is further increased if the exposure is prolonged over a year [12].

A French study (CESAM) on 19,486 patients with IBD showed an increased tendency of getting NMSC in Caucasians, over 65 years of age, with the hazard ratio (HR) of 5.9 for patients still in treatment phase and the HR of 3.9 in patients who discontinued the drug [13].

Information about the tendency of getting melanoma in these patients is still very scarce. Only 3 cases of melanoma on combined immunosuppression (azathioprine plus corticosteroids) have been described so far [14].

Clearly, for patients treated with thiopurines there is the need for regular dermatologic examination once a year. Rigorous sun protection (avoidance of direct sun exposure, especially between 11 a.m. and 4 p.m.; sun protective clothes and the use of broad spectrum sun protection factor (SPF) 50 sunscreens is also mandatory and should be emphasized [15].

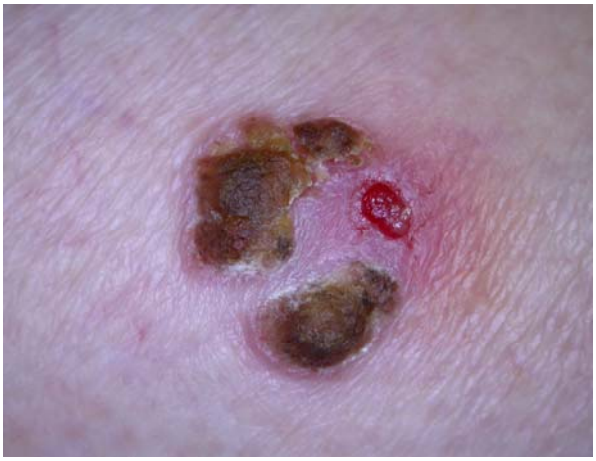

Fig. 5 − Basal cell carcinoma in a 48-year-patient with ulcerative colitis treated with mesalazine and azathioprine for 3 years. The patient was presented with erythematous patch with ulcerations and crusting on the left shoulder. Complete excision was done.

Methotrexate

MTX is anti-metabolite drug which inhibits DNA, RNA and protein thymidylate synthesis. The mechanism of immune suppression induced by MTX is accumulation of adenosine, which inhibits the activation of T lymphocytes. Cutaneous reactions to MTX are nonspecific maculopapular exanthems, (15%), alopecia (8%), photosensitivity (5%) and urticaria (4%). Erosions, ulceration, hemorrhagic bullae of the oral and vaginal mucosa, the knees and the back are the result of the MTX cytotoxic effect the regions with the fast epithelial turnover [16].

Only 10 cases of cutaneous lymphomas induced by MTX have been described so far, Epstein Barr virus (EBV) associated multifocal B lymphomas being the most frequent. In the majority of cases, they spontaneously regress with MTX treatment discontinuation [17]. Also, a case of cutaneous lymphoma at the site of intramuscular injection, which spontaneously regressed after discontinuation of MTX has been described [18].

There are several case reports on melanoma developed while on MTX treatment, most commonly in rheumatologic patients. Also, in a study a 3-fold increased risk for melanoma was described in these patients. Similar results, however, have not been published for IBD patients. Further investigations in this filed are needed, until then yearly dermatological checkups are recommended [19].

Cyclosporine

Cyclosporine is still used in Serbia in treatment of the most severe forms of ulcerative colitis, although in most countries after advent of drugs from the group of anti-tumor necrosis factor (TNF), its use has been drastically reduced. Cyclosporine inhibits translocation of nuclear transcription factor [nuclear factor of activated T lymphocytes (NFAT)] which leads to reduced secretion of proinflammatory cytokines by T cells [20]. Cutaneous lesions develop in 7% of patients and are manifested as hirsutism, acne and gingival hyperplasia. Also, cases of cutaneous T-cell lymphoma are described, especially in prolonged use in transplant patients. Since cyclosporine is used in IBD for a short period of time (usually 3 months) these side effects have not been described in IBD patients [21].

Biologic era

For the last 15 years in Serbia, biological therapy of IBD primarily involves the use of monoclonal antibodies to TNF, which is one of the dominant secreting proinflammatory cytokines in the pathogenesis of IBD. Although these drugs are also used in the treatment of psoriasis and other dermatoses, inflammatory dermatoses can appear as a paradoxical side-effect of these class of drugs. New drugs called antiadhesive antibodies have not yet been registered in Serbia, and hereby were not reviewed.

Anti-TNF drugs

TNF-α is a proinflammatory cytokine, and one of the main inflammatory mediators involved in the pathogenesis of IBD. TNF-α inhibitors led to the revolutionary advance in the treatment of patients with IBD. Today there are 4 products of this group on the market: infliximab (Remicade®), adalimumab (Humira®), golimumab (Simponi®) and certolizumab (Cimzia®). TNF inhibitors lead to the fast control of IBD symptoms with a maximum mucosal healing, reduce the need for hospitalization and surgery, and the cost of treatment of these patients [22, 23]. Combination of anti-TNF inhibitors with thiopurine or with MTX is considered the most effective therapy in the treatment of IBD. Treatment with TNF inhibitors is, however, accompanied with frequent cutaneous side effects, including the occurrence of skin infections and inflammatory dermatoses in about 12−20% of patients.

Local site reactions (Figure 6) with the application of anti-TNF drugs are frequent and are described in the form of erythema and edema at the site of the injection. These changes are observed only during the application of adalimumab.

Skin infections are also described in the literature as common side effects of TNF inhibitors: *cellulitis*, herpes simplex infection and reactivation, staphylococcal skin infection, *tinea corporis*, *pityriasis versicolor*. Infections are not related to the particular TNF inhibitor, and are specific for the entire class of these drugs. Overall, they do not exceed 1.5% in biologics exposed patients, and are second most common after acute respiratory infections. However, coadministration of anti-TNF with thiopurine is leading up to 3−4 times higher incidence of opportunistic infections. The risk is highest for the simultaneous administration of at least two of immunosuppressants and in patients older than 50 years. They are mostly milder infections as oral candidiasis but life-threatening infections are also possible [24].

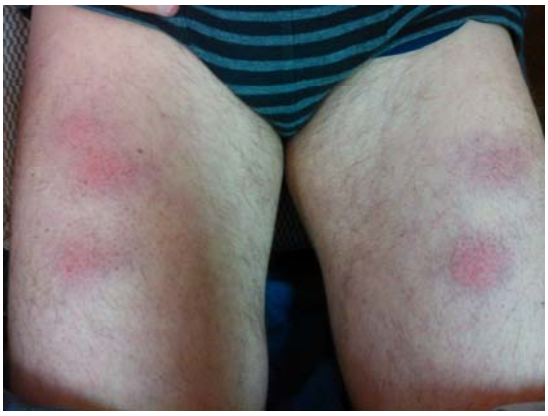

Fig. 6 − Injection site reaction in a 26-year-old patient with small bowel Crohn's disease on treatment with adalimumab. Skin reaction presented immediately after starting adalimumab therapy. The patient was treated with topical corticosteroids until resolution. Therapy was switched to infliximab.

Development of anti-nuclear antibodies (ANA) and lupus-like syndrome are rare, and they are most frequently described in rheumatologic patients. In IBD patients it is described as case reports. In a study that analyzed 105 drug-induced lupus erythematosus cases on anti-TNF therapy, ten of them were patients with Crohn disase [25], so the literature is scarce. Malar rash and photosensitivity are the most common presentation, followed by discoid cutaneous lesions, while lupus nephritis is rare. TNF itself prevents the formation of autoantibodies and its blockade creates the opposite effect. Also, treatment with TNF inhibitors decreases clearance of antibodies, enables the shift from Th1 to Th2-type cytokines, leading to the production of ANA [26].

Psoriasis is a chronic inflammatory skin disease with different manifestations. In about 10% of cases it is associated with other inflammatory diseases, and IBD is one of them. TNF inhibitors are used for the treatment of psoriasis and psoriatic arthritis, but paradoxically the use if these drugs can be associated with *de novo* psoriasis onset, irrespective of the type of TNF inhibitor. The frequency of psoriasis in TNF inhibitor treated patients is about 3%, it is more often in women (70%) and it usually develops after 2−6 months from the application of this type of treatment. It was shown that anti-TNF-alpha therapy induces higher levels of

interferon (IFN)-alpha, that is known as a factor capable of inducing or aggravating existing psoriasis, through the induction of proinflammatory cytokines, such as IL-12 and IL-23 [27]. That is why it is recommended to switch the patients with anti-TNF induced psoriasis to ustekinumab anti IL-12/23 antibody [28].

A number of patients also show exacerbation of previously existing psoriasis, while the highest number gets *de novo* changes. The changes are most frequently manifested in the form of palmoplantar pustulosis which is nowadays considered as a separate entity from psoriasis (Figure 7). Also, it can be manifested in the form of guttate (Figure 8) and pustular psoriasis. Scalp lesions with infiltration and thickened scale, sometimes in the form of *tinea amiantacea* can also be present [29], as frequently as the palmoplantar lesions. Completely atypical lesions are also described, where psoriasiform reaction was confirmed by histopathological analysis. These cutaneous lesions quickly regress after discontinuation of TNF inhibitor [30].

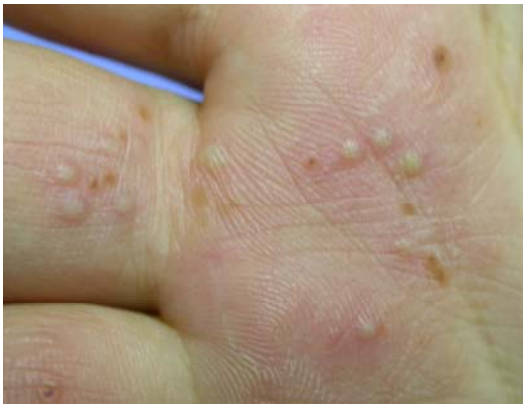

Fig. 7 − Palmoplantar pustulosis in a 35-year-old patient with inflamatory bowel disease on infliximab/azathioprine therapy. After a year of the therapy the patient presented with sterile pustules on erythematous background on the palms and soles, and was successfully treated with acitretin 25 mg a day without interruption of the biologic therapy. Trough levels were checked, at that point that were high, so optimisation with lowering the infliximab dose was done.

Treatment depends on the severity of skin lesions, mainly does not require discontinuation of anti-TNF preparations and is well controlled with application of topical corticosteroids, keratolytics, vitamin D analogs. In severe cases, addition of MTX, retinoids or cyclosporine is necessary. Also, phototherapy can be very useful in most cases. If there is no success in treatment it should be discontinued with the implementation of anti-TNF preparations or possibly replace one anti-TNF preparation with another, although it is accompanied with a large (52%) percentage of relapse [31]. Consultation of dermatologist to diagnose psoriasis, psoriasiform eruption or other inflammatory dermatosis is necessary in order to choose the appropriate and most effective treatment combination.

Localized or generalized eczema, sometimes typical of atopic dermatitis is well-known in IBD patient on anti-TNF therapy. However, in a case control study [32] it was shown that atopic manifestations, particulary eczema were more frequently reported in patients with Crohn's disease in comparison to control patients. Anti-TNF therapy can aggravate atypical form dermatitis manifestations, with erythema, desquamation and itching usually manifested in popliteal and antecubital fossa, but more severe erythema, oozing and severe itching can occur on other flexural areas, and can progress to erytroderma (Figure 9). Treatment involves application of local therapy, but if the lesions are generalized systemic corticosteroids are necessary with intensive local treatment with emollients, topical corticosteroids and topical calcineurin inhibitors. In the most severe cases, discontinuation of therapy with a possibility to switch to another form of biological therapy is necessary. Close collaboration with the dermatologist is mandatory to diagnose and adequately treat these patients.

More rare changes observed in the application of these therapies involve alopecia which may occur shortly after the start of treatment, or after a few months/years of anti-TNF treatment. Alopecia areata, androgenetic alopecia and diffuse alopecia were all described. Discontinuation of the drug is generally sufficient for withdrawal symptoms but the reintroduction of these products can repeatedly induce alopecia [33]. Vasculitis is present in 5% of gastroenterology patients treated with anti-TNF therapy over 30 years, but this percentage

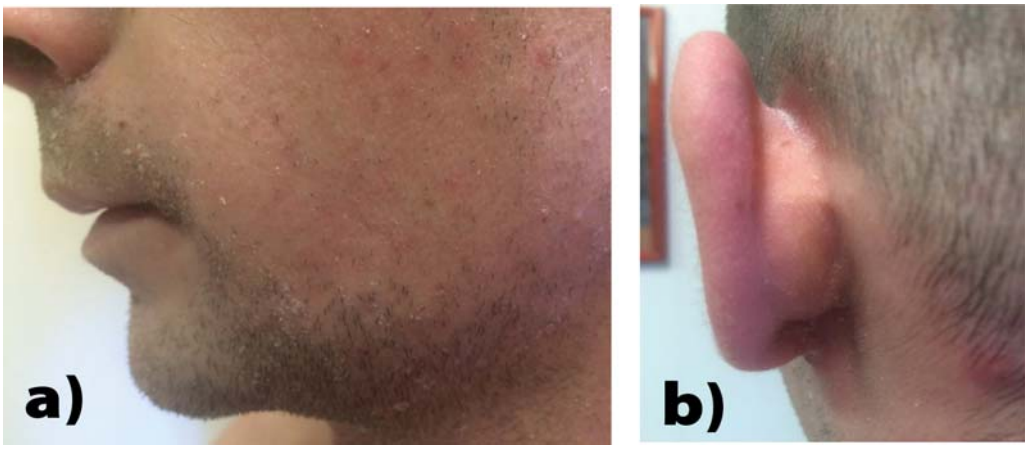

Fig. 8 − Psoriasiform reaction during treatment with infliximab: a) Guttate papules on the face; b) Slightly indurated plaques with whitish adherent scales in the retroauricular region in a 24-year-old patient with Crohn's colitis presented with skin lesions 8 months following starting the therapy. The patient was treated with topical corticosteroids and a combination of calcipotriol/betamethasone.

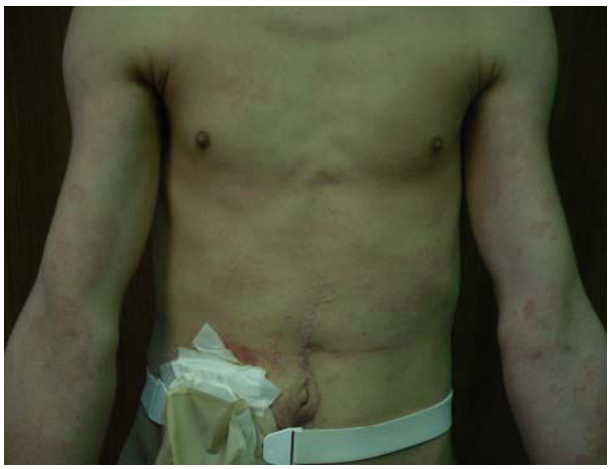

Fig. 9 − Generalized eczema developed during the treatment with adalimumab. A 36-year-old patient with small bowel Crohn's disease treated with adalimumab presented after the 3rd dose with generalized eczema. The patient was treated with systemic corticosteroids and intensive topical corticosteroid and emollient therapy. Skin lesions resolved and biologic therapy was switched on infliximab. At follow-up no further skin lesions developed, and the disease was in stable remission.

also includes patients with vasculitis as a manifestation on the underlying disease. Cutaneous vasculitis usually starts as palpable purpura, hemorrhaagic bullae and skin ulcers can further develop [34]. Erythema nodosum is one of the most common extraintestinal manifestations of IBD, but in IBD patients it was described as a complication of infliximab and certolizumab treatment [2]. Non-melanoma skin cancer develops in 0.3−1.4% on mono anti-TNF therapy [35]. These skin tumors were specific for the application of thiopurine medications but given that in practice the most common use is the combination therapy of thiopurine/anti-TNF, it can sometimes lead to a confusion of which of administered medicationsis a specific cause of these complications.

More recent meta-analysis, including patients with mono application of anti-TNF preparations, have established HR for development of NMSC of 2.02, indicating that these cancers are rare but clearly possible consequence during anti-TNF treatment [12]. It is important to familiarize patients with complications and refer them to implement preventive actions (protection and covering of the skin, avoiding open exposure to UV radiation, regular control), thereby reducing the incidence of NMSC. Regular self-skin examination every month is advisable and dermatological consultation if new or changing lesions appear is necessary. If NMSC is suspected, surgical excision is advised, and there is no need to discontinue the therapy. The risk of lymphoma is also present during anti TNF treatment and HR is approximately 4.4, with or without concomitant use of thiopurine medications and usually manifests itself in the form of systemic lymphoma, without cutaneous manifestations, but one case report of cutaneous lymphoma on infliximab therapy was described [36].

The risk of melanoma in IBD patients and relation to the used anti-TNF medication at least more than 1 year conveyed with almost 2-fold risk for getting this malignancy [37]. Long et al. [38] in a retrospective nested case control study reveal that IBD patients have an increased risk for melanoma, and that therapy with biologics further increases the risk. Risk was significant for Crohn's disease but not for ulcerative colitis [12]. Therefore, regular and rigorous sun protection, self skin examination and dermatologic consultations for new and changing lesions are necessary. Patients on combination therapy have the greatest risk of NMSC with the HR of 5.85 while the HR, in application of only thiopurine medications, is 3.56 and the lowest one in mono therapy with anti-TNF preparations, 2.07 [38]. This clearly indicates the synergistic effect of a combination, where the risk is particularly increased with thiopurine combination with corticosteroids or anti-TNF medication. Similar associations were seen in the incidence of skin lymphoma in IBD population. In thiopurine monotherapy the risk of developing cutaneous lymphoma is 1.4, in anti-TNF monotherapy 1.7, but in combination with anti-TNF preparations the risk is substantially increased and the HR is 6.6. [39]. Contrary to these published results are those from study of Soh et al. [40] where the most significant observation is that the concomitant use of AZA/6 MP at the time of introducing anti-TNF agents decreases the risk of adverse skin lesions in both univariate ($p = 0.008$) and multivariate ($p = 0.006$) analysis. Moreover, the cumulative probability of the incidence of adverse skin lesion was the lowest in the group with concomitant use of azathisprine/6 mercaptopurine at the start of anti-TNF treatment. Further studies and careful statistical interpretation of results are necessary to draw the final conclusion. Meanwhile, regular skin check-ups are necessary in these patients.

What gastroenterologists have to know?

Gastroenterologists are not trained to identify early premalignant skin lesions or even NMSC and are not trained to stratify patients based on their risk factors. Evidence based guidelines for primary and secondary prevention are lacking in IBD population. Torres et al. [41] propose the algorithm for all the patients who are starting on azathioprine/anti-TNF in an attempt to avoid the adverse effects of this drug on the skin and to timely recognize skin lesions that may be malignant, and it is presented in Figure 10. It is recommended that all patients starting with immunosuppressive therapy must be examined by the dermatologist, which would reduce the risk of serious skin lesions. At the same time it is recommended that the control and monitoring continues even after cessation of immunosuppressive therapy.

Conclusion

Inducing effective immunosuppression as early as possible is a goal of effective treatment in IBD, since it will beneficially affect the course of the disease and prevent development of severe disease and its complications. But this immunosuppression and immune modulation, could, on the other hand, lead to diverse dermatologic manifestations which could severely affect the quality of life of patients and compromise further treatment. Although some of the cutane-

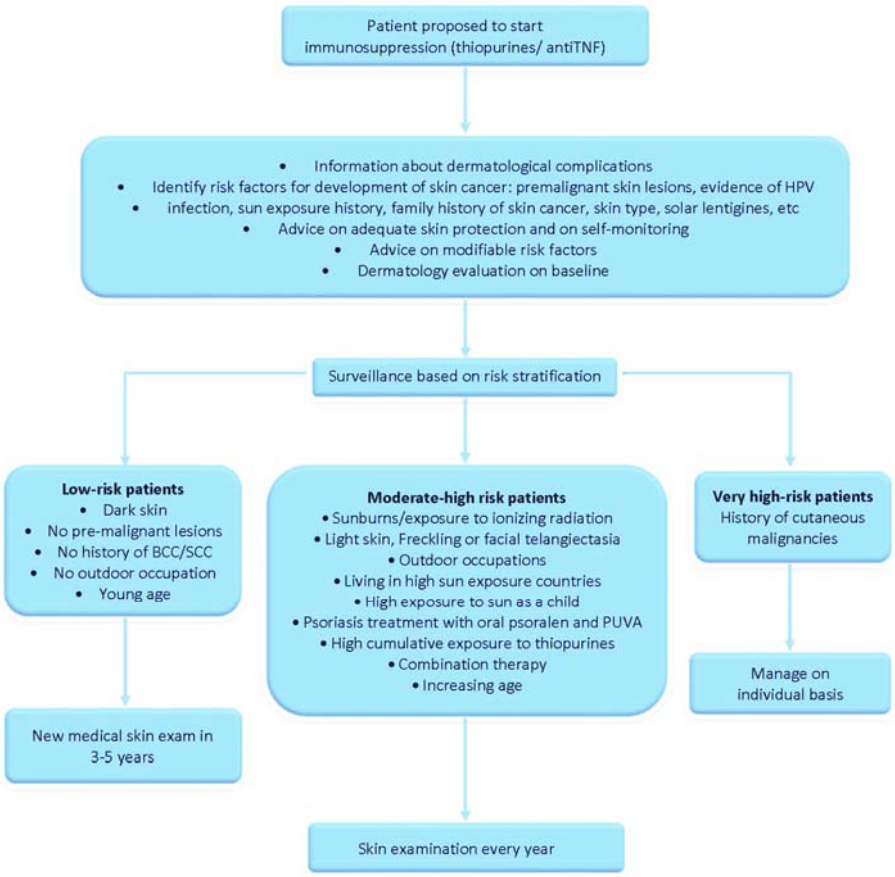

Fig. 10 − Modified algorithm [41] where yearly skin exams were recommended.
TNF – tumor necrosis factor; HPV – human papilloma virus; BCC – basal cell carc inoma; SCC – squamous cell carcinoma; PUVA – psoralen and ultraviolet A.

ous manifestations are not frequent, it is necessary to know their pathogenesis, prevention, and how to treat them.

The most important suggestion is that these changes should be seen and treated by a team, where the role of dermatologist is very important and unavoidable. Recommendations and adequate approach in this field is necessary to be established at the international level and make them available to the physicians from the primary care level to high-end subspecialists.

R E F E R E N C E S

1. *Pearl RK, Nelson RL, Prasad ML, Orsay CP, Abcarian H.* Serious complications of sulfasalazine. Dis Colon Rectum 1986; 29(3): 201−2.
2. *Ward SK, Roenigk HH, Gordon KB.* Dermatologic manifestations of gastrointestinal disorders. Gastroenterol Clin North Am 1998; 27(3): 615−36, vi.
3. *Sartor RB, Sandborn WJ.* Kirsner's Inflammatory Bowel Diseases. 6th ed. New York, NY: Saunders; 2004.
4. *Toruner M, Loftus EV, Harmsen SW, Zinsmeister AR, Orenstein R, Sandborn WJ*, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. Gastroenterology 2008; 134(4): 929−36.
5. *Gupta G, Lautenbach E, Lewis JD.* Incidence and risk factors for herpes zoster among patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2006; 4(12): 1483−90.
6. *Rahier JF, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y,* et al. Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. J Crohns Colitis 2014; 8(6): 443−68.
7. *El-Azhary RA, Brunner KL, Gibson LE.* Sweet syndrome as a manifestation of azathioprine hypersensitivity. Mayo Clin Proc 2008; 83(9): 1026−30.
8. *McNally A, Ibbetson J, Sidhu S.* Azathioprine-induced Sweet's syndrome: A case series and review of the literature. Australas J Dermatol 2015; doi: 10.1111/ajd.12383. (In Press)
9. *de Fonclare AL, Khosrotehrani K, Aractingi S, Duriez P, Cosnes J, Beaugerie L.* Erythema nodosum-like eruption as a manifestation of azathioprine hypersensitivity in patients with inflammatory bowel disease. Arch Dermatol 2007; 143(6): 744−8.
10. *O' Donovan PO, Perrett CM, Zhang X, Montaner B, Xu YZ, Harwood CA*, et al. Azathioprine and UVA light generate mutagenic oxidative DNA damage. Science 2005; 309(5742): 1871−4.
11. *Patel AA, Swerlick RA, McCall CO.* Azathioprine in dermatology: The past, the present, and the future. J Am Acad Dermatol 2006; 55(3): 369−89.
12. *Long MD, Herfarth HH, Pipkin CA, Porter CQ, Sandler RS, Kappelman MD.* Increased risk for non-melanoma skin can-

cer in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2010; 8(3): 268−74.
13. *Peyrin-Biroulet L, Khosrotehrani K, Carrat F, Bouvier AM, Chevaux JB, Simon T,* et al. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. Gastroenterology 2011; 141(5): 1621−8. e1−5.
14. *Guenova E, Lichte V, Hoetzenecker W, Woelbing F, Moehrle M, Roecken M,* et al. Nodular malignant melanoma and multiple cutaneous neoplasms under immunosuppression with azathioprine. Melanoma Res 2009; 19(4): 271−3.
15. *Wolff T, Tai E, Miller T.* Screening for skin cancer: An update of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2009; 150(3): 194−8.
16. *Harrison PV*. Methotrexate-induced epidermal necrosis. Br J Dermatol 1987; 116(6): 867−9.
17. *Maruani A, Wierzbicka E, Machet M, Abdallah-Lotf M, Muret A, Machet L.* Reversal of multifocal cutaneous lymphoproliferative disease associated with Epstein-Barr virus after withdrawal of methotrexate therapy for rheumatoid arthritis. J Am Acad Dermatol 2007; 57(5 Suppl): S69−71.
18. *Giard C, Avenel-Audran M, Croué A, Verret J, Martin L.* Primary cutaneous Epstein-Barr virus-associated B-cell lymphoma arising at the site of subcutaneous injections of methotrexate. J Clin Oncol 2010; 28(34): e717−8.
19. *Buchbinder R, Barber M, Heuzenroeder L, Wluka AE, Giles G, Hall S*, et al. Incidence of melanoma and other malignancies among rheumatoid arthritis patients treated with methotrexate. Arthritis Rheum 2008; 59(6): 794−9.
20. *Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G,* et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med 1994; 330(26): 1841−5.
21. *Krupp P, Monka C.* Side-effect profile of cyclosporin A in patients treated for psoriasis. Br J Dermatol 1990; 122(Suppl 36): 47−56.
22. *Colombel J, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R*, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: The CHARM trial. Gastroenterology 2007; 132(1): 52−65.
23. *Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J*, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005; 353(23): 2462−76.
24. *Kerbleski JF, Gottlieb AB.* Dermatological complications and safety of anti-TNF treatments. Gut 2009; 58(8): 1033−9.
25. Ramos-Casals M, Brito-Zerón P, Soto MJ, Cuadrado MJ, Khamashta MA. Autoimmune diseases induced by TNF-targeted therapies. Best Pract Res Clin Rheumatol 2008; 22(5): 847−61.
26. *Gonnet-Gracia C, Barnetche T, Richez C, Blanco P, Dehais J, Schaeverbeke T.*Anti-nuclear antibodies, anti-DNA and C4 complement evolution in rheumatoid arthritis and ankylosing spondylitis treated with TNF-alpha blockers. Clin Exp Rheumatol 2008; 26(3): 401-7.
27. *Fiorino G, Danese S, Pariente B, Allez M.* Paradoxical immune-mediated inflammation in inflammatory bowel disease patients receiving anti-TNF-α agents. Autoimmun Rev 2014; 13(1): 15−9.
28. *Tillack C, Ehmann LM, Friedrich M, Laubender RP, Papay P, Vogelsang H,* et al. Anti-TNF antibody-induced psoriasiform skin lesions in patients with inflammatory bowel disease are characterised by interferon-γ-expressing Th1 cells and IL-17A/IL-22-expressing Th17 cells and respond to anti-IL-12/IL-23 antibody treatment. Gut 2014; 63(4): 567−77.
29. *Ettler J, Wetter DA, Pittelkow MR.* Pityriasis amiantacea: A distinctive presentation of psoriasis associated with tumour necrosis factor-α inhibitor therapy. Clin Exp Dermatol 2012; 37(6): 639−41.
30. *Seneschal J, Lepreux S, Milpied B, Schaeverbeke T, Taïeb A.* Psoriasiform eruptions during anti TNF-alpha treatment: Psoriasis or not. Arch Dermatol 2007; 143(12): 1593−5.
31. *Ko JM, Gottlieb AB, Kerbleski JF.* Induction and exacerbation of psoriasis with TNF-blockade therapy: A review and analysis of 127 cases. J Dermatolog Treat 2009; 20(2): 100−8.
32. *Włodarczyk M, Sobolewska A, Wójcik B, Loga K, Fichna J, Wiśniewska-Jarosińska M.* Correlations between skin lesions induced by anti-tumor necrosis factor-α and selected cytokines in Crohn's disease patients. World J Gastroenterol 2014; 20(22): 7019−26.
33. *El Shabrawi-Caelen L, La Placa M, Vincenzi C, Haidn T, Muellegger R, Tosti A.* Adalimumab-induced psoriasis of the scalp with diffuse alopecia: A severe potentially irreversible cutaneous side effect of TNF-alpha blockers. Inflamm Bowel Dis 2010; 16(2): 182−3.
34. *Jarrett SJ, Cunnane G, Conaghan PG, Bingham SJ, Buch MH, Quinn MA*, et al. Anti-tumor necrosis factor-alpha therapy-induced vasculitis: Case series. J. Rheumatol 2003; 30(10): 2287−91.
35. *Devos SA, Van Den Bossche N, De Vos M, Naeyaert JM.* Adverse skin reactions to anti-TNF-alpha monoclonal antibody therapy. Dermatology (Basel) 2003; 206(4): 388−90.
36. *Mariette X, Tubach F, Bagheri H, Bardet M, Berthelot JM, Gaudin P*, et al. Lymphoma in patients treated with anti-TNF: Results of the 3-year prospective French RATIO registry. Ann Rheum Dis 2010; 69(2): 400−8.
37. *Singh S, Nagpal SJ, Murad MH, Yadav S, Kane SV, Pardi DS,* et al. Inflammatory bowel disease is associated with an increased risk of melanoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2014; 12(2): 210−8.
38. *Long MD, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD.* Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. Gastroenterology 2012; 143(2): 390−9.e1.
39. *Siegel CA, Marden SM, Persing SM, Larson RJ, Sands BE.* Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: A meta-analysis. Clin Gastroenterol Hepatol 2009; 7(8): 874−81.
40. *Soh JS, Yun WJ, Kim K, Won CH, Park SH, Yang D,* et al. Concomitant use of azathioprine/6-mercaptopurine decreases the risk of anti-TNF-induced skin lesions. Inflamm Bowel Dis 2015; 21(4): 832−9.
41. *Torres J, Buche S, Delaporte E, Colombel JF.* Skin side effects of inflammatory bowel disease therapy. Inflamm Bowel Dis 2013; 19(5): 1086−98.

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CASE REPORTS

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Intravenous lipid emulsion in treatment of cardiocirculatory disturbances caused by glyphosate-surfactant herbicide poisoning

Primena intravenske emulzije masti u lečenju kardiocirkulatornih poremećaja prouzrokovanih trovanjem glifosat-surfaktant herbicidom

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Abstract

Introduction. Glyphosate is the first widely used herbicide against weed in genetically modified crops. Though glyphosate itself has a low toxicity, commercial products are more dangerous because of increased toxicity due to surfactants addition. There is no specific antidote for the poisoning with glyphosate-surfactant (Gly-SH). In recent times, the efficacy of intravenous lipid emulsion (ILE) administration for the treatment of acute poisoning caused by Gly-SH has been investigated. **Case Report.** A 50-year-old man was admitted 3 h after self-poisoning with herbicide containing glyphosate and polyoxyethyleneamine, as a surfactant. On admission, the patient was in a coma, hypotensive (80/50 mmHg) and without spontaneous breathing. Electrocardiogram showed wide-complex tachycardia, and arterial blood gas (ABG) revealed acidosis (pH 7.07). Conventional treatment included mechanical ventilation, intravenous fluids, bicarbonate and dopamine. As there was no improvement, ILE was started. The patient received 100 mL of 20% Intralipid® bolus followed by infusion of 400 mL over 20 minutes. Prior to expiration of infusion, a gradual rise in blood pressure was noted, and within 2 hours sinus rhythm was restored. **Conclusion.** This case report suggests that the use of ILE may be an additional option for the treatment of cardiocirculatory disturbances caused by commercial products of glyphosate herbicide.

Key words:
herbicides; poisoning; fat emulsions, intravenous; cardiovascular system; treatment outcome.

Apstrakt

Uvod. Glifosat je prvi herbicid namenjen za uništavanje korova na genetski modifikovanim usevima. Sam glifosat je malo toksičan, ali komercijalni proizvodi sadrže i surfaktante koji povećavaju njegovu otrovnost. Za lečenje trovanja ovim preparatima ne postoji specifičan antidot, a u novije vreme se ispituje mogućnost primene intravenskih lipidnih emulzija (ILE). **Prikaz bolesnika.** Bolesnik, star 50 godina, primljen je 3 časa nakon samotrovanja preparatom koji sadrži glifosat i surfaktant polioksietilenamin. Na prijemu je bio u stanju kome, bez spontanog disanja, hipotenzivan 80/50 mmHg, sa tahikardijom širokih kompleksa na elektrokardiogramu. Analize arterijske krvi pokazale su postojanje acidoze (pH 7.07). Primenjena je konvencionalna terapija koja je uključivala mehaničku ventilaciju, intravensku primenu tečnosti, bikarbonata i dopaminsku stimulaciju. Kako nije dovela do popravljanja hemodinamskih poremećaja, primenjen je Intralipid® 20%, 100 mL u bolusu, a zatim 400 mL u infuziji tokom 20 minuta. Pre isteka infuzije došlo je do postepenog porasta krvnog pritiska, a u roku od 2 sata i uspostavljanja sinusnog ritma. **Zaključak.** Prikaz ovog bolesnika potvrđuje da bi primena intravenskih emulzija masti mogla biti dodatna terapijska opcija u lečenju kardiocirkulatornih poremećaja prouzrokovanih trovanjem komercijalnim preparatima herbicida glifosata.

Ključne reči:
herbicidi; trovanje; masne emulzije, intravenske; kardiovaskularni sistem; lečenje, ishod.

Introduction

Glyphosate is in focus of not only professionals, but also the general population, because it is the first herbicide against which crops have been genetically modified to increase their tolerance. It is the one of the most widely used general-purpose herbicide in the world. Animal experiments have shown that glyphosate is a relatively low toxic herbicide [1], but it turned out that commercial preparations containing it could cause severe poisoning with fatal outcome in humans [2, 3]. These products are commonly available as glyphosate-surfactant (Gly-SH) mixture, made of glyphosate in the form of isopropylamine salt dissolved

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in surfactants which seem to significantly contribute to the acute toxicity of formulations [4].

Clinical picture of severe Gly-SH poisoning usually includes irritant, or even corrosive lesions on the gastrointestinal tract, hypotension refractory to fluid resuscitation, inotropes or vasopressors, cardiac arrhythmias, respiratory distress, impaired consciousness, hepatic injury, renal failure, metabolic acidosis and hyperkaliemia [5, 6].

Treatment of glyphosate-surfactant toxicity is mainly supportive. In recent years, potential therapeutic effects of intravenous lipid emulsion (ILE) in the patients with acute Gly-SH poisoning has been examined [7]. We report the unique case of acute Gly-SH poisoning with refractory wide complex tachycardia and hypotension successfully treated with ILE.

Case report

A 50-year-old man ingested 250 mL of herbicide which contained 48% glyphosate as an isopropylamine salt dissolved in polyoxyethyleneamine surfactant and water. He phoned the ambulance shortly after, so he was brought to local hospital. The patient was alert and agitated at presentation one hour post-ingestion. However, his condition rapidly deteriorated. He became drowsy, dyspnoeic and hypotensive with blood pressure of 80/40 mmHg. Despite the supportive treatment with intravenous fluids, his blood pressure decreased to undetectable level. The patient was intubated, infusion of dopamine was initiated and after consultation with the National Poison Control Centre in the Military Medical Academy in Belgrade, he was transferred to the toxicology ward of the Centre.

On admission, 3 hours after herbicide ingestion, the patient was in a coma, with a Glasgow coma scale score of 3. His pupils were dilated and unresponsive. He had no spontaneous breathing, and blood pressure was 80/50 mmHg. Electrocardiogram revealed wide complex rhythm (Figure 1). The results of the arterial blood gas analysis were as follows: pH 7.07; PaO2 53 mmHg, $PaCO_2$ 27 mmHg, lactates 7.2 mol/L and bicarbonates 7.8 mmol/L. Complete blood count, serum electrolyte concentrations and other chemistry tests results were within the normal range. A chest radiograph showed normal findings.

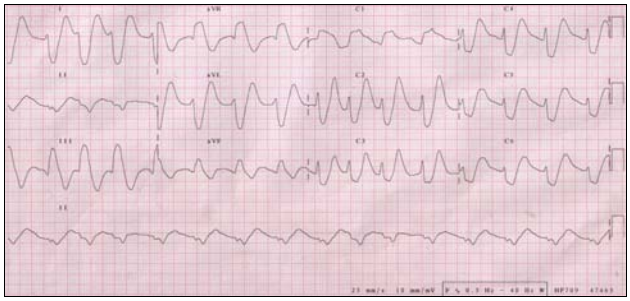

Fig. 1 – Electrocardiogram (ECG) on admission at 11.50 h.

The patient was admitted into intensive care unit and placed on mechanical ventilation. Despite fluid resuscitation, sodium bicarbonate administration and continuous infusion of dopamine (10 μg/kg/min), the patient remained comatose and hypotensive.

Because the patient responded poorly to conventional therapy, about 2.5 h after admission ILE was started. He received 100 mL of 20% Intralipid® (ILE) bolus followed by an infusion of 400 mL over 20 minutes. At a moment when ILE was initiated, the patient's blood pressure was 70/40 mmHg, and there was no improvement of ECG (Figure 2). Elevation of blood pressure was noticed about 15 min after the start of infusion. Within following 2 h sinus rhythm was re-established (Figure 3), blood pressure reached the value of 120/75 mmHg and the patient started to response to stimuli. He remained stable, so dopamine infusion was ceased. Seven hours post-admission the patient was alert, with normal clinical findings except for sore throat and incipient mild left lung pneumonia. After 2 days the patient was transferred to the psychiatric ward for further psychiatric treatment.

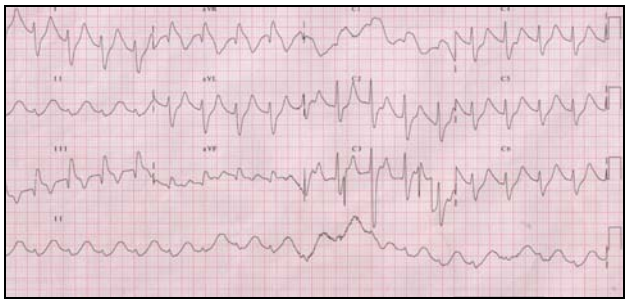

Fig. 2 – Electrocardiogram (ECG) after bicarbonate and before intravenous lipid emulsion (ILE) at 14.15 h.

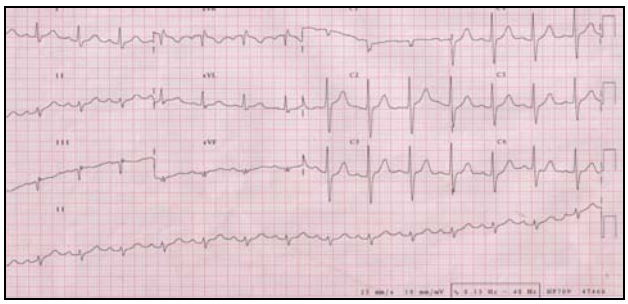

Fig. 3 – Electrocardiogram (ECG) after intravenous lipid emulsion (ILE) at 15.52 h.

Discussion

In massive ingestion of Gly-SH herbicide fatal outcome may occur despite early recognition of severity of the poisoning and intensive treatment. On the basis of published cases, it can be concluded that after large ingestion of Gly-SH, marked sedation, refractory hypotension, respiratory failure, metabolic acidosis, dysrhythmia, elevated creatinine, and hyperkalemia are important risk factors for fatal outcome [8]. In our patient, many of those factors were present, including coma, respiratory failure, metabolic acidosis, refractory hypotension and wide complex tachycardia.

The mainstay of treatment for Gly-SH toxicity is decontamination, supportive and symptomatic therapy. Our patient was admitted 3 hours after the herbicide ingestion, in critical condition, so gastric lavage was not indicated for decontamination. Haemodialysis may be effective as a method of decontamination in GlySH poisoning [9] and could be performed even before acute renal failure developed [10]. However, this type of treatment could add to hypotension and thus be very hazardous. In our case,

haemodialysis was not considered because the patient was haemodynamically instable and had life-threatening arrhythmia. Conventional treatment of hypotension with dopamine in our patient was not effective, as there was no elevation of blood pressure and urine output. Sodium bicarbonate was indicated for two reasons – correction of acidosis and treatment of arrhythmia. As there was no effect on cardiac rhythm, ILE was used as a rescue therapy. Before the second hour from ILE application expired, sinus rhythm was re-established and haemodynamic stability was achieved. As the result, the patient's condition improved and further complications, like renal failure, were thus prevented.

In recent years, ILE has been demonstrated to be effective in treatment of cardiocirculatory toxic effect caused by many liposoluble agents [11, 12]. Its beneficial effect is first and foremost explained by "lipid sink theory" suggesting that a lipophilic agent may be shifted from tissue and captured by an extended lipid compartment in the blood [13]. Except for that mechanism, ILE may recover cardiac contractility by increasing fatty acid content and calcium level in cardiomyocytes [14, 15]. Experimental and anecdotal evidence indicated the lipid solubility of toxic agent may be a crucial factor in determining the efficacy of ILE. Glyphosate is a water-soluble compound with low solubility in lipids, and it is unlikely that the "lipid sink" mechanism acts on the toxicity of glyphosate itself. On the other hand, the first well documented case of life saving effect of a lipid emulsion in a patient with refractory hypotension caused by Gly-SH herbicide was published in 2010 [16]. Authors explanation was that commercial product contained non-ionic surfactant polyoxyethylene amine (POEA) which contributed to the toxicity and was capable of solubilising fats, so the whole mixture was liposoluble.

ILE was for the first time introduced for the treatment of cardiotoxic effects caused by bupivacaine [17], local anaesthetic able to generate depression of cardiac conduction by blocking the fast inward sodium channels in myocardium [18]. Wide complex tachycardia is usually caused by the same mechanism. In the present case, like in the case of propranolol cardiotoxicity we had reported earlier, [19] ILE was effective in abolition of this type of arrhythmia.

Conclusion

This case suggests that intravenous lipid emulsion could be a new, additional option for treatment of acute poisoning by commercial products of glyphosate-surfactant herbicide, characterized by haemodynamic and heart rhythm disturbances.

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REFERENCES

1. *European Comission. Health & Consumer Protection Directorate-General.* Review report for the active substance glyphosate. 2002. Available from: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf
2. *Talbot AR, Shiaw MH, Huang JS, Yang SF, Goo TS, Wang SH,* et al. Acute poisoning with a glyphosate-surfactant herbicide ('Roundup'): a review of 93 cases. Hum Exp Toxicol 1991; 10(1): 1−8.
3. *Stella J, Ryan M.* Glyphosate herbicide formulation: a potentially lethal ingestion. Emerg Med Australas 2004; 16(3): 235−9.
4. *Sawada Y, Nagai Y, Ueyama M, Yamamoto I.* Probable toxicity of surface-active agent in commercial herbicide containing glyphosate. Lancet 1988; 331(8580): 299−300.
5. *Bradberry SM, Proudfoot AT, Vale J.* Glyphosate poisoning. Toxicol Rev 2004; 23(3): 159−67.
6. *Potrebic O, Jovic-Stosic J, Vucinic S, Tadic J, Radulac M.* Acute glyphosate-surfactant poisoning with neurological sequels and fatal outcome. Vojnosanit Pregl 2009; 66(9): 758−62. (Serbian)
7. *Gil H, Park J, Park S, Hong S.* Effect of intravenous lipid emulsion in patients with acute glyphosate intoxication. Clin Toxicol 2013; 51(8): 767−71.
8. *Lee C, Shih C, Hsu K, Hung D, Lin C.* The early prognostic factors of glyphosate-surfactant intoxication. Am J Emerg Med 2008; 26(3): 275−81.
9. *Garlich FM, Goldman M, Pepe J, Nelson LS, Allan MJ, Goldstein DA,* et al. Hemodialysis clearance of glyphosate following a life-threatening ingestion of glyphosate-surfactant herbicide. Clin Toxicol (Phila) 2014; 52(1): 66−71.
10. *Moon JM, Min YI, Chun BJ.* Can Early Hemodialysis Affect the Outcome of the Ingestion of Glyphosate Herbicide. Clin Toxicol (Phila) 2006; 44(3):329−32.
11. *Cave G, Harvey M, Graudins A.* Intravenous lipid emulsion as antidote: a summary of published human experience. Emerg Med Australas 2011; 23(2): 123−41.
12. *Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, Chauny J.* Lipid emulsions in the treatment of acute poisoning: a systematic review of human and animal studies. Clin Toxicol 2010; 48(1): 1−27.
13. *Weinberg G.* Lipid rescue resuscitation from local anaesthetic cardiac toxicity. Toxicol Rev 2006; 25(3): 139−45.
14. *van de Velde M, Wouters PF, Rolf N, Van AH, Flameng W, Vandermeersch E.* Long-chain triglycerides improve recovery from myocardial stunning in conscious dogs. Cardiovasc Res 1996; 32(6): 1008−15.
15. *Huang JM, Xian H, Bacaner M.* Long-chain fatty acids activate calcium channels in ventricular myocytes. Proc Natl Acad Sci USA 1992; 89(14): 6452−6.
16. *Han SK, Jeong J, Yeom S, Ryu J, Park S.* Use of a lipid emulsion in a patient with refractory hypotension caused by glyphosate-surfactant herbicide. Clin Toxicol 2010; 48(6): 566−8.
17. *Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ.* Pretreatment or Resuscitation with a Lipid Infusion Shifts the Dose-Response to Bupivacaine-induced Asystole in Rats. Anesthesiology 1998; 88(4): 1071−5.
18. *Clarkson CW, Hondeghem LM.* Mechanism for bupivacaine depression of cardiac conduction: fast block of sodium channels during the action potential with slow recovery from block during diastole. Anesthesiology 1985; 62(4): 396−405.
19. *Jovic-Stosic J, Gligic B, Putic V, Brajkovic G, Spasic R.* Severe propranolol and ethanol overdose with wide complex tachycardia treated with intravenous lipid emulsion: A case report. Clin Toxicol 2011; 49(5): 426−30.

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CASE REPORT

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Case report of Mikulicz`s disease – a modern concept of an old entity

Prikaz bolesnika sa Mikuličevom bolesti – savremeni koncept starog entiteta

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Abstract

Introduction. Modern knowlegde defines Mikulicz´s disease as a part of immunoglobulin G4-related disease. The main feature is the presence of lymphoplasmacytic infiltrates, immunoglobulin G4 plasma cells positivity, distinctive storiform fibrosis and moderate eosinophilia. **Case report.** A 59-years old male presented with a mild keratoconjuctivitis sicca and enlarged lacrimal and salivary glands during the last two years. Althought clinical presentation of the patient was typical, earlier testing did not pinpoint Mikulicz´s disease. By typical clinical presentation, elevated serum immunoglobulin G4 level and histopathological finding of lacrimal glands tissue we diagnosed Mikulicz´s disease successfully treated with corticosteroid therapy. **Conclusion.** We reported the first case of IgG4-related Mikulicz´s disease in Serbia. Our report highlights IgG4-related Mikulicz`s disease as an important differential diagnosis with Sjögren`s syndrome and lymphoproliferative disease in rheumatological practice.

Key words:
mikulicz' disease; diagnosis, differential; diagnosis; lacrimal apparatus; salivary glands; immunoglobulin G; histological techniques; glucocorticoids; treatment outcome.

Apstrakt

Uvod. Savremena saznanja svrstala su Mikuličevu bolest u grupu bolesti posredovanih imunoglobulinom G4, čija je glavna odlika histološki nalaz limfoplazmocitnih infiltrata, imunoglobulin G4 pozitivnih plazma ćelija, uz storiformnu fibrozu i umerenu eozinofiliju. **Prikaz bolesnika.** Prikazan je 59-godišnji bolesnik koji je dve godine imao umeren suvi keratokonjuktivitis i uvećane pljuvačne i suzne žlezde. Iako je klinička slika bila karakteristična, ranijim ispitivanjem nije se došlo do dijagnoze Mikuličeve bolesti. Na osnovu tipičnog kliničkog nalaza, visoke serumske koncentracije imunoglobulina G4, uz patohistološki nalaz biopsije tkiva suznih žlezda, dijagnostikovali smo Mikuličevu bolest. Primenjena terapija kortikosteroidima bila je efikasna. **Zaključak.** Prikazali smo prvog bolesnika u Srbiji sa Mikuličevom bolesti posredovanim imunoglobulinom G4. Našim prikazom istakli smo značaj poznavanja Mikuličeve bolesti posredovane IgG4, kao i diferencijalnu dijagnozu sa Sjögrenovim sindromom i limfoproliferativnim bolestima u reumatološkoj praksi.

Ključne reči:
mikuličeva bolest; dijagnoza, diferencijalna; dijagnoza; suzni aparat; pljuvačne žlezde; IGG; histološke tehnike; glukokortikoidi; lečenje, ishod.

Introduction

Immunoglobulin G4 (IgG4)-related diseases (IgG4-RD) are new clinical entity of fibro-inflammatory conditions, characterized by the tendency to form tumorous lesions, dense lymphoplasmacytic infiltration abudant of IgG4-positive plasma cells and storiform fibrosis in relevant organs and often, but not always, elevated serum IgG4 levels [1]. The concept is based on the discovery of increased serum IgG4 levels in patients with sclerosing pancreatitis [2]. IgG4-RD can affect various organs, pancreas more often than the others as well as hepatobiliary tract, salivary and lacrimal glands, orbits and lymph nodes.

Very little data exists on the incidence and prevalence of IgG4-RD. Most epidemiological studies come from Japan and they are focused on autoimmune pancreatitis. It was estimated that the incidence of new cases with IgG4-RD is 2.63–10.2/million, with newly diagnosed 336–1,300 patients *per* year [3].

Mikulicz´s disease (MD) is characterized by symmetrical, painless enlargement of lacrimal, parotid and submandibular salivary glands. Based on histological similarites, this disease was considered for a long time as a subtype of Sjögren´s syndrome (SS). A recent research of Japanese authors has discovered increased serum IgG4 levels in pati-

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ents with MD and defined histopathological findings in glands tissue, presenting MD as part of IgG4-RD [4].

Today, the focus of interest is how to differentiate the diagnosis of MD and SS. MD is usually observed in patients older than 50 years, both males and females. In spite of the persistent swelling of salivary and lacrimal glands, their function is not significantly reduced. The number of positive antinuclear antibody (ANA) is small, while anti-Ro and anti-La antibodies are negative. Patients with SS are mostly females around 50 years old. Although gland swellings in SS are intermittent, *keratoconjunctivitis sicca* is present. Serum tests in most patients show positive ANA, 70% of them have anti-Ro and 30% anti-La antibodies. High serum level of IgG4 is specific for MD, but is usually not seen in SS. The basics for MD diagnosing is a histopathological feature. Although the lymphocytic infiltrates are typical for MD and SS, their influence is different. In MD lymphoid follicules are placed around the duct protecting it while in SS they create lymphoepithelial lesions and destroy the duct. This explains less frequent *keratoconjunctivitis sicca* in MD in spite of significant gland swellings. Infiltration of IgG4 positive plasma cells is the main difference between MD and SS. The ratio of IgG4 positive cells to IgG positive cells is higher than 40%. Steroid therapy is efficient in MD, but partially in SS. In spite of certain clinical similarities, MD and SS are two different diseases [5, 6].

We presented the first patient with proven IgG4-related MD in our country.

Case report

A 59-year-old man was admitted to our hospital due to chronic dacryoadenitis and sialadenitis, with suspicion of the existence of lymphoproliferative disease. The patient had dry mouth and painless swelling in the area of parotid salivary glands during two years. A year before hospitalization he noticed swollen eyelids. His other problem was nasal obstruction. The diagnosis of *keratoconjunctivitis sicca* was made in other hospital a year before. There was no evidence for the existence of SS (Schirmer`s test was 7 and 8 mm; scintigraphy showed mildly reduced accumulative and excretory function of salivary glands; ANA, anti-Ro and anti-La antibodies were negative; ultrasound showed enlarged parotid and submandibular salivary glands; biopsy of the minor labial salivary gland was negative). The computed tomography (CT) scan showed enlarged lacrimal glands more on the left side (Figure 1). Biopsy of the both glands was performed. The results showed chronic dacryoadenitis followed by significant polyclonal proliferation of the plasmocites. Lymphoproliferative disease was suspected.

On admission the patient was obese, body mass index (BMI) of 34 kg/m^2 (normal range 18.5–25 kg/m^2), with enlarged, painless parotid, submandibular and lacrimal glands (Figure 2).

Blood tests showed erythrocytes sedimentation rate (SER) of 70 mm/h (the normal range is 0–22 mm/h form men), C-reactive protein (CRP) of 7.61 mg/L (normal range: less than 10 mg/L), polyclonal hipergamaglobulinemia IgG of 28.3 g/L (normal range: 5–16 g/L) and IgE of 1,060 UI/mL. Serological markers including ANA, anti-Ro, anti-La and RF were negative. The serum IgG4 level was 15.7 g/L (normal range: 0–1.3 g/L). Inflammatory pseudotumor of the lacrimal gland with signs of storiform fibrosis revealed after histopathological and immunohistological analysis. The IgG4 positive plasma cells infiltration was enormous, once microscopically enlarged where ratio IgG4+/ IgG+ was at le-

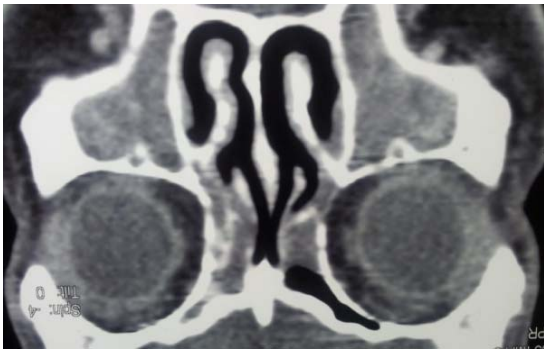


Fig. 1 – Computed tomography (CT) images reveal enlargement of the lacrimal glands.

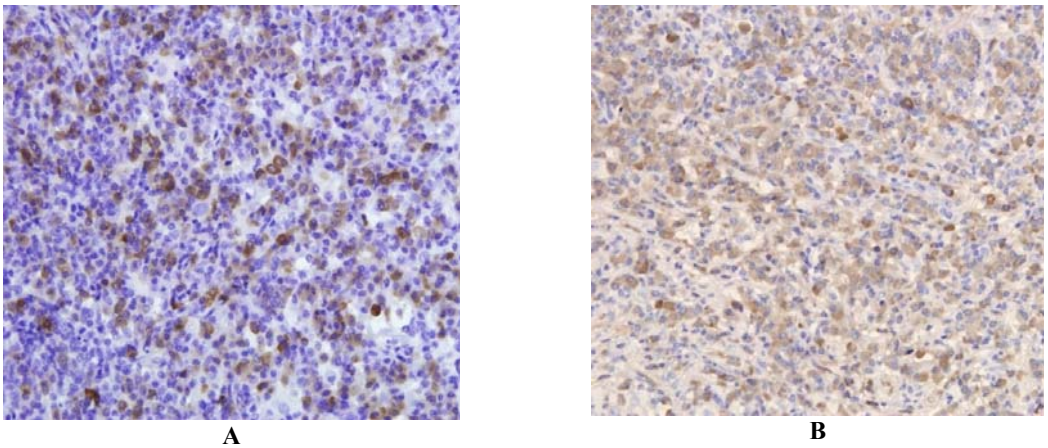
A **B**

Fig. 2 – A) Dacryoadenitis manifests as bilateral swelling of upper eyelieds, and B) Sialadenitis manifests as bilateral swelling of parotid regions in the same patient.

ast 40% (Figure 3). There was no histological criteria for a lymphoproliferative neoplasm.

Additional tests were done including myelogram and analysis of clonality in bone marrow lymphocytes. Using polymerase chain reaction method, no lymphoproliferative disease was found.

On the seventh hospital day, the patient showed signs of compressive, peripheral paresis of the left facial nerve. It was the reason for urgent corticosteroid therapy with prednisone 0.5 mg/kg/day. Two weeks later, swelling disappeared completely and the facial nerve recovered its function. The corticosteroid dosage was tapering gradually 2.5 mg *per* week. After a 3-mount follow-up, the patient was still without any complaints. He continued to receive prednisolone 10 mg *per* day as maintenance therapy.



A **B**

Fig. 3 – Histological findings of lacrimal gland biopsies. Immunohistochemical staining showing: A) IgG4-positive plasma cells, and B) IgG-positive plasma cells in the lacrimal gland section of the patient (×200).

Discussion

By the end of the 19th century, Johan von Mikulicz-Radecki described a patient with symmetrical swellings of the lacrimal, submandibular and parotid glands with massive infiltration of the glands by mononuclear cells [7]. Later, these clinical features were observed in patients with tuberculosis, sarcoidosis and lymphomas [8]. Schafer and Jacobsen [9] formed a group of patients with typical clinical features known as Mikulicz`s syndrome. In 1933, Henrik Sjögren [10] described histopathological lymphocyte infiltrates in salivary gland of patients with *keratoconjunctivitis sicca* and swollen main salivary glands. But his findings were forgotten until the mid 20th century, when Morgan and Castelman observed that salivary glands tissues in MD and SS are similar [11]. Since then, MD is considered as a subtype of SS chronical form of dacryoadenitis and sialadenitis of an autoimmune etiology [11].

The research of Japanese authors in the 21st century shows elevated serum IgG4 levels and histopathologically abundant infiltration of IgG4 positive plasma cells in lacrimal and salivary glands of patients with MD. This is the creation of a modern clinical concept clearly differentiating MD from SS, putting MD to the group of IgG4-RD [12]. The Japanese Society for Sjögren`s Syndrome has published diagnostic criteria for IgG4-related MD. According to them, IgG4-related MD defines with persistent (longer than 3 months) symmetrical swellings of at least 2 pairs of lacrimal, parotid or submandibular glands, elevated serum IgG4 levels (> 135 mg/dL) or histopathologically marked infiltration of IgG4 positive plasma cells with a ratio of IgG4 /IgG > 40%, with typical tissue fibrosis or sclerosis [13]. So, patients with swelling of salivary and lacrimal glands, elevated serum IgG4 levels and significant infiltration of IgG4 positive plasma cells in lacrimal gland tissue fulfil all the criteria for IgG4-related MD.

The clonality of lymphocytes is necessary to be tested due to histopathological similarities between IgG4-related MD and lymphoma. In the early diagnosing of B-cell lymphomas, they are predominantly represented by B-cell infiltrates unlike primary T-cell infiltrates in IgG4- RD [14].

Allergic rhinitis and bronchial asthma are more frequent in MD than in SS. A high occurrence of allergic conditions is explained by domination of type 2 helper T (Th2) cells immune response, which raises the concentration of IgG4 and IgE [15].

Corticosteroids are the standard first-line treatment for IgG4-related MD. They rapidly reduce swellings of lacrimal and salivary glands, recover their functions and reduce IgG4 concentration in serum [16].

A number of patients with IgG4-related MD was described in Japan with just a few individual cases in the western world. In spite of low prevalence of IgG4-related MD, it is necessary to have it in mind when dealing with patients presenting with swollen lacrimal and salivary glands.

Conclusion

In suspicion for Mikulicz´s disease serum levels of immunoglobulin G4 as well as biopsies from glands with immunohistochemical evaluation should be assessed. It is nessesery to distinguish immunoglobulin G4-related Mikulicz´s disease from other distinct disorders, including Sjögren´s syndrome and lymphoproliferative disease. Therapy with corticosteroids is efficient and recommended for a longer period of time.

R E F E R E N C E S

1. *Khosroshahi A, Stone JH*. A clinical overview of IgG4-related systemic disease. Curr Opin Rheumatol 2011; 23(1): 57−66.
2. *Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T,* et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001; 344(10): 732−8.
3. *Hamano H, Arakura N, Muraki T, Ozaki Y, Kiyosawa K, Kawa S*. Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. J Gastroenterol 2006; 41(12): 1197−205.
4. *Yamamoto M, Takahashi H, Sugai S, Imai K*. Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy). Autoimmun Rev 2005; 4(4): 195−200.
5. *Guma M, Firestein GS*. Best Practice. Res Clin Rheumatol 2012; 26(4): 425−38.
6. *Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H*, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol 2006; 16(6): 335−40.
7. *Mikulicz JH*. Uber eine eigenartige symmetrische Erkrankung der Tranen- und Mundspeicheldrusen. Stuttgart: Beitr Chir Fortschr; 1892.
8. *Napp O*. Über die Beziehungen der Mikuliczschen Erkrankung Tuberkulose. Stschr Augenheilk 1907; 17: 513.
9. *Schaffer AJ, Jacobsen AW*. Mikulicz's syndrome: a report of ten cases. Am J Dis Child 1927; 34(3): 327−46.
10. *Sjögren HS*. Zur kenntnis der keratoconjunctvitis sicca (Keratitis folliformis bei hypofunktion der tranendrusen). Acta Opthalmol (Copenh) 1933; 2: 1−151.
11. *Morgan WS*. The probable systemic nature of Mikulicz's disease and its relation to Sjögren's syndrome. N Engl J Med 1954; 251(1): 5−10.
12. *Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T,* et al. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. Mod Rheumatol 2012; 22(1): 1−14.
13. *Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T,* et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. Mod Rheumatol 2012; 22(1): 21−30.
14. *Stone JH, Zen Y, Deshpande V*. IgG4-related disease. N Engl J Med 2012; 366(6): 539−51.
15. *Masaki Y, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M,* et al. Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis 2009; 68(8): 1310−5.
16. *Khosroshahi A, Stone JH*. Treatment approaches to IgG4-related systemic disease. Curr Opin Rheumatol 2011; 23(1): 67−71.

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C A S E R E P O R T

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Color Doppler imaging features in patients presenting central retinal artery occlusion with and without giant cell arteritis

Karakteristike kolor dopler snimanja kod bolesnika sa okluzijom centralne retinalne arterije sa i bez arteritisa džinovskih ćelija

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Abstract

Introduction. Central retinal artery obstruction (CRAO) represents an abrupt diminution of blood flow through the CRA that is severe enough to cause ischemia of the inner retina with permanent unilateral visual loss. We presented the role of color Doppler imaging (CDI) of orbital vessels and of extracranial duplex sonography (EDS) in the etiological diagnosis of CRAO in two patients with clinical suspicion of unilateral CRAO. **Case report.** Patients were examined following the protocol which included CDI of orbital vessels and EDS. Both patients had no emboli visible on ophthalmoscopy. The B-scan ultrasound evaluation of the first patient found a small round, moderately reflective echo within the right optic nerve, 1.5 mm behind the optic disc (emboli of cholesterol). CDI of retrobulbar vessels revealed the normal right ophthalmic artery (OA) hemodynamic parameters, but the first patient had no arterial flow signal on CDI at the distance of 1.5 mm behind the right optic disc. In contrast, the left eye had the normal aspect on CDI of retrobulbar vessels. The right internal carotid artery EDS identified a severe stenosis at its origin as CRA's emboli source. The second patient had characteristic CDI findings for giant cell arteritis (GCA) with eye involvement: severe diminished blood flow velocities, especially end-diastolic velocities, in both CRAs. Less abnormalities were observed in the posterior ciliary arteries, and in the ophthalmic arteries. The second patient had no systemic symptoms or signs of GCA. **Conclusion.** In the presented cases, the ultrasound investigation enabled prompt differentiation between central retinal artery occlusion of embolic mechanism and CRAO caused by GCA.

Key words:
retinal artery occlusion; ultrasonography, doppler, color; giant cell arteritis; diagnosis, differential.

Apstrakt

Uvod. Opstrukcija centralne retinalne arterije (OCRA) predstavlja naglo smanjenje protoka krvi kroz CRA koje može da izazove ozbiljnu ishemiju unutrašnje retine trajnim jednostranim gubitkom vida. Cilj rada bio je da se proceni uloga kolor dopler snimanja (KDI) orbitalnih sudova i ekstrakranijalne dupleks sonografije (EDS) u etiološkoj dijagnostici OCRA. **Prikaz bolesnika.** Dva bolesnika sa kliničkom sumnjom na jednostrani desni OCRA ispitana su primenom složenog protokola uključujući i KDI orbitalnih sudova. Nisu ustanovljene embolije vidljive na oftalmoskopiji. Na B-sken ultrazvučnoj evaluaciji prvog bolesnika pronađen je mali krug, umereno reflektujući eho unutar desnog očnog živca, 1,5 mm iza optičkog diska. KDI retrobulbarnih krvnih sudova prikazao je normalne hemodinamičke parametre oftalmičke arterije (OA), bez prisustva signala arterijskog protoka na KDI na rastojanju od 1,5 mm iza desnog optičkog diska. Nasuprot tome, levo oko imalo je normalan KDI aspekt retrobulbarnih sudova. Ultrazvučnim pregledom na početnom delu desne *a. carotis intenae* identifikovana je velika stenoza, kao izvor CRA embolije. Drugi bolesnik imao je karakterističan KDI nalaz za arteritis džinovskih ćelija (GCA) sa učešćem oka: visok indeks otpora u svim retrobulbarnim sudovima (sa izrazitim smanjenjem brzine protoka krvi, posebno end-dijastolne brzine, u pogođenoj desnoj CRA). Bolesnik nije imao sistemske simptome, ni znake GCA. **Zaključak.** Ultrazvučna dijagnostika omogućuje brzu orijentaciju u pogledu digitalne dijgnoze između OCRA embolijskog mehanizma nastanka i OCRA izazvane prisustvom GCA.

Ključne reči:
okluzija retinalne arterije; ultrasonografija, dopler, kolor; arteritis, džinovske ćelije; dijagnoza, diferencijalna.

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Introduction

Central retinal artery obstruction (CRAO) is the result of an abrupt diminution of blood flow in the central retinal artery (CRA), severe enough to cause ischemia of the inner retina with permanent unilateral visual loss [1–3]. Frequently, the blockage is located within the optic nerve substance and for this reason, it is generally not visible on ophthalmoscopy [1–3]. We presented the role of color Doppler imaging (CDI) of orbital vessels and extracranial duplex sonography (EDS) in the etiological diagnosis of CRAO in two patients with clinical suspicion of acute unilateral right CRAO.

Case report

Two patients were examined at presentation in our ophthalmology and neurology departments in January 2012 with the following protocol: collection of detailed history of all previous or current systemic diseases, including arterial hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation (AF), valvular diseases, ischemic heart disease, stroke, carotid artery disease, systemic coagulopathies (including thrombophilias), and vasculitis, including giant cell arteritis (GCA); complete physical examination, including the temporal arteries (Tas), was performed by a neurologist and an internist in order to detect eventual temporal arteritis as part of GCA; comprehensive ophthalmic evaluation, conducted by an ophthalmologist by recording visual acuity with the Snellen visual acuity chart, visual fields with a Goldmann perimeter, relative afferent pupillary defect, intraocular pressure, slit-lamp examination of the anterior segment, lens and vitreous, direct ophthalmoscopy, and color fundus photography; laboratory workup, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), factor V Leiden mutation, etc; cranial computed tomography (CT) scanning, in order to identify whether stroke was associated with CRAO; CT-angiography (CT-A), performed at presentation, which allowed analysis of the arterial wall and the endoluminal part of the aorta and its branches; ECG, and transthoracic echocardiography (TTE), to detect eventual cardiac source of emboli; CDI of retrobulbar (orbital) vessels, performed with an ultrasound (US) equipment (Logic 500, GE) with a 9 MHz linear probe for detecting and measuring orbital vessel blood flow in the ophthalmic arteries (OAs), the CRAs, the superior ophthalmic veins, and the posterior ciliary arteries nasal and temporal (PCAs) [4, 5], EDS, performed with an US equipment (My Lab50 Esaote) with a 7.5–10 MHz linear array transducer to determine the carotid source of emboli and with a 10 MHz linear probe for the examination of the TAs. All CDI of retrobulbar vessels and EDS examinations were performed by the first two authors of the study. One investigator, who was unaware of the patients' diagnoses, looked only for detecting and measuring orbital and extracranial vessels blood flow. If their results disagreed, then the investigators would have examined the results together and would have reached a consensus on the findings; temporal artery biopsy (TAB) was assessed at 1 day after presentation when GCA was suspected, for the second case.

Case 1 – Central retinal artery obstruction with embolic mechanism

A 73-year old hypertensive woman presented with sudden and painless visual loss in the right eye. She had visual acuity of 20/20 in her left eye, and saw only hand movements in the right eye. Anterior segment examination was normal in both eyes. The fundus of the affected right eye presented ischemic whitening of the retina, cherry-red spot in the center of the retina, and the site of obstruction of the right CRA was not visible on ophthalmoscopy (no embolus was found).

B-scan ultrasound evaluation found a small round, moderately reflective echo within the right optic nerve, 1.5 mm behind the optic disc. This image suggested a cholesterol structure of the embolus (Figure 1a). CDI of retrobulbar vessels revealed normal right OA hemodynamic parameters, but the patient had no blood flow signal on CDI on the surface of 1.5 mm behind the right optic disc (Figure 1b). The arterial flow signal stopped at the level of emboli, and could not be recorded in front of it (right CRAO). In contrast, the left eye had the normal aspect on CDI of retrobulbar vessels, including left CRAO.

Right internal carotid artery (ICA) EDS examination, and CT-A identified a severe stenosis at its origin. TTE, the sonography of the TAs, and laboratory data were all normal, the only exception being an increased ESR (40 mm/hr). After eleven months, a diminished arterial flow signal could be detected at the level of the right CRA (Figure 1c).

Case 2 – Central retinal artery obstruction with vasculitis mechanism, due to occult giant cell arteritis

A 71-year old hypertensive man presented with CRAO of the right eye, with the abrupt painless severe loss of vision of the right eye (visual acuity 0.1), with normal anterior segment examination in both eyes, and a fundus of the right

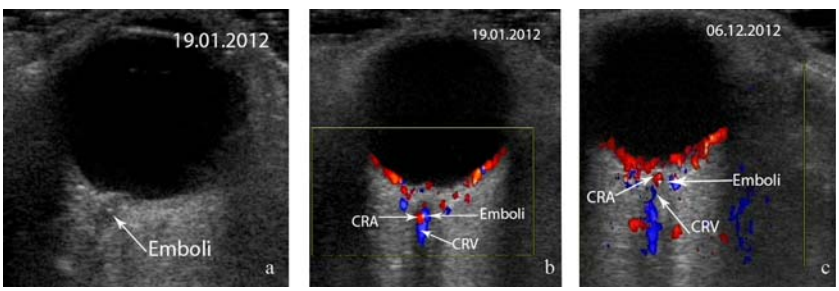

Fig. 1 – First patient: a) B-scan ultrasound evaluation of the right eye; b) Color Doppler imaging (CDI) of the right central retinal artery; c) CDI of the right central retinal artery after 11 months.

eye with ischemic whitening of the retina, and a cherry-red spot in its center. The site of obstruction of the right CRA was not visible on ophthalmoscopy.

The patient developed moderate right temporal headache, one week before presentation in our departments. The superficial TAs were normal at clinical examination, including TA's pulsation. He did not present associated systemic symptoms: fever, fatigue, and/or malaise.

A normal ESR (8 mm/hr), and the elevated CRP (6.4 mg/L) were revealed in this patient; the other laboratory data were all normal.

EDS investigated almost completely the whole length of the common superficial TAs, including the frontal and parietal branches [6, 7], and found only dark hypoechoic circumferential wall thickening (halo) around the lumen of a segment of the frontal branch of the right TA. Normal US patterns were found in all the other branches of the two external carotid arteries and for the other extracranial vessels (facial arteries, etc). TAB was guided by Doppler US of the TAs at the level of the affected segment of the frontal branch of the right TA. We observed characteristic lessions for GCA: intimal thickening, internal limiting lamina fragmentation, and chronic inflamatory infiltrate with giant cells [8].

Spectral Doppler analysis of retrobulbar vessels revealed in this case severely diminished blood flow velocities especially end-diastolic velocities (EDV) in both CRA (Figures 2a and 2b), normal values: peak systolic velocity (PSV) 17.3 ± 2.6 cm/s; EDV 6.2 ± 2.7 cm/s [4, 5], despite the fact that the left eye had the normal aspect at ophthalmoscopy. Less abnormalities were observed in the PCAs (Figures 2c and 2d), (normal values for temporal PCA: PSV: 13.3 ± 3.5 cm/s; EDV: 6.4 ± 1.5 cm/s; normal values for the nasal PCA: PSV: 12.4 ± 3.4 cm/s; EDV: 5.8 ± 2.5 cm/s) [4, 5], and in the OAs (normal values: PSV: 45.3 ± 10.5 cm/s; EDV: 11.8 ± 4.3 cm/s) [4, 5]. CT-A, and TTE were normal in this case. CT-scanning excluded strokes in both presented patients.

Discussion

Since there are no functional anastomoses between choroidal (nasal, and temporal PCAs) and retinal circulation (CRA), CRAO determines severe and permanent loss of vision, as mentioned in different studies [1–3, 9–16]. Therefore, it is very important to identify the cause of CRAO, in order to protect the contralateral eye [1–3, 9–16]. According to Gonzales-Gay [8], and Gonzales-Gay et al. [16] the majority of GCA patients with CRAO develop the classic clinical symptoms of GCA: new moderate bitemporal headache, scalp tenderness, and abnormal TAs on palpation (tender, nodular, swollen, and thickened arteries). However, in the case at hand, the second patient presented developed only new moderate right temporal headache.

Gonzales-Gay [8] and Gonzales-Gay et al. [16], along with Duker et al. [3], Connolly et al. [10], and Foroozon et al. [15] continue to argue that most of the patients with GCA and CRAO present systemic symptoms: fever, fatigue, malaise, and weight loss. Contrary to what they found, the second patient with CRAO due to GCA did not show systemic symptoms. Nevertheless, a study of Gonzales-Gay et al. [16] show that 21% of the patients with positive TAB for GCA have no systemic symptoms or signs and the only presenting sign was visual loss. He named this type of GCA occult GCA [8, 16], which matched the profile of our second patient.

Lopez-Diaz et al. [17] note that the ESR is often very high in GCA, with the levels more than 50 mm/hr (fairly suggestive of this disease). In interpreting the ESR, he observes that the levels of 40 mm/hr may be normal in the elderly [17] (as we found in our first case with CRAO due to embolic mechanism) and cases of biopsy-proven GCA have been reported in patients with ESR levels lower than 30 mm/hr [17]. In his study, approximately 20% of the patients who have a positive TAB for GCA present a normal ESR [17] (like in our second case). Lopez-Diaz et al. [17] concluded that "normal" ESR does not rule out GCA. CRP is generally raised in GCA (the normal range is < 5mg/L) [6, 8, 16]. It generally runs parallel with ESR, and may be helpful when the ESR is equivocal [6, 8, 16]. However, in some cases, Gonzales-Gay [8] and Gonzales-Gay et al. [16] demonstrated ESR elevation but not CRP. In their opinion, the combination of ESR and CRP together gives the best specificity (97%) for detection of GCA [8, 16].

Schmidt et al. [7], and Arida et al. [18] demonstrate that EDS examination of the TAs in temporal arteritis has garnered con-

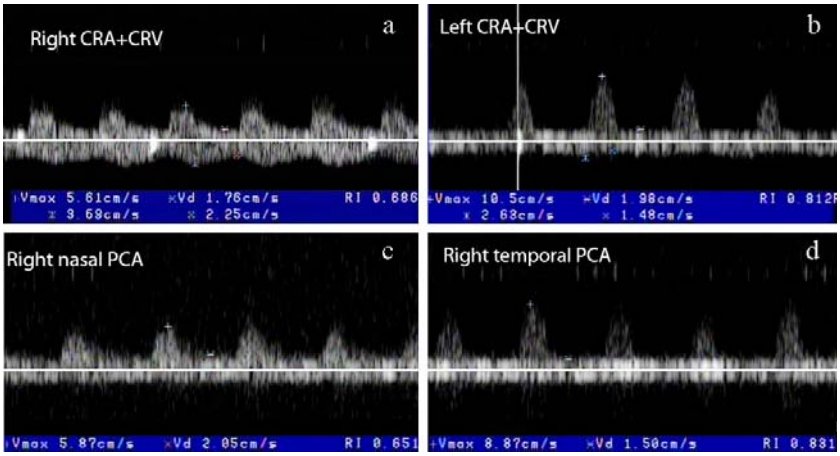

Fig. 2 – Second patient: a-d) Spectral Doppler analysis of retrobulbar vessels.

siderable interest as a GCA diagnosis tool, because it indicates segmental inflammation of TAs. A meta-analysis of Arida et al. [18] confirm that the halo sign in US is useful in diagnosing GCA. US may also detect inflamed TAs in patients with clinically normal TAs [6, 7, 18], as we observed in our second case.

Schmidt et al. [7] compared the results of TAs EDS examinations with the occurrence of visual ischemic complications (CRAO, arteritic anterior ischemic optic neuropathies, etc) in patients with newly diagnosed active GCA. However, findings of TAs EDS did not correlate with eye complications. For this reason, CDI of retrobulbar vessels is of critical importance. In Foroozon et al.'s [15] opinion, this technique is able to detect certain orbital vascular abnormalities in patients with CRAO, because it indicates the direction of blood flow, and allows calculation of the PSV, EDV, and the mean velocities of flow, and estimation of the resistence index (RI) of these vessels. These abnormalities are not detected by the standard diagnostic modalities now used to evaluate permanent monocular blindness [6, 15]. In the first case, diagnostic imaging (including CT-A, neurosonological investigations, ECG, TTE, etc) revealed a large-artery atherosclerosis etiology (ICA's severe stenosis) for CRAO [2, 3, 10, 11, 13, 15, 19].

According to Duker [3], less than one third of CRAO results from emboli. We did not perform prolonged cardiac monitoring in both cases for detection of paroxysmal AF, because, according to the Rabinstein study, there were no risk factors for paroxysmal AF detection (left dilatation on TTE, frequent premature atrial complexes on ECG, etc) [20].

Platelets and fibrin are the materials found in cardiac emboli [3, 19], which was not the case of our first patient (emboli of cholesterol). Duker [3] noted that cholesterol emboli typically emanate from atheromatous plaques of the ipsilateral ICA. In the first preseted case (CRAO with artery to artery embolism), detecting by B-scan US evaluation the retrobulbar embolic material interrupting the pixels of color of the right CRA was helpful in eliminating the diagnosis of GCA with eye involvment [15]. When CDI localizes retrobulbar embolus, the patient does not have to be subjected to high-dose corticosteroids, even if the ESR is elevated, like in the first case [3, 11, 15]. The patient received antiplatelet aggregating agents and statins before right carotid endarterectomy. In the second case (CRAO with vasculitic mechanism, due to GCA), the patient had the normal ESR without systemic/clinical symptoms, even a swollen TA (occult GCA) [8, 16]. His spectral Doppler analysis of the orbital vessels revealed characteristic CDI findings for GCA (severe diminished blood flow velocities, especially EDV, in retrobulbar vessels, especially in CRAs) [6, 11, 15]. In patients with CRAO due to occult GCA prompt recognition and early corticotherapy are crucial to prevent further visual loss in the controlateral eye [3, 6, 8, 10–15].

Conclusion

In the presented cases, ultrasound investigation enabled prompt differentiation (when no emboli are visible on ophthalmoscopy in the retinal circulation) between central retinal artery occlusion of embolic mechanism due to severe stenosis of the ipsilateral internal carotid artery and central retinal artery occlusion caused by vasculitis from ocult giant cell arteritis.

REFERENCES

1. *Hayreh SS*. Vascular disorders in neuro-ophthalmology. Curr Opin Neurol 2011; 24(1): 6−11.
2. *Ahuja RM, Chaturvedi S, Eliott D, Joshi N, Puklin JE, Abrams GW*. Mechanisms of retinal arterial occlusive disease in African American and Caucasian patients. Stroke 1999; 30(8): 1506−9.
3. *Duker JS*. Retinal arterial obstruction. In: *Yanoff M, Duker JS, Augsburger JJ,* editors. Ophtalmology. 2nd ed. St. Louis, MO: Mosby; 2004. p. 856−63.
4. *Pichot O, Gonzalvez B, Franco A, Mouillon M*. Color Doppler ultrasonography in the study of orbital and ocular vascular diseases. J Fr Ophtalmol 1996; 19(1): 19−31. (French)
5. *Lieb WE, Cohen SM, Merton DA, Shields JA, Mitchell DG, Goldberg BB*. Color Doppler imaging of the eye and orbit. Technique and normal vascular anatomy. Arch Ophthalmol 1991; 109(4): 527−31.
6. *Jianu DC, Jianu SN*. Chapter 5. Giant Cell Arteritis and Arteritic Anterior Ischemic Optic Neuropathies. In: *Sakkas LI, Katsiari C,* editors. Updates in the Diagnosis and Treatment of Vasculitis. Rijeka: Intech; 2013. p. 111−30.
7. *Schmidt WA, Kraft HE, Vorpahl K, Völker L, Gromnica-Ihle EJ*. Color duplex ultrasonography in the diagnosis of temporal arteritis. N Engl J Med 1997; 337(19): 1336−42.
8. *Gonzalez-Gay MA*. The diagnosis and management of patients with giant cell arteritis. J Rheumatol 2005; 32(7): 1186−8.
9. *Hayreh SS, Zimmerman BM*. Fundus changes in central retinal artery occlusion. Retina 2007; 27(3): 276−89.
10. *Connolly BP, Krishnan A, Shah GK, Whelan J, Brown GC, Eagle RC*, et al. Characteristics of patients presenting with central retinal artery occlusion with and without giant cell arteritis. Can J Ophthalmol 2000; 35(7): 379−84.
11. *Jianu DC, Jianu SN*. Chapter 6-The role of Color Doppler Imaging in the study of central retinal artery obstruction. In: *Jianu DC, Jianu SN*, editors. Color Doppler Imaging. Neuro-ophthalmological correlations. Timisoara, Romania: Mirton; 2010. p. 125−42. (Romanian)
12. *Jianu DC, Jianu SN, Petrica L*. Color Doppler Imaging of retrobulbar vessels findings in large giant cell arteritis with eye involvement. J US-China Med Sci 2011; 8(2): 99−108.
13. *Jianu DC, Jianu SN, Petrica L, Serpe M*. Chapter 16-Large giant cell arteritis with eye involvement. In: *Amezcua-Guerra LM*, editor. Advances in the diagnosis and treatment of vasculitis. Rijeka, Croatia: Intech; 2011. p. 311−30.
14. *Jianu SN, Jianu DC*. Color Doppler imaging and central retinal artery occlusion. Oftalmologia 2011; 55(4): 55−65. (Romanian)
15. *Foroozan R, Savino PJ, Sergott RC*. Embolic central retinal artery occlusion detected by orbital color Doppler imaging. Ophthalmology 2002; 109(4): 744−7.
16. *González-Gay MA, García-Porrúa C, Llorca J, Hajeer AH, Brañas F, Dababneh A*, et al. Visual manifestations of giant cell arteritis. Trends and clinical spectrum in 161 patients. Medicine (Baltimore) 2000; 79(5): 283−92.
17. *Lopez-Diaz MJ, Llorca J, Gonzalez-Juanatey C, Peña-Sagredo JL, Martin J, Gonzalez-Gay MA*. The erythrocyte sedimentation rate is associated with the development of visual complications in

biopsy-proven giant cell arteritis. Semin Arthritis Rheum 2008; 38(2): 116−23.

18. *Arida A, Kyprianou M, Kanakis M, Sfikakis PP*. The diagnostic value of ultrasonography-derived edema of the temporal artery wall in giant cell arteritis: a second meta-analysis. BMC Musculoskelet Disord 2010; 11(1): 43−52.
19. *Sacco RL, Toni D, Brainin M, Mohr JP*. Chapter 4. Classification of ischemic stroke. In: *Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA*, editors. Stroke: pathophysiology, diagnosis and management. 4th ed. Philadelphia, Pa, USA: Elsevier Saunders; 2004. p. 61−70.
20. *Rabinstein AA*. Prolonged cardiac monitoring for detection of paroxysmal atrial fibrillation after cerebral ischemia. Stroke 2014; 45(4): 1208−14.

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CASE REPORT

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Solitary extramedullary plasmacytoma of the duodenum and pancreas – A case report and review of the literature

Solitarni ekstramedularni plazmocitom duodenuma i pankreasa

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Abstract

Introduction. The extramedullary plasmacytomas (EMPs) are rare tumors of plasma cell disorders which are rarely found in the duodenum. We presented a case of solitary EMPs involving the duodenum and pancreas successfully treated by surgical resection after failure of chemotherapy. **Case report.** A 55-year-old female with previously diagnosed solitary EMP of the duodenum was admitted to our institution after failure of three cycles of vincristine, adriablastine, dexamethasone (VAD) chemotherapy regimen with an upper gastrointestinal obstruction. On admission computed tomography of the abdomen showed tumor in the region of the second part of duodenum and uncinate process of the pancreas with a complete duodenal obstruction. Intraoperatively a tumor formation was in the region of the second duodenal part, originated from the wall of duodenum with the total diameter of 7 × 5 cm, covering the entire circumference of duodenal wall leaded to a narrowing of duodenal lumen to the thigh gap with an upper gastrointestinal obstruction. Infiltration in the head of the pancreas and uncinate process were also found. The Whipple's procedure was performed but postoperative course was complicated by rapidly refilling chylous ascites which was resolved 4 days after the surgery. **Conclusion.** Each patient with gastrointestinal EMPs should be considered separately and in timely manner, thus adequate treatment could provide local disease control.

Key words:
plasmacytoma; duodenum; pancreas; intestinal obstruction; surgical procedures, operative; postoperative period; ascites; treatment outcome.

Apstrakt

Uvod. Ekstramedularni plazmocitomi (EMP) su retki tumori koji pripadaju plazmaćelijskim oboljenjima sa retkom lokalizacijom u duodenumu. Prikazali smo bolesnicu sa solitarnim EMPs i zahvatanjem duodenuma i pankreasa uspešno izlečenu hirurškom resekcijom, nakon neuspeha hemioterapije. **Prikaz bolesnika.** Bolesnica, starosti 55 godina, sa prethodno dijagnostikovanim solitarnim EMP duodenuma i pankreasa primljena je u našu ustanovu nakon neuspeha u lečenju sa tri ciklusa hemioterapije po vinkristin, adriablastin, deksametazon (VAD) protokolu, sa visokom gastrointestinalnom opstrukcijom. Na prijemu, kompjuterizovanom tomografijom abdomena otkriven je tumor u predelu drugog dela duodenuma i uncinatnog nastavka pankreasa, sa potpunom opstrukcijom duodenuma. Intraoperativno, nađen je tumor u predelu drugog dela duodenuma, koji je poticao iz zida duodenuma, sa prečnikom od 7 × 5 cm, koji je zahvatao kompletnu cirkumferenciju zida duodenuma dovodeći do sužavanja lumena duodenuma na usku pukotinu sa visokom gastrointestinalnom opstrukcijom. Takođe, nađena je i infiltracija glave pankreasa i uncinatnog nastavka. Primenjena je Viplova procedura, ali se postoperativni tok komplikovao nastankom obilnog hiloznog ascita, što je sanirano četiri dana nakon operacije. **Zaključak.** Svakog bolesnika sa gastrointestinalnom lokalizacijom EMP trebalo bi posmatrati zasebno, a blagovremeno i adekvatno lečenje može omogućiti lokalnu kontrolu bolesti.

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Ključne reči:
plazmocitom; duodenum; pankreas; creva, opstrukcija; hirurgija, operativne procedure; postoperativni period; ascit; lečenje, ishod.

Introduction

Plasmacytoma derives from clonal proliferation of plasma cells and can be classified as an osseous disease or extraosseous tumor. It appears in three clinical forms: multiple myeloma (MM), medullary plasmacytoma (MP) and extramedullary plasmacytoma (EMP) [1].

Solitary EMPs are rare tumors constituting fewer than 5% of all plasma cell tumors. These tumors could originate in a variety of anatomical sites, but most often, approximately

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90% of cases, have been reported from the upper respiratory tract. Less than 10% of EMPs occur in the gastrointestinal tract, whereas the stomach is the most frequent site of its occurrence [2, 3]. There are only several cases described in the literature of EMP in the duodenum and few cases involving the pancreas or both duodenum and pancreas [4–18].

We presented a case with solitary EMPs in the duodenum and pancreatic head infiltration with an upper gastrointestinal obstruction, surgically treated after failure of chemotherapy.

Case report

A 55-year-old Caucasian female was admitted to our institution due to deterioration of the general condition, persistent vomiting and weight loss of 5 kg a month before the hospitalization. On admission all laboratory findings including bilirubin were in normal ranges with the exclusion of the erythrocyte sedimentation rate of 58 mm/h (normal range: 0–20 mm/h), alkaline phosphatase of 281 U/L (normal range: 20–140 U/L) and lactate dehydrogenase of 344 U/L (normal range: 140–280 U/L).

Almost a year before, the patient was admitted to our hospital also with severe abdominal pain, whereas abdominal ultrasound showed tumor formation in the upper right region with the diameter of 10 × 5 cm. Computed tomography (CT) scan showed enlargement of the head of pancreas and stenosis of duodenum in the region distally of the duodenal papilla of the length of 7 cm. In the same hospitalization esophagogastroduodenoscopy showed normal duodenal mucosa but narrowed duodenal lumen of 6 cm in length in the region of the second and the third part of the duodenum. Biopsies from that region showed chronic lymphoproliferative plasma cell infiltration which corresponded to solitary EMPs of duodenum, immunoglobulin A (IgA) lambda chain. The application of diagnostic criteria, including bone marrow biopsy, did not show the existence of MM. Considering the tumor size and localization, the patient's age, and propensity for gastrointestinal occlusion, it was decided that the patient begin receiving VAD (vincristine, adriablastine, dexamethasone) chemotherapy regimen protocol.

After the third cycle of VAD chemotherapy regimen, multislice CT abdominal scan did not show any improvement in the reduction of tumor mass (Figure 1). Due to impairment of the patient general condition (vomiting and weight loss) and evident failure of chemotherapy, it was decided to perform surgical intervention.

With the open surgical approach and medial laparotomy we found intraoperatively a tumor formation in the region of the second duodenal portion, the first and the second duodenal knee, originated from the wall of the duodenum with the total diameter of 7 × 5 cm. The tumor covered the entire circumference of the duodenal wall leading to duodenal lumen narrowing to a tight gap with upper gastrointestinal obstruction and moderate *gastrectasia*. The tumor consistency was solid with the infiltration of head of the pancreas and uncinate process in the anterior segments, but without involvement of the portal vein and superior mesenteric vessels (Figures 2 and 3).

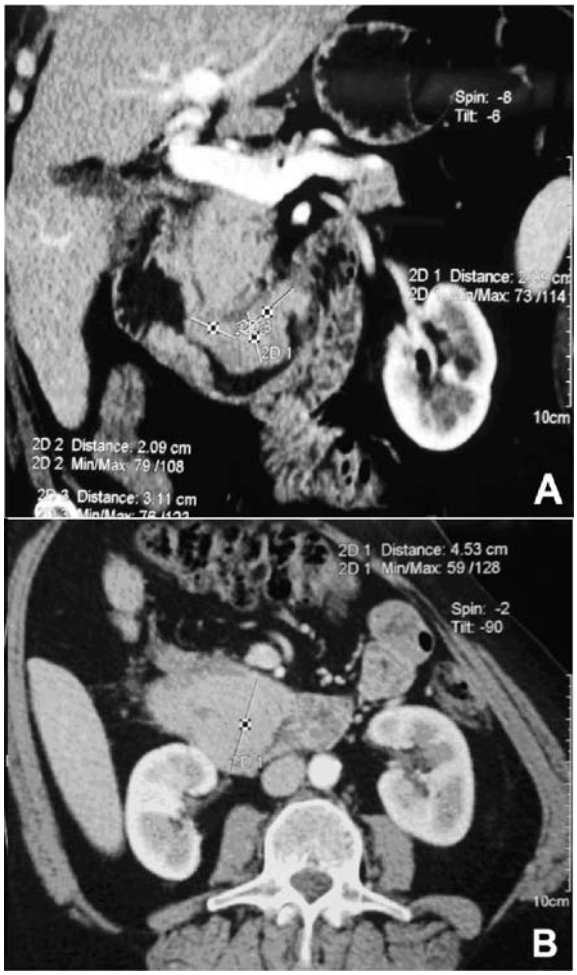

Fig. 1 – A) Multislice computed tomography abdominal scan showing tumor in the wall of the second part of the duodenum (frontal section); B) tumor in the region of the second duodenal knee and uncinate process with complete duodenal obstruction (transversal section).

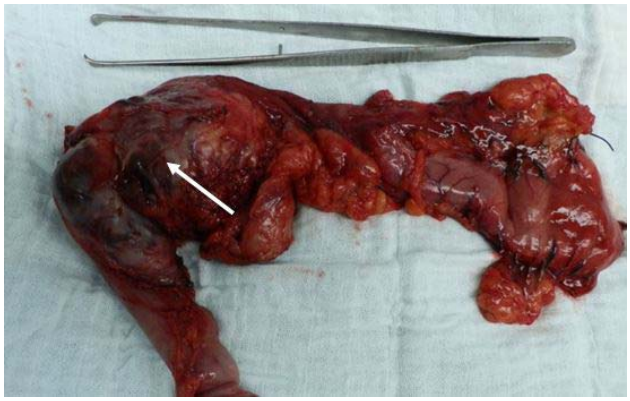

Fig. 2 – Tumor resection specimen, the duodenum, a part of the stomach, pancreatic head and the first jejunal limb (arrow shows the tumor in the second duodenal portion and the head of the pancreas).

No other findings were observed during the surgery. We performed the Whipple's procedure with one bore drain placement. Postoperatively, the patient presented with chylous ascites approximately 1,500 mL daily that diminished and resolved on the postoperative day 4 (Figure 4). However, on the postoperative day 6 a low-output external

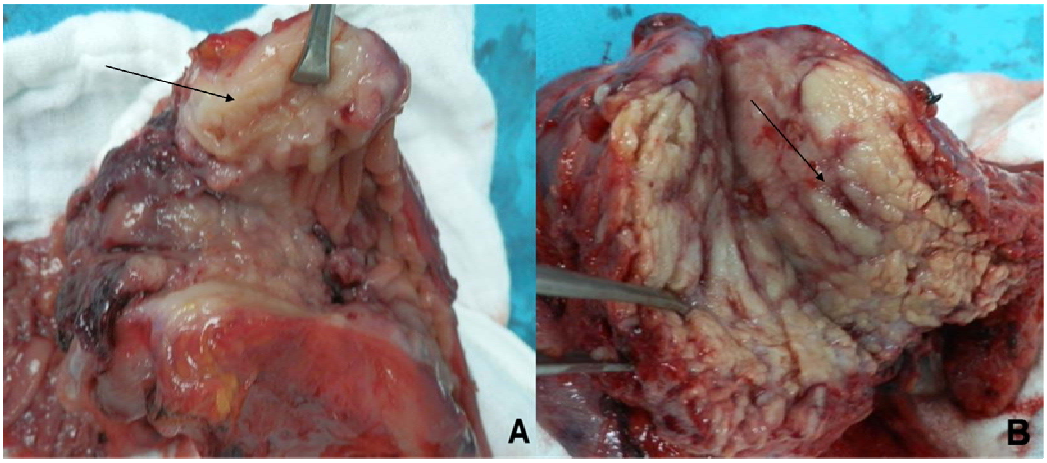

Fig. 3 – A) Longitudinally transected duodenum with infiltration in the duodenal wall by the tumor (arrow); B) The transected pancreatic head shows infiltration in the pancreas by the tumor (arrow).

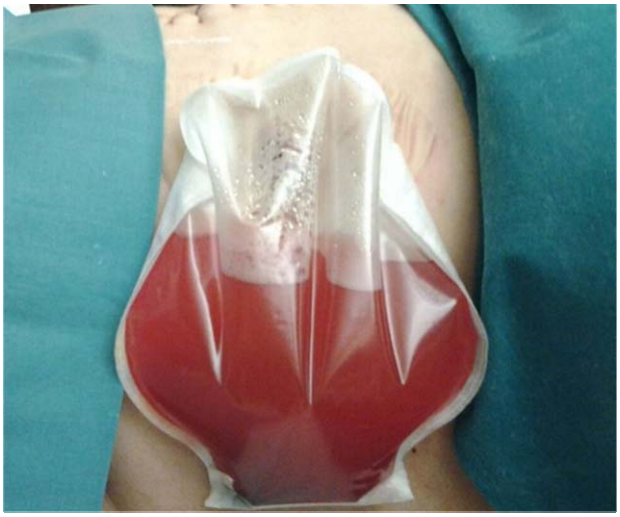

Fig. 4 – Chylous ascites in the drainage bag on the day 2 postoperatively.

pancreatic fistula occurred, and resolved during conservative treatment on the postoperative day 10. The postoperative treatment was based on the control of infection process and low-fat oral diet with medium-chain triglycerides after the introduction of *per* oral food intake on the day 5 after the surgery. The drain was removed on the postoperative day 12, and patient was discharged two weeks after the surgery.

Histopathological examination showed diffuse neoplastic proliferation of plasma cells in duodenal submucosa and pancreatic tissue, with tumor cells positivity for MUM-1, CD138 and λ light chain expression on immunohistochemical studies (Figure 5).

On follow-up 3 months later the patient had no gastrointestinal problems, disease recurrence, nor activity on positron emission tomography scan.

Discussion

The diagnostic criteria of EMPs are: 1) solitary plasma cell tumor; 2) histological confirmation; 3) normal bone marrow, taken from a distant site with less than 5% of plasma cells; and 4) low concentration of monoclonal paraprotein [1, 3]. The upper respiratory tract including oropharynx, nasopharynx, nasal cavity, sinuses and larynx are the most common sites of solitary EMPs. Approximately 7% of all solitary EMPs can be found in gastrointestinal tract [2, 3, 11]. The most affected organs include the stomach, small bowel, while the colon and esophagus are involved rarely [11]. There are only 11 cases in the literature with duodenal localization of solitary EMP, but only 3 cases with pancreas involvement and two cases with duodenal and pancreas involvement until now [4–14, 19]. In all described cases with solitary EMP involving both duodenum and pancreas or just pancreas, patients had obstructive jaundice that was not present in our case. In our patient a plasma cell infiltrate has spread in the area out of the Vater's ampulla and the main pancreatic duct, mainly in the anterior segments of pancreatic head what was the reason of the jaundice absence. However, nausea, pain, vomiting, upper gastrointestinal bleeding or weight loss occurred in almost all described cases. Also, the presented patient was the first one with upper gastrointestinal obstruction caused by EMPs. Excluding malignant tumors originating from the stomach, duodenum, pancreas and biliary tree there are not many pathological conditions reported to cause upper ileus [20].

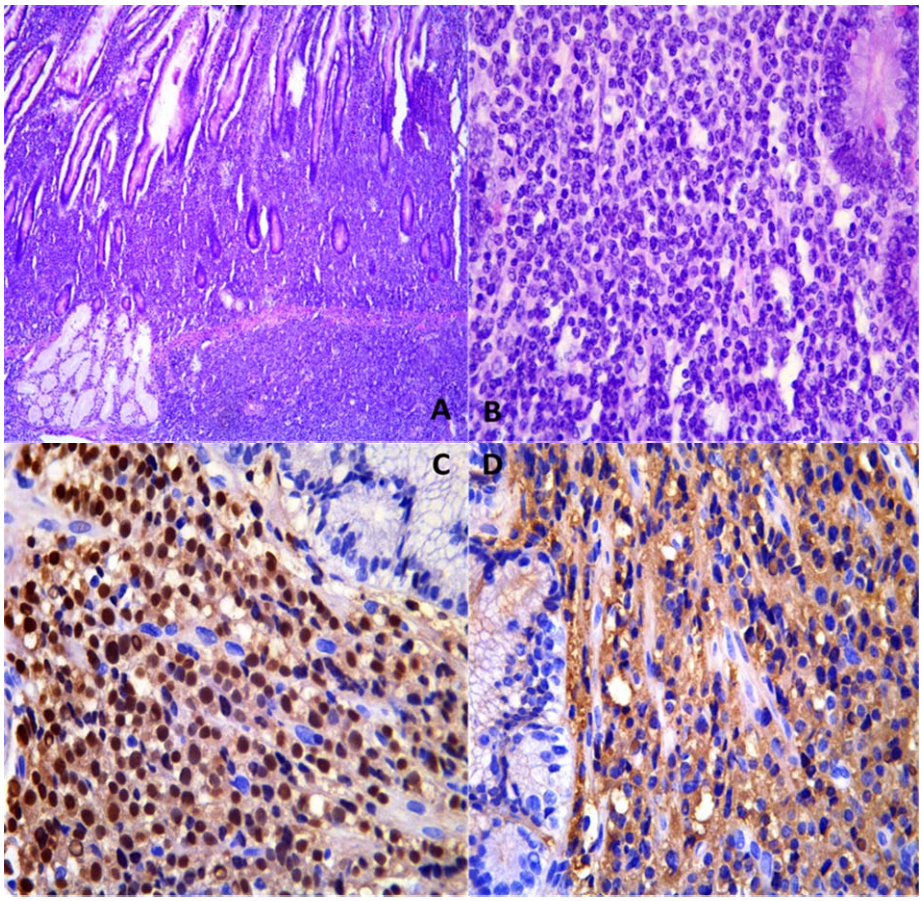

Fig. 5 – A, B) The tumor forms a submucosal duodenal nodule with diffuse proliferation of mildly pleomorphic plasma cells [(A) HE, × 10; B) HE, ×20)]; C) The tumors cells demonstrating strong diffuse positivity for MUM-1 (× 40); D) Diffuse cytoplasmic λ light chain expression in primary duodenal plasmacytoma and CD138 positivity (× 40).

The diagnosis of EMPs comprises in fact the exclusion of other two from plasmacytoma, both MM and MP. A localization and extent of the tumor could be evaluated by the radiological imaging procedures which include CT scan and magnetic resonance imaging. Interestingly, our case was the third reported with imaging of duodenal involvement by solitary EMPs in the literature data so far [14, 17]. The first case was published by Magagnoli et al. [17], and a recently published case by Karam et al. [14] was the second reported with imaging of duodenal localization of EMPs with the explanation that the most of the published cases appeared in gastroenterology journals. Therefore, our case was the first one reported with duodenal and pancreatic localization of solitary EMP with both imaging and specimen figures. With regard to neoplastic proliferation of plasma cells in the stomach, primary plasmacytoma should always be distinguished from the other lymphoproliferative disorders with plasmacytic differentiation. Immunohistochemical analysis of the resected specimen in the presented patient showed that tumor cells were diffusely CD138, MUM-1, and IgA positives, and CD5 negative.

Therapeutic strategies for solitary plasmacytoma and EMP include surgery, chemotherapy, radiotherapy, along with combined approaches [2]. Although it was showed that solitary plasmacytoma are highly chemoradiosensitive [3, 21, 22], many cases of EMP are reported to be insensitive to radiotherapy and chemotherapy [2, 19, 23]. In eligible patients, radiation therapy in the dose of 40–50 Gy has been suggested providing an excellent locoregional disease control [24]. However, in that study the majority of patients were with EMPs in the region of the neck and head, and in only two patients EMPs was localized in the abdominal cavity. A relatively small number of inconclusive data are available for analysis of the natural history and treatment for EMPs of the gastrointestinal tract. Some reported cases showed poor sensitivity of EMPs to radiochemotherapy [2, 19, 23], suggesting aggressive course of the disease. Others favor radiation therapy over chemotherapy in the treatment of solitary plasmacytoma [25], and in cases of extramedullary spread high dose chemotherapy followed by stem-cell transplantation have been suggested as standard treatment [11, 14]. However, it was considered that gastrointestinal involvement of MM has a very poor prognosis even with aggressive treatment [26], and surgical resection should be implemented only in cases with local complications by the mass itself [11]. As radiation therapy was used in the past for EMPs treatment, and the standard first line chemotherapy regimen for EMPs failed, in addition to occurrence of local complications in our patient, we decided to perform surgical intervention. In the literature data there are only few patients with EMP localized in the region of duodenum and pancreatic head who underwent surgery. Our case was the third patient

reported with EMP of this localization in whom radical pancreaticoduodenectomy (Whipple's procedure) was performed [4, 9]. In case of the three other patients, one was treated with local excision succumbing 15 months later to MM [7], the second one only with gastrointestinal by-pass without resection [5], whilst the third case was operated with no data regarding the surgical procedure [18].

The ascites and pleural effusion occur in approximately one-third of patients with plasma cell disorder. Those clinical features could be found solitary or as a part of POEMS or Crow-Fukase syndrome in patients with plasma cell disorders, predominantly in patients with MM [27]. The pathogenesis of this syndrome, mainly edema, pleural effusions and ascites development, is attributed to markedly increased plasma and serum levels of vascular endothelial growth factor (VEGF) in patients with MM. The VEGF is secreted from plasma cells and platelets promoting vascular permeability, angiogenesis and monocyte/macrophage migration. There are no literature data regarding etiopathogenesis of the ascites and/or pleural effusion occurrence in patients who underwent surgery for EMP although a case was reported with plasmacytoma of the ovary and stormy postoperative course complicated by rapidly refilling ascites and pleural effusion [28]. Such complications may be attributed to angiogenesis, especially to lymphangiogenesis, in the field of plasma cell disorder, VEGF hypersecretion and operative trauma. The drainage of chylous ascites in postoperative course burdened our patient also, however, it was diminished and resolved shortly after the surgery. Unfortunately, we had no possibility to evaluate the drainage fluid due to technical circumstances. In addition to the aforementioned case of plasmacytoma of the ovary, our patient was another with this unusual complication but the first one with duodenal localization of EMPs.

The most effective therapy for gastrointestinal EMPs cannot be determined from this small case series. It is obvious that some of these patients with duodenal plasmacytoma are insensitive to irradiation and/or chemotherapy, whereas surgery could provide local disease control not only in cases of complications. When the first line chemotherapy fails, surgical procedure should be considered timely to prevent the development of possible complications.

Conclusion

Solitary EMPs are rare tumors of plasma cell disorders and even more rarely with duodenal and pancreas involvement. The presented patient did not respond to chemotherapy and was operated on with success. The Whipple's procedure is the optimal surgical procedure in cases of EMPs in the duodenum and pancreas. Each patient with gastrointestinal EMPs should be considered separately and timely providing him/her an adequate treatment which can assure local disease control.

REFERENCES

1. *Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H,* et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: International Agency for Research on Cancer (IARC); 2008.
2. *Yoshida T, Soda K, Yamada S, Nakahara M, Nishida J, Kametaka M,* et al. Biclonal extramedullary plasmacytoma arising in the peritoneal cavity: report of a case. Surg Today 2004; 34(4): 379−82.
3. *Nolan KD, Mone MC, Nelson EW*. Plasma cell neoplasms. Review of disease progression and report of a new variant. Surg Oncol 2005; 14(2): 85−90.
4. *Schoretsanitis G, Livingstone JI, El-Japour JN , Watkins N, Wastell C*. Duodenal plasmacytoma: a rare extramedullary localization simulating carcinoma of the head of the pancreas. Postgrad Med J 1994; 70(823): 378−9.
5. *Hefferman A*. Plasmacytoma of pancreas and duodenum causing acute intestinal obstruction. Lancet 1947; 249(6461): 910.
6. *Derechin MM, Goldberg LS, Herron L*. Extraosseous Plasmacytomas Causing Extrahepatic Cholestasis and Cardiac Tamponade: A Unique Case of Multiple Myeloma. Scand J Haemtol 1970; 7(5): 318−21.
7. *Pentimone F, Camici M, Cini G, Levorato D*. Duodenal plasmacytoma. A rare primary extramedullary localization simulating a carcinoma. Acta Haematol 1979; 61(3): 155−60.
8. Hirakawa T, Fukuda Y, Tabata M, Hatanaka Y, Aihara K, Suzuki T, et al. Primary duodenal submucosal plasmacytoma - an autopsy case. Nihon Rinsho 1978; 36(9): 3278−9. (Japanese)
9. *Mannell A*. Primary isolated extramedullary plasmacytoma of the duodenum. Aust NZ J Surg 1979; 49(5): 577−80.
10. *Spalding DR, Isla AM, Thompson JN, Williamson RC*. Pancreas-sparing distal duodenectomy for infrapapillary neoplasms. Ann R Coll Surg Engl 2007; 89(2): 130−5.
11. *Telakis E, Tsironi E, Tavoularis G, Papatheodorou K, Tzaida O, Nikolaou A*. Gastrointestinal involvement in a patient with multiple myeloma: A case report. Ann Gastroenterol 2009; 22(4): 287−90.
12. *Gradishar W, Recant W, Shapiro C*. Obstructing plasmacytoma of the duodenum: first manifestation of relapsed multiple myeloma. Am J Gastroenterol 1988; 83(1): 77−9.
13. *Siddique I, Papadakis KA, Weber DM, Glober G*. Recurrent bleeding from a duodenal plasmacytoma treated successfully with embolization of the gastroduodenal artery. Am J Gastroenterol 1999; 94(6): 1691−2.
14. *Karam AR, Semaan RJ, Buch K, Shankar S*. Extramedullary duodenal plasmacytoma presenting with gastric outlet obstruction and painless jaundice. J Radiol Case Rep 2010; 4(8): 22−8.
15. *Carneiro FP, Sobreira MN, Maia LB, Sartorelli AC, Franceschi LE, Brandão MB,* et al. Extramedullary plasmocytoma associated with a massive deposit of amyloid in the duodenum. World J Gastroenterol 2009; 15(28): 3565−8.
16. *Fowell AJ, Poller DN, Ellis RD*. Diffuse luminal ulceration resulting from duodenal plasmacytoma. Gastrointest Endosc 2007; 65(4): 707−8.
17. *Magagnoli M, Pedicini V, Rahal D, Santoro A*. Solitary plasmacytoma of the duodenum. Am J Hematol 2004; 76(2): 185−6.
18. *Chen CH, Yang CC, Yeh YH, Kuo CL*. Duodenal plasmacytoma. Gastrointest Endosc 2001; 54(6): 753.
19. *Ali R, Ozkalemkas F, Ozcelik T, Ozkan A, Ozkocaman V, Ozturk H,* et al. Extramedullary plasmacytoma involving the abdominal vessels and pancreas. Dig Dis Sci 2007; 52(11): 3199−201.
20. *Bezmarević M, Mirković D, Perisić N, Cerović S, Panisić M, Micković S,* et al. Sclerosing mesenteritis as a rare cause of upper ileus. Vojnosanit Pregl 2013; 70(3): 326−30.
21. *Kadokura M, Tanio N, Nonaka M, Yamamoto S, Kataoka D, Kushima M,* et al. A surgical case of solitary plasmacytoma of rib

origin with biclonal gammopathy. Jpn J Clin Oncol 2000; 30(4): 191−5.
22. *Das-Gupta EP, Sidra GM, Bessell EM, Lush RJ, Byrne JL, Russell NH.* High-dose melphalan followed by radical radiotherapy for the treatment of massive plasmacytoma of the chest wall. Bone Marrow Transplant 2003; 32(8): 759−61.
23. *Damaj G, Mohty M, Vey N, Dincan E, Bouabdallah R, Faucher C,* et al. Features of extramedullary and extraosseous multiple myeloma: a report of 19 patients from a single center. Eur J Haematol 2004; 73(6): 402−6.
24. *Liebross RH, Ha CS, Cox JD, Weber D, Delasalle K, Alexanian R.* Clinical course of solitary extramedullary plasmacytoma. Radiother Oncol 1999; 52(3): 245−9.
25. *Hirata S, Yamaguchi K, Bandai S, Izumo A, Chijiiwa K, Tanaka M.* Secondary extramedullary plasmacytoma involving the pancreas. J Hepatobiliary Pancreat Surg 2002; 9(1): 111−5.
26. *Talamo G, Cavallo F, Zangari M, Barlogie B, Lee C, Pineda-Roman M,* et al. Clinical and biological features of multiple myeloma involving the gastrointestinal system. Haematologica 2006; 91(7): 964−7.
27. *Dispenzieri A, Gertz MA.* POEMS syndrome. Orphanet encyclopedia. Available from: http://www.orpha.net/data/patho/GB/uk-POEMS.pdf. [cited 2005 June 10].
28. *Shakuntala P, Praveen S, Shankaranand B, Rajshekar K, Umadevi K, Bafna U.* A rare case of plasmacytoma of the ovary: A case report and literature review. Ecancermedicalscience 2013; 7: 288.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

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References

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

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

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

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

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Primeri referenci:

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

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

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

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

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